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RESEARCH

Potential Application of Polysaccharide-based Aerogel Scaffolds for Bone Tissue Engineering

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ABSTRACT

In recent times, there has been a significant increase in bone-related diseases, posing a pressing challenge in the field of medicine. While bone tissues possess a natural self-healing capability, severe injuries can lead to a loss of this regenerative potential. Traditional transplantation approaches, despite being billion-dollar industries, are riddled with issues such as a scarcity of organ donors, a high risk of infections, and post-transplant complications. To address this issue, tissue engineering has demonstrated to be a possible alternative for wound remodeling and organ transplantation. Recently, biopolymer-based aerogel has caught tremendous attention as a result of its exceptional qualities in the field of biomedical engineering. This review aims to provide comprehensive information on the properties and recent research regarding the use of polysaccharides like chitosan, cellulose, alginate, hyaluronic acid, and starch-based aerogels in bone tissue engineering. It highlights the potential of these aerogels in addressing bone-related issues and discusses the obstacles and future prospects of polysaccharides in tissue engineering applications.

KEYWORDS

Bone regeneration, Aerogel, Chitosan, Alginates, Polysaccharides.

INTRODUCTION

Bone is a living tissue which provides structural foundation, and protection to our crucial organs. With the help of the local osteoprogenitor cells, bone tissues have a remarkable capacity for self-repair and spontaneous regeneration [**1**]. However, because of multiple factors, including injury, trauma, and several bone disorders such as osteoporosis, osteopenia, osteogenesis, arthritis, and bone cancer, it loses that property and may lead to serious health issues [**2**].

The National Osteoporosis Foundation report states that around 44 million people in America suffer from low bone density and 10 million have osteoporosis, putting them at higher risk [**3**]. It has also been reported that postmenopausal women, after the age of 45 or 50, are more likely than men to develop bone disease because estrogen levels, a hormone that protects bones, drop dramatically, resulting in bone loss [**4**]. Due to the situation, treatment of the injured areas is required to promote tissue regeneration. Traditional tissue restoration methods, including the use of bone grafts including autografts (bone tissue retrieved from the patient body) and allografts (bone tissue from a donor), have their limitations and associated drawbacks like donor site morbidity, the need for numerous surgeries, a high risk of infection, uncomfortable procedures, a scarcity of donors, and the potential for rejection [**5**]. Even though the supply of other treatments, such as medical equipment, pharmacological therapy, synthetic prostheses, and surgical reconstruction, is not constrained, they do have significant issues [**6**].

Tissue engineering technology offers new therapeutic possibilities and has proven to be a fantastic alternative to surgery and organ transplantation for the treatment of injured tissues and organs [**7**]. It is a novel interdisciplinary field of science that deals with the maintenance, restoration, and growth of tissue and organs. Bone tissue engineering (BTE) recently put forth as a substitute to standard therapies

for massive non-healing bone lesions, and it holds great possibilities for increasing bone regrowth and repair without the associated downsides. By incorporating autologous cells, tissue engineering (TE) eliminates the hazards of immunological reactions such as rejections (hyperacute and delayed), as well as pathogenic infections. The incorporation of three-dimensional polymeric scaffolds grafted at a tissue defect site is a common component of these techniques.

In the method of tissue engineering, the introduction of porous structures called scaffolds is an essential part because they can serve as a template for tissue development and maintain a surrounding to distribute substances such as metabolites and nutrients to cells [**8**]. Scaffold works as a supporting structure or mimics an extracellular matrix (ECM) to intensify the adhesion, cell proliferation, and cell differentiation at the site of impaired body tissues and organs. Precisely, the designed structure may affect the recipient after implantation by secreting osteogenic as well as vasculogenic growth factors (for example, by using a scaffold that releases growth factors, one that contains growth factor or their analogues, or one that is implanted with platelet-enriched plasma), or through housing cells that have been genetically modified to release growth factors or do so inherently. Several attempts have been made up to this point to create biomaterials that resemble the ECM by replicating its biological architecture and chemical characteristics [**9-11**].

Aerogel is classified as solid, light-weight, consistent open porous framework of roughly packed, entangled particles or nanoscale filaments that are produced from a gel after the pore fluid is removed without significantly changing the gel's structural integrity [**12**]. However, since its invention in 1931 by Kistler [**13**], aerogel research has been limited to a few specific formulations for about 70 years, including silica, various non-silica oxides, carbonised-RF (CRF) aerogel, resorcinol formaldehyde (RF) aerogel, and composites of aerogel [**14**]. At the commencement of the 21st century, aerogels attracted a tremendous amount of interest in the discipline of tissue engineering as it possesses various properties that are essential for tissue regeneration. It has several distinctive qualities that make it unique from the rest of other materials, including low thermal conductivity, transparency, agility, outstanding porosity, lightweight, density, vast surface area, strong mechanical strength, and an extremely low dielectric constant [**15**], as shown in **Fig. 1**. In addition to tissue engineering, its unique features make it useful for a range of other biomedical applications, which includes drug delivery, wound healing, biosensors, and diagnostics [**15**].

Synthetic polymer-based aerogels, including polycaprolactone (PCL), polylactic acid/polyglycolic acid, polyethylene oxide, and polybutylene terephthalate, have been employed to create porous, fibre, or matrix substrates that are biodegradable, but the main disadvantage of synthetic components is the absence of cell recognition response for tissue engineering; as a result, it is frequently more developed to employ natural materials that can mimic the characteristics of most tissues.

Aerogels made of polysaccharides belongs to a family of special functional materials that have various applications. Biobased aerogels offer a wide potential in the field of interdisciplinary and diverse scientific study because of their shared functions (bioactivity, biocompatible, biodegradability, and environmental friendliness) [**16**]. Freeze-drying, phase separation, particle leaching, and gas foaming are the various technologies which have been successfully used for the construction of aerogel scaffolds [**17**]. Although there has been a tremendous advancement in polysaccharide-based aerogels, on the road to BTE becoming an exact clinical reality, numerous significant obstacles still need to be overcome. The following review critically considers recent research work on aerogel-based scaffolds for bone tissue regeneration and challenges with polysaccharide-based scaffolds for BTE.

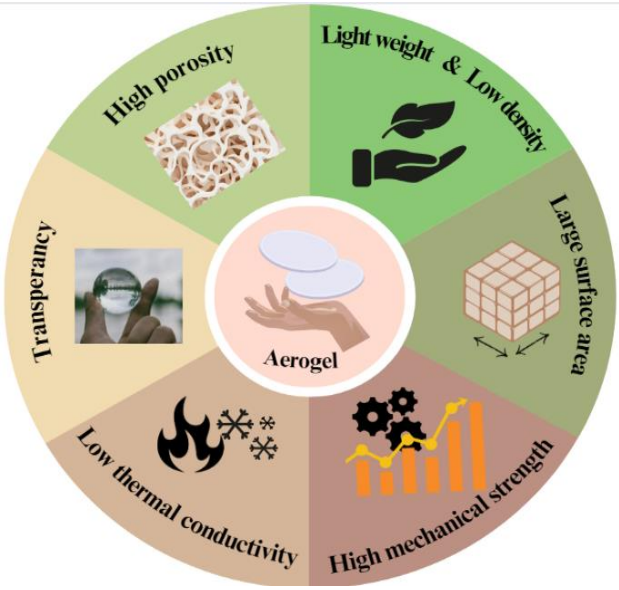

Fig. 1. Aerogel possesses several qualities that set it apart from other materials, for the use of applications such as biomedical, waste water treatment, cosmetic industry etc.

BONE TISSUE ENGINEERING

Tissue engineering attempts to repair tissues together with the development of new organs. With the rapid evolution of tissue engineering technology during the recent years, bone tissue engineering has arose as a promising strategy to repairing bone defects [**18**]. The goal of bone tissue engineering is to develop functional, living bone tissue which can integrate with the surrounding tissue and substitute the structure and functionality of the damaged bone. Research in bone tissue engineering strives to create materials that perform better than bone autografts and allografts.

The main objective of the whole BTE is to utilizing patient's body cells to treat bone defects. Strategies of bone regeneration through tissue engineering majorly require-tissue scaffolds, a temporary matrix for bone development, osteoinductive growth factors, and osteogenic cells, that will respond to the growth signals. In clinical settings, the success of BTE approaches is heavily reliant on the cells, scaffold material, and signalling stimuli added to the cell-scaffold mix and/or present in the healing defect's microenvironment [**19**].

Biology and structural composition of natural bone tissue

The majority of the body's connective tissue mass is made up of bone. In addition to supporting muscles and protecting internal organs, bones also produce blood, maintain calcium homeostasis, buffer acids, and bases, and transmit sound [**20**]. Just as any other biological tissue, bone is comprised of distinct cell types (osteoblasts, osteocytes, and osteoclasts) that are embedded in an extracellular matrix (ECM) that contains or is created by biologically active substances. Although it may seem inert, bone is an extremely dynamic organ that is constantly being resorbed by osteoclasts and neo formed by osteoblasts [**21**]. Based on their basic shape, they are divided into four categories: long bones (the femur and tibia), short bones (the wrist and ankle), flat bones (the cranial vault), and bones with irregularities [**22**].

Bone tissue is an extremely organized composite material containing 65% minerals (predominantly hydroxyapatite [Ca10(PO4)6(OH)2]), 25% organic matter (predominantly type I collagen, which is a triple-helical molecule with α1 and α2 chain), and 10% water [**23**]. In mammals, collagen is the most ample protein which accounts for approximately one-third of the body's protein tissue mass [**24**]. There are numerous types of collagens found in the human body however, collagen type I is primarily formed in bone by osteoblasts, which also have the responsibility of controlling the synthesis of hydroxyapatite from accumulated calcium and phosphate salts [**25**].

When compared to the inorganic phase (compact bone), which contributes approximately 65%-70% of bone's wet weight and gives hardness and resistance to mechanical stress, the organic phase (spongy bone) adds flexibility and elasticity [**26**] and makes up around 20% of the wet weight of bone. Bone strength or fragility is correlated with the architecture and elemental distribution in the bone [**27**]. Spongy bone exhibits a trabecular structure (75-85% porosity) containing bone marrow, while the compact bone is made up of osteons and haversian canals enclosing tiny blood veins [**28**]. The techniques that are frequently employed to examine bone composition include coherent-scatter computed tomography (CSCT) [**29**], Raman spectroscopy [**30**], Fourier transform infrared (FTIR) spectroscopy [**31,32**] and other related techniques. **Fig. 2** illustrate the detailed structure, characteristics and components of a natural bone.

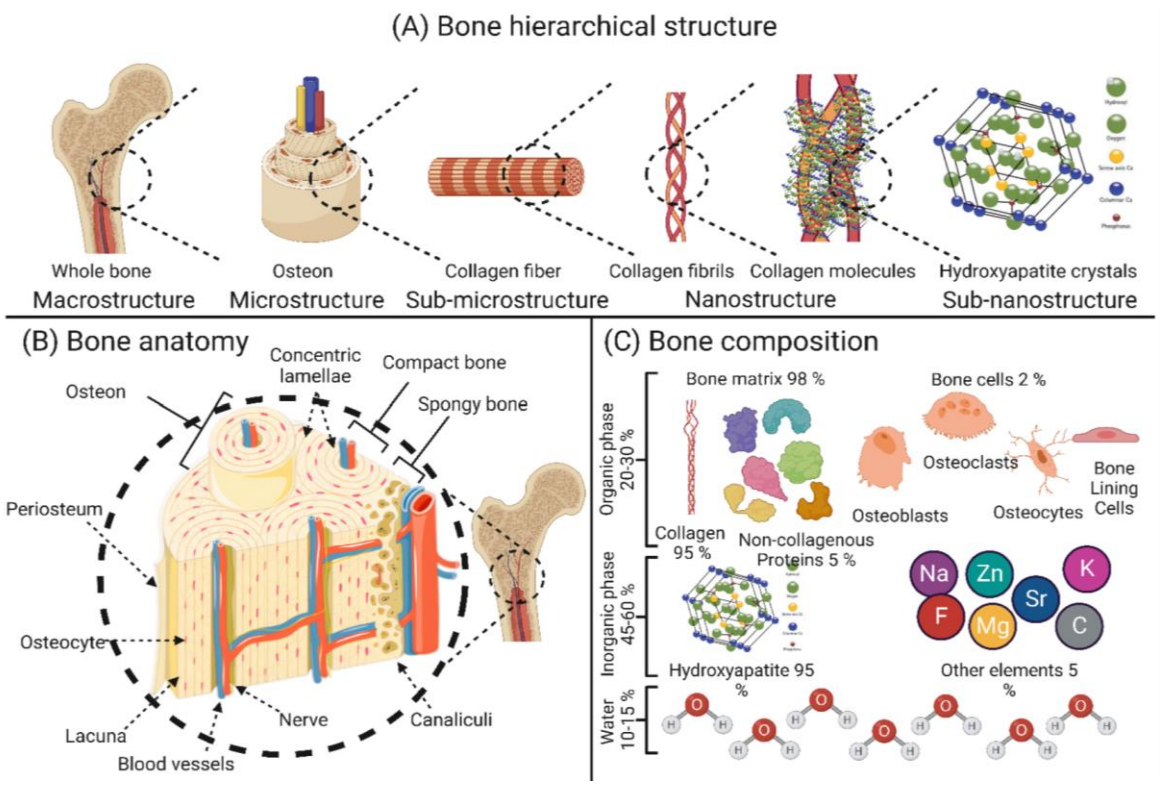

Fig. 2. Schematic illustration of structural composition of natural bone tissue: (A) organisation of bone hierarchy from macrostructure to sub-nanostructure; (B) characteristics of the bone's anatomy; (C) Components present in bone [**33**].

The primary objective of the whole BTE is to use the patient's body cells to treat bone defects. Strategies of bone regeneration through tissue engineering majorly require-tissue scaffolds, a temporary matrix for bone development, osteoinductive growth factors, and osteogenic cells, that will respond to the growth signals. In clinical settings, the success of BTE approaches is heavily reliant on the cells, scaffold material, and signalling stimuli added to the cell-scaffold mix and/or present in the healing defect's microenvironment [**19**]. In the next section, we have described all the essential elements required for BTE in detail and highlighted in **Fig. 3**.

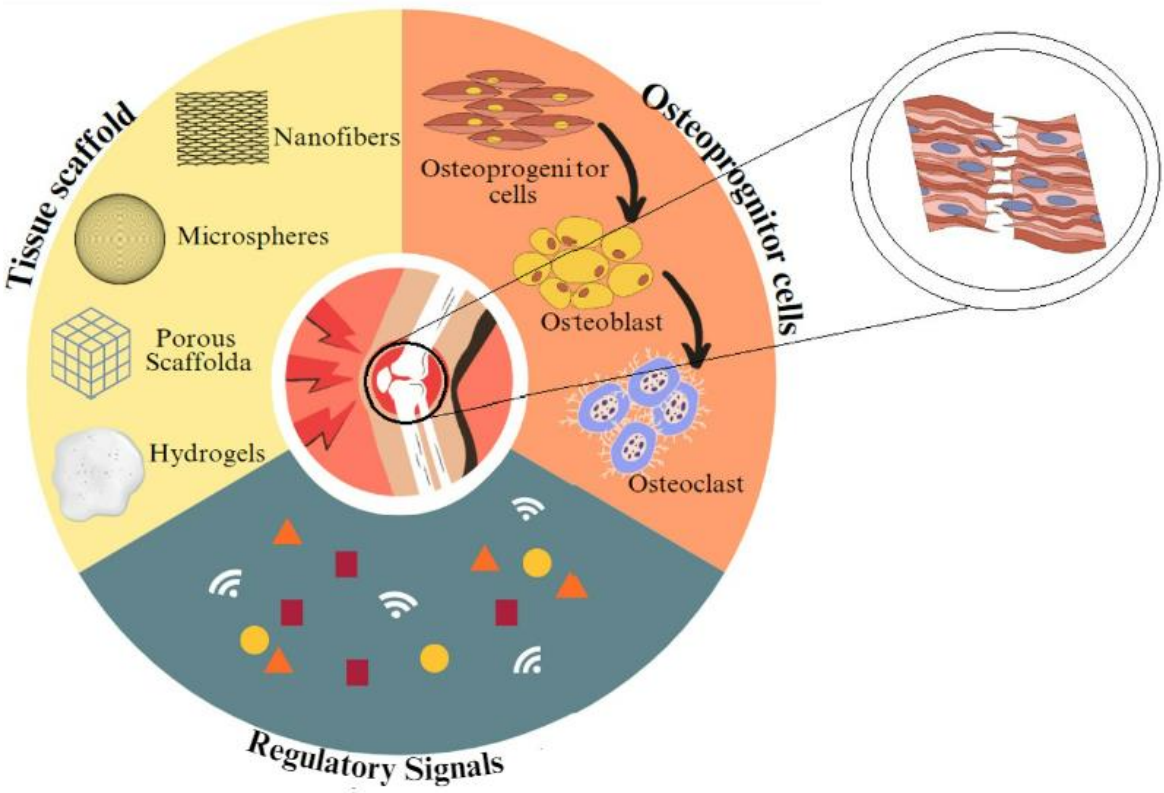

Fig. 3. Key elements for Bone tissue engineering like tissue scaffolds, regulatory signals and osteoprogenitor cells with enlarged view of fracture healing process, as these elements work in harmony to facilitate bone repair and regeneration.

Elements requires for BTE

Tissue scaffolds

Tissue scaffolds offer a structural framework to which cells introduced into the matrix can cling and eventually populate it [**34**]. An ideal scaffold can function as a storage space for growth factors, cytokines, and nutrients for cell proliferation. An important aspect of the tissue scaffold's design is its architecture since this has an impact on cell attachment, migration, modification of vital cell nutrient diffusion, and control of cell phases that exert mechanical and biological influences [**35**]. A viable scaffold must meet certain biological, mechanical, and structural requirements to satisfy these components. The porous structure is one of the most important prerequisites for the precise diffusion of nutrients and gases as well as the removal of waste product of metabolite produced by the action of the cells that had meanwhile inserted themselves within the scaffold [**36**]. A porous surface also enhances mechanical interaction between the implanted biomaterial and the surrounding native bone, resulting in increased mechanical stability at this crucial interface [**37**]. For appropriate cell development, cell migration, nutrient flow, vascularization, and improved spatial organisation for cell growth and ECM synthesis, scaffold pore structure that is, pore size, volume, and interconnectedness must be considered. The minimally acceptable size is approximately 100 μm, but a pore size of ≥300 μm is typically needed to promote new bone growth and vascularization [**38**]. Furthermore, the structural capabilities and endurance of scaffolds depend on mechanical properties like tensile strength, elastic modulus, and stiffness [**17**]. However, scaffolds must also not be overbuilt to the point where they are too stiff compared to the surrounding and regenerating tissues [**39**]. The mechanical qualities of scaffold framework can be modified by thoughtfully selecting the materials, creating significant composite structures, and altering the overall porosity [**40**]. A perfect scaffold must be biocompatible, biodegradation, can create the extracellular matrix (ECM) and offers an environment that encourages cell adhesion, proliferation, and differentiation. There are various methods that can be used to fabricate tissue scaffold

ranging from 3D printing, electrospinning, and decellularization of natural tissues. Once the scaffold is fabricated, cells can be introduced onto the matrix and cultured in vitro or implanted into the body to promote tissue regeneration.

Growth factors

Growth factors (GFs) are essential for the communication of information among various cell types [**41**]. In general, GFs are the key component to achieving neo-tissue. They are proteins that are naturally present in the body and regulate a wide range of cellular processes. They bind with their specific transmembrane receptors available on the outer membrane of target cells and stimulate a further cascade of events of cell growth and proliferation. The releasing rate of GFs depends upon multiple factors like the polymer's degradation profile employed in the scaffold, various methods of infusing growth factors, and the crosslinking temperature of different polymers used in the scaffold [**42**]. Thus, understanding the fundamental concept behind the bone-repairing cascade and manipulating biomaterials and growth factors is critical for the development of efficient tissue engineering strategies for optimal regeneration and repair of bone [**43**]. Many different growth factors are used in tissue engineering, each with its specific biological function. Some of the widely used growth factors in tissue engineering are listed in **Table 1** which involve and modulate bone cell activities and help in bone regeneration. At this time only two that is BMP (recombinant BMP-2 (rhBMP-2) and rhBMP-7) [**44**] and PDGF (PGDF-BB) are FDA approved for bone regeneration [**45**].

Table 1: Major families of GFs associated with bone regeneration.

Growth Factors (GFs)	Role in Bone Tissue Engineering	References
Vascular Endothelial Growth Factor (VEGF)	Significantly increase osteogenesis marker, promote angiogenesis, and increases expression of COL1 and RUNX2	**[46, 47]**
Platelet derived growth factor (PDGF)	Promote angiogenesis, involve in bone generation through osteogenic differentiation	**[48]**
Bone morphogenic proteins (BMP)	Induce osteogenic differentiation, Improves bone density, bone volume & bone density	**[49, 50]**
Fibroblast growth factor (FGF)	Bone homeostasis, promote vascular regeneration, promote proliferation of PDCs (Periosteum derived cells), regulate chondrogenesis	**[51-53]**
Transforming growth factor (TGF)	Regulate bone remodeling, Cartilage differentiation, multiplication of BMSCs (Bone marrow-derived mesenchymal stem cells)	**[54, 55]**

These growth factors are capable of being utilised in combination with tissue scaffolds and further biomaterials to encourage the regeneration of damaged or diseased tissues. The choice of growth factor(s) used will depend on the specific tissue being regenerated and the desired cellular response.

Osteogenic cells

Osteogenic cells are those that can transform into bone-forming cells, known as osteoblasts. Any approach for bone tissue engineering must include osteoprogenitor cells. They are bone stem cells that have a major effect on bone growth and healing. The main characteristics of osteogenic cells are their self-renewal capacity. Osteoprogenitor cells must be attracted through a complex and tightly controlled interplay between signalling from the systemic and local biomechanical as well as biophysical environment for new bone to grow [**56**]. There are several types of osteogenic cells that can be used in bone tissue engineering, including mesenchymal stem cells (MSC), osteoblasts, osteocytes, and periosteal cells. One of the common seeding cells used for bone production are BMSCs, which have produced positive outcomes [**57,58**]. The ability of mesenchymal stem cells (MSC) to self-renew and specialise in osteoblastic cells when cultured in a medium has made them a popular choice for bone repair [**59**]. In bone tissue engineering, osteogenic cells are typically combined with a scaffold material and growth factors to promote the formation of new bone tissue. The scaffold provides a temporary framework for the cells to grow and organize themselves, while the growth factors stimulate cell proliferation and differentiation. The use of osteogenic cells in bone tissue engineering has the potential to improve the treatment of bone defects and fractures, as well as other bone-related disorders.

Chronological development of BTE

Bone tissue engineering is relatively recent discipline that has developed over the past few decades**.** The concept of bone tissue engineering was first introduced by W.T. Green when he and his team performed a series of research investigation to create artificial cartilage using chondrocytes seeded onto bone spicules and implanted them in naked mice. Although he was unsucessful with his experiment but acurately concluded that the development of novel biocompatible materials would make it possible to grow new tissue by seeding live cells onto suitably designed scaffolds [**60**]. Ever since, up to 1980’s researchers began to explore the application of bone transplants and synthetic biomaterials, like hydroxyapatite, tricalcium phosphate, and other ceramics to stimulate bone growth [**61-64**]. These materials were biocompatible, osteoconductive, and could be shaped to fit the defect site. The biodegradability of those synthetic materials, however, was the main concern, and that's when biopolymers based scaffolds entered the area. In 1980’s natural polysaccharides, such as chitosan, colagen and, hyaluronic acid were explored for their potential in tissue engineering applications. The first attempt of seeding cells using polysccharide based 3D gel was developed by Yasui N *et. al.* in 1982. The author

successfully grown strenal cartilage of embroyonic chick on 3D collagen gel without any alteration in their cartilage phenotype [**65**]. Until the 20th century was over, researchers began to investigate the use of polysaccharide along with synthetic polymer based scaffold for bone tissue engineering [**66-68**].

Between late 2000s and early 2010s, researchers started looking into the use of 3D printing to make specialised scaffolds for bone tissue engineering [**69,70**]. **Table 2** highlighted the historical development of bone tissue engineering over time.

The utilisation of induced pluripotent stem cells (iPSCs) and innovative biomaterials are two significant advancements in bone tissue engineering techniques that were established over the recent years. In general, the discipline of bone tissue engineering is still expanding and growing with the aim of creating cures for diseases and disorders that affect the bones.

Table 2. The chronological evolution of bone tissue engineering.

Year	Scaffold Type	Type of cells	Remark	Reference
1970's	Spicules of bone	Cartilage	Chrondrocytes seeded onto bone spicules and implanted in naked mice	[71]
1977	Ceramic made of calcium phosphate	Cancellous tissue	Scaffold materials is safe and useful in replacing or suplementing bone graft	[72]
1979	Dense calcium hydroxylapatite (CHA)	Dental root	CHA implants served as ankylosed natural roots.	[63]
1983	Chitosan-beta-tricalcium phosphate	Bone marrow stromal cells	BMSCs showed good adhesion to scaffold	[73]
1995.	Poly(propylene-fumarate) (PPF) composite	Trabecular bone substitute	The temporary replacement of human trabecular bone with composite material was suitable.	[74]
1997	Poly(lactic-co-glycolic acid) 3-D foams	Rat stromal osteoblast	In vitro calcified bone like tissue can form on 3-D porous poly(lactic-coglycolic acid)	[75]
2002	Titanium foams	Simulated body fluid (SBF)	SBF showed exhibited promising capacity for generating bone like apatite layer	[76]
2005	hydroxyapatite ceramic (IP-CHA)	Rabbit femoral condyle	Compressive strenght increases after 9 weeks of implantation	[77]
2008	Chitosan/hydroxyapatite (HA) composite	preosteoblast cell line	Cell migration and growth were supported by the scaffold	[78]
2009	Collagen nano-hydroxyapatite(nHA) composite scaffold	Bone tissue	Concentration of nHA effects modulus	[79]
2012	PCL/nanoclay scaffold	human mesenchymal stem cells (hMSCs)	Precise cytoskeletal arrangement formed on scaffold	[80]
2013	chitosan/gelatin/nSiO2 composite	Bone tissue	Reduced degradation rate	[81]
2016	Collagen with poly(glycolic acid) (PGA)	Bone tissue	Concluded suitable for non-union fractures	[82]
2018	calcium silicate (CS)/sodium alginate/silk fibroin	Bone regeneration	Pore size changes by changing CS propotion	[83]
2019	Mesoporous bioactive glass/ silk fibroin (MBG/SF) composite	Human Bone Marrow Mesenchymal Stem Cell (hBMSCs)	Seeded scaffold transplanted in nude mice	[84]
2020	Human-like collagen (HLC)/nano-hydroxyapatite (n-HA) crosslinked scaffold	Osteoblast cells	Characteristics of scaffold varied with different concentrations of DEO	[85]
2021	3-D alginate-gelatin composite hydrogel scaffold	Simulated Body Fluid (SBF)	Using BioFabX4 3D-printer	[86]
2022	Poly (glutamic acid) filled poly (ε-caprolactone)-modified cellulose nanocrystals (PCL/PGlu-NCC)	Human mesenchymal stem cells (hMSCs)	Scaffold showed low toxicity	[87]
2023	Poly (ε-caprolactone) (PCL)/Gelatin/TiO_2 nanofibrous scaffolds	Bone tissue	Cell toxicity increased by incresing TiO_2 concentration	[88]

PRINCIPAL OF BONE REGENERATION

Understanding the intricate physiological procedure of bone repairing is necessary for effective injury treatment. Bone tissue engineering is an interdisciplinary approach that involves the application of principles and methods from engineering, biology, and medicine to regenerate bone tissue as depicted in **Fig. 4**. The natural process of bone regeneration involves highly defined processes: endochondral ossification (ECO), intramembranous occification (IMO) or a mixture of both. The existence or absence of the cartilaginous phase is the key distinction

between these procedures [**89**]. The classical BTE approach is intended to induce bone repair through cell-based bone tissue engineering through the IMO route, which induces mesenchymal stem cells (MSCs) to undergo osteogenic differentiation, resulting in the development of a bone-like matrix [**90**]. Yet, it has been discovered that the creation of a calcified matrix during such graft in vitro culture can limit their vascularisation in vivo by acting as a barrier to invading blood vessels, ultimately leading to implant failure [**91**]. To overcome these limitations, researchers have come up with another alternative approach using the ECO pathway, especially in the case of long bone formation from cartilage templates [**92-94**]. Both IMO and ECO based bone tissue generation come under the bio-based approach, whereas reproducing bone using synthetic or bio-based material comes under the engineering-based approach for BTE [**95**].

The engineering-based approach provides a more sustainable, long-term treatment strategy for bone reconstitution and involves the fabrication of implants using a combination of scaffolds, cells, and mechanical or soluble factors [**96**]. The main aim of BTE is to understand bone structure and function to create new, healthy bone tissues. This process involves the isolation of mesenchymal-derived stem cells (MSCs) from adult tissues like adipose tissue, bone marrow, and dental tissues, followed by the implantation of proliferation factors and a three-dimensional biocompatible porous scaffold to support the growth and differentiation of bone cells. Due to their multipotency, MSCs have the capability to differentiate into bone, cartilage, ligament, and tendon. In the final step, the scaffold is reimplanted in the host and integrates with host bone tissue. Over time, the scaffold gets fully degraded, leaving healthy bone formation.

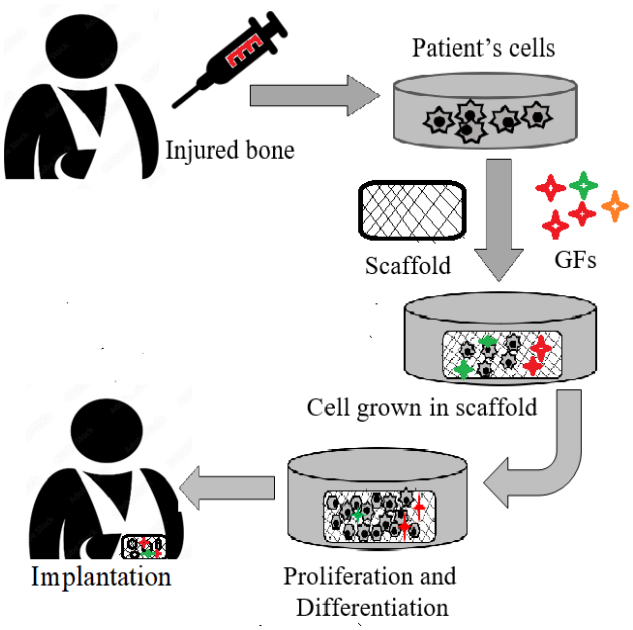

Fig. 4. A schematic illustration of the Bone Tissue Engineering (BTE) process depicts the key steps involved in regenerating bone tissue.

DESIRABLE PROPERTIES OF POLYSACCHARIDES AEROGEL SCAFFOLDS FOR BONE TISSUE ENGINEERING

Polysaccharides are complex carbohydrates that have unique properties, outlined in **Fig. 5**, that make them attractive for use as scaffolds in bone tissue engineering. These are polymers that are comprised of monomer units bonded through glycosidic linkage. From linear to highly branched, polysaccharides can have several structural types [**97**], and their physical properties, such as solubility, viscosity, gelling potential, and/or surface and interfacial properties, are determined by differences in their monosaccharide composition, linkage types and patterns, chain shapes, and molecular weight [**98**]. They typically serve a structural or storing purpose in the living organism. A large percentage of polysaccharides are naturally occurring substances that are affordably and easily retrieved from plants, animals, and microorganisms. They are well known for having the capacity to self-organise or self-assemble into certain physical shapes or structures [**99**] and can be easily modified according to the purpose. Compared with synthetic materials, polysaccharide-based materials can encourage cell adhesion, proliferation, and differentiation while having good biocompatibility and an acceptable host response [**100**]. These properties of polysaccharides are mentioned in figure 4, which attracted researchers' attention to using them for tissue engineering [**101**]. The production of bio-aerogels employs a wide range of polysaccharides. Chitosan, alginate, and cellulose are regarded as the numerous polysaccharides employed in the manufacture of safe and economical aerogels that can serve as tissue scaffolds.

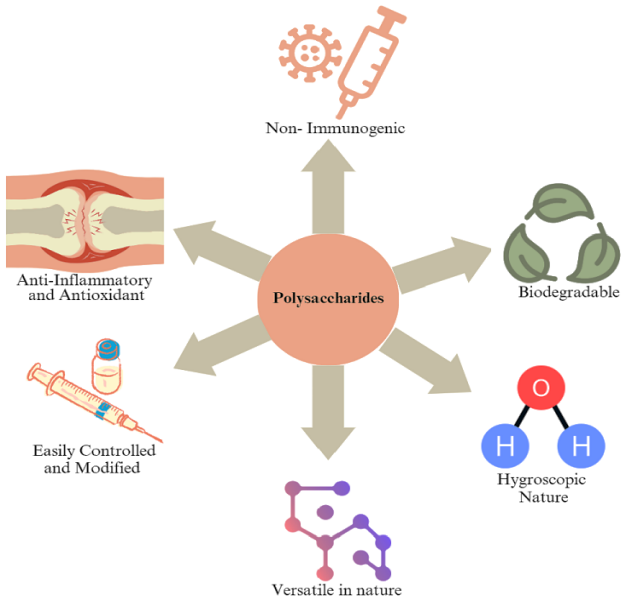

Fig. 5. Schematic illustration of the exceptional physiochemical and biological properties of polysaccharides showcases the diverse and remarkable characteristics.

Chitosan

Chitosan's history began in the 19th century, when Rouget wrote about its deacetylated form [**102**]. It is a highly flexible biopolymer that is extracted through the alkaline hydrolysis of chitin (CI), which is a natural polymer that can be mainly retrieved from the constituents of crustacean shells, insect exoskeletons, and the cell walls of some fungi and yeasts [**103,104**]. The depiction of the chitosan extraction process can be observed in **Fig. 6**. The structure of CS consists of randomly arranged β-(1-4) linked 2-amino-2-deoxy-β-D-glucopyranose [**105**]. Pure chitosan comes in a variety of forms with varying molecular weights and degrees of deacetylation (DD). The DD of chitosan is computed as the ratio of D- glucosamine to the total of D-glucosamine and N-acetyl D-glucosamine units [**106**].

It is the second most widely used polysaccharide obtained from biomass after cellulose and is a cost-effective and sustainable product [**107**]. Other than its low cost, CS is a bioactive, biodegradable, and biocompatible product that induces a limited response to foreign bodies and fibrous encapsulation [**108**]. The structure of CS resembles glycosaminoglycans (GAGs), which are an essential component of the bone matrix and influence the function and accessibility of several osteoclastic and osteogenic agents [**109**], therefore facilitating their use in BTE [**110**]. Moreover, chitosan is an extremely versatile polymer that can easily be engineered into a variety of morphologies, including nanofibers, films, beads, and sponges for bone tissue repair [**111-114**].

Table 3. Different materials used for modifying chitosan's properties to prepare scaffold for BTE.

Polymer	Modifying material and type of scaffold	Technique	Type of tissue	Result	Ref.
Chitosan	Chitosan tricalcium Phosphate fucoidan scaffold	Freeze Drying	hMSCs	Scaffold support osteogenic differentiation	[115]
	Chitosan- HA hydrogel	3-D bio printing	MC3T3-E1 pre-osteoblast cells	Hydrogel showed calcification and differentiated osteogenically with maximal expression of early and late stages of osteogenic markers	[116]
	Chitosan/nHAp/n ZrO2	Freeze Drying	mMSCs	The differentiation of osteoblasts was aided by scaffolds.	[117]
	Strontium-modified chitosan/ montmorillonite (Sr-C/MMT) sponge	Freeze Drying	Human osteoblasts	Sr2+ alteration of MMT-chitosan enhances the scaffold's characteristics	[118]
	Chitosan/ hydroxyapatite scaffolds	Freeze Drying	Simulated Body Fluid (SBF)	Scaffolds properties, bioactivity, and biocompatibility were all greatly enhanced by the inclusion of nano-HAp	[119]
	EO-loaded CS/dextrin	Ice template-assisted freeze-drying	NRD	Demonstrate antioxidant and antifungal activity	[120]
	Chitosan-modified halloysite nanotubes (mHNTs) hydrogel	Sol-gel transition	Mesenchymal stem cells	Chitosan modification of HNTs boosted loading capacity and entrapment efficiency	[121]

*NDR- no data reported

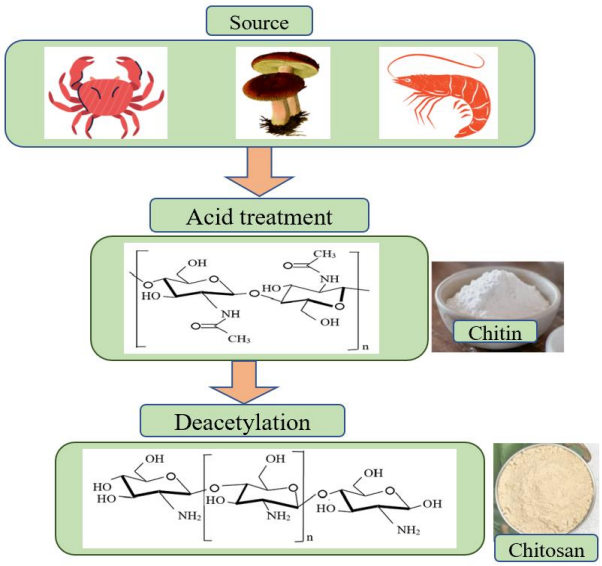

Fig. 6. The extraction of Chitosan from natural sources, such as crab shells, crustaceans, fungi, etc., consists of two main steps: acid treatment and deacetylation.

The relationship between the chemical makeup of chitosan compounds and their possible applications in numerous fields of science, such as tissue engineering is determined by their physicochemical and biological properties. The structural characteristics of CS can vary with the amount of deacetylation and molecular weight. Depending on the source and processing, chitosan DD significantly differs from 60 to 100%, and its molecular weight normally ranges from 200 to 1200 kDa [**122**]. For instance, Yuan et al. employed a different source of CS in their investigation and deacetylated it with 45% sodium hydroxide under specific time and temperature conditions and concluded that processing conditions elevated DD, which affects physicochemical and biological parameters [**123**]. The molecular mass of chitosan is corelated with the number of monomer units in a biopolymer, and both molecular weight and degree of deacetylation affect other properties of chitosan like viscosity, solubility, crystallinity, degradation, cell growth, and proliferation [**108,124**]. In recent research work, Sukul et al. evaluated the in vitro response of human osteoblasts on chitosan

sponges with different DD and MW. The author reported that cell migration and ALP (an osteogenic marker) increased with an increase in DD, while low DD increased the release of substances that promote octeoclastogenesis. Regarding MW, they observed that high molecular weight caused the production of substances that promoted angiogenesis and bone remodelling [**125**]. Another study by Grigoriev T. *et. al.* examined the effects of MW and DD of chitosan on the characteristics of the chitosan/beta-glycerophosphate situ-forming gel, revealing that the storage modulus increases when chitosan DD decreases and chitosan with a higher molecular weight displays less cytotoxicity [**126**]. Furthermore, CS undergoes significant protonation in aqueous media because it contains amine groups, which means it is cationic in nature, which enables electrostatic contact with anionic macromolecules, components, and biological sites and affects the action of cytokines and growth factors and can be used in BTE **[127, 128**]. Moreover, other natural or synthetic polymers, metals, and ceramics are mixed with CS to improve its qualities for BTE applications, such as structural integrity and mechanical strength [**103**]. Various materials employed to alter the properties of chitosan for the creation of scaffolds are listed in **Table 3**.

Excluding its desirable physiochemical properties, CS also have biological properties including biocompatibility [**129**], antimicrobial activity [**130**], anti-inflammatory [**131**], biodegradability [**132**], and low toxicity [**133**], which suggests that it is an encouraging biomaterial.

CS can degrade by dissolving bonds between molecules such as glucosamine-glucosamine, glucosamine-N-acetyl-glucosamine, and N-acetyl-glucosamine-N-acetyl-glucosamine units [**134**]. Previous studies reported that there are some physiological enzymes in the human body that can break down chitosan without releasing any toxic products [**135,136**]. During depolymerization, chitosan produces monomeric products (glucosamine), which are either metabolised by the body or eliminated from it. These bioactive chito-oligosaccharides have outstanding antimicrobial activities. Because of this, CS is biodegradable and excellently biocompatible with practically all biological tissues [**137**].

In addition to this, Martel-Estrada determined in his work that a composite of chitosan enhances the ability of osteoblasts to multiply and differentiate without cytotoxicity. As of now, various studies have proven that a derivative of CS, carboxymethyl chitosan (CMC), improves the characteristics of bone-growth scaffolds when combined with other composite materials [**138**]. A CMC-based nanoparticle shows tremendous antimicrobial activity and support the proliferation and cell adhesion [**139**]. Modification of a CS-based scaffold with other materials also upgrades its biological properties. Chen and team prepared a CS/HA composite and modified it with arginine-glycine-aspartic acid (RGD) for bone tissue regeneration. The prepared scaffold showed high biocompatibility, cytocompatibility, and histocompatibility. Furthermore, osseointegrative property examinations were done on rabbit model, which revealed complete bone tissue formation after 8 weeks of implantation [**140**].

Cellulose

Cellulose is among the most prevalent polymeric substance in the environment and can be considered as an essentially endless resource of raw material for the rising need for environmentally and biologically suitable products [**141**].

The flexible structuring of cellulose through a range of avenues of modification, including both physical and chemical techniques, has allowed its usage in a broad spectrum of applications ranging from food industries, cosmetics, paints, ceramics, paper, textile clothing, printing inks, and pharmaceuticals [**142,143**]. In addition to some types of algae and bacteria, cellulose can be produced by both wooden and non-wooden plants (cotton, bast plants, wood, and bamboo) [**144**]. Its structural elements consist of a continuous linear chain of (1-4) D-glucose connected through glycosidic linkages to a disaccharide repeat unit called cellobiose [**145**]. In nature, this biodegradable polymer is mostly found as microfibrils located in the plants cell wall and trees, the tissues of algae, and the membrane of tunicate epidermal cells [**146**]. Besides having the ability to be a green product, cellulose also has a variety of uses and transformational applications because of its distinctive and diverse structure. Due to their flexible chemical and structural characteristics and outstanding mechanical properties such as source abundance, non- nontoxicity, immunogenicity, and low production cost [**146**], derivatives of cellulose such as bacterial cellulose (BC) [**147**], fibrillated cellulose (CNF) [148], and crystalline cellulose (CC) [**149**] are commonly used in scaffolds for tissue regeneration.

Cellulose and their derivatives possess numerous favourable properties that make them highly versatile and widely used in tissue regeneration application [**150**]. Carboxymethylcellulose (CMC) has numerous carboxyl groups, strong solubility in water, and is an encouraging material for BTE [**151**]. In their study, Gaihre and Jayasurya fabricated CMC-based microparticles to assess their potential. The author reported that murine pre-osteoblasts (OB-6) were very well attached and differentiated on the surface of the microparticle [**152**]. Similarly, in other research work, researchers synthesised hybrid material-based hydrogels with CMC and hydroxyapatite (HA) and observed that the combination of both materials increased metabolic activity in extracellular mineralized matrix synthesis. Like CMC, nanocellulose (NC) has great hydrophilicity, superior mechanical strength, low density, and variable surface functionalization [**153**]. And all three (BC, FC, and CC) are primary forms of NC. Out of other celluloses, bacterial cellulose (BC) has been widely used for tissue scaffolds as it is free of lignin and hemicellulose and possesses a unique 3D network structure [**154**]. It is generally manufactured by growing *Gluconacetobacter xylinus* (also known as *Acetobacter*

xylinum in older publications) in a static suspension in a liquid medium [**155**]. It has high porosity, which helps in gaseous and fluid exchange; biocompatibility; and the capacity to store huge volumes of water that maintain moisture in the whole environment [**155**]. All these properties favour osteogenesis. Yang et al., in their experimental work, fabricated a BC-based scaffold and characterised it using different techniques. They observed that mechanical testing like Young's modulus, compressive strength and maximum load revealed a considerable increase in mechanical properties [**38**]. Additionally, a team of scholars synthesised a BC-based hydrogel scaffold filled with inorganic calcium to analyse its physiochemical properties. A significant increase in swelling ability with compressive strength like trabecular bone was observed, along with cell viability [156]. Hence, due to its high tensile strength and stiffness, cellulose is advantageous for bone tissue engineering since it may give the newly produced tissue structural support.

Table 4. Different materials used for modifying cellulose properties to prepare scaffold for BTE.

Polymer	Modifying material and type of scaffold	Technique	Type of tissue	Result	References
Cellulose	Cellulose/Iron Acetate Nanofibers	Electrospinning	human fetal-osteoblast cells (hFOB)	Cellulose/Iron Acetate mat showed biocompatibility and supported cell attachment and proliferation	**[157]**
	BC modified with Gelatin and HA coating	Freeze-drying technique	hBMSCs	Cells showed great intracellular communication with high proliferation	**[38]**
	Pullulan/cellulose acetate fibrous scaffolds	Electrospinning	Human Osteogenic Sarcoma Cell Line (Saos-2)	Scaffold with ratio P_{50}/CA_{50} showed best cytocompatibility	**[158]**
	Cellulose-graft-polyacrylamide/ nano-HA scaffolds	Freeze-drying technique	Stimulated body fluid (SBF)	Apatite layer formed on scaffold	**[159]**
	Deacetylated porous cellulose acetate microspheres modified with polydopamine suspension of hydroxyapatite	One-step in-situ method	MC3T3-E1 cell line	Cells were able to differentiate	**[160]**

Both cellulose and its derivatives are sustainable and biodegradable, with significant potential for bone tissue engineering. A cellulose-based scaffold enhances cell proliferation [**161**], shows excellent biocompatibility [**162**], and supports osteogenic differentiation [**161**]. Shaheen et al. fabricated scaffolds with different contents of cellulose nanocrystals (CNC) using the freeze-dry technique. The findings suggested that the CNC-containing scaffold has encouraging cell growth and cell adhesion, and as a result, it is anticipated to have a strong potential for applications in bone tissue development [**163**]. In another study, a group of researchers incorporated cellulose nanofibers (CNF) on a polyhydroxybutyrate scaffold. The scaffold showed a pertinent rate of degradation, outstanding biomineralization, and impressive osteoblast cell growth and migration in the presence of CNF [**164**]. It has been demonstrated that the biological characteristics of cellulose can be enhanced when combined with other biomaterials [163, 165, 166]. For example, Maharjan and his team prepared chitosan hydrogel integrated with cellulose nanofibers. Authors observed that scaffolds displayed improved pre-osteoblast cell (MC3T3-E1) viability, adhesion, and proliferation in addition to higher biomineralization [**167**]. Similarly, Huang et al. modified BC scaffolds with gelatine and hydroxyapatite and developed their biocompatibility and osteoinductivity [**167**]. Some examples of other modifying materials are mentioned in **Table 4**. Therefore, cellulose can be altered to improve its properties and processed into several forms of scaffolds for BTE applications.

Alginate

Alginate is another kind of natural polysaccharide that exists abundantly in brown seaweed. In the early 1880s, Stanford patented an crude alginate salt as an industrial product [**168**]. It can be describe as linear polymer composed of α-L-guluronic acid monomers (G) and β-D-mannuronic acid (M) linked with β (1-4) linkage [**169**] and is frequently obtained from brown algae (Phaeophyceae), such as *Laminaria japonica*, *Laminaria hyperborea*, *Laminaria digitata*, *Macrocystis pyrifera* and *Ascophyllum nodosum*, by treating them with aqueous alkali solutions, most frequently NaOH. Calcium chloride is mixed in to the filtrate after the extract has been filtered in order to precipitate alginate [**170**]. Alginate has been majorly used in tissue engineering because of its exceptional qualities in terms of non-antigenicity, biocompatibility, chelating ability and biodegradability [**171**]. It is suitable for the trapping of delicate materials because it readily gels with multivalent cations in mild circumstances. They easily get crosslinked when the divalent cations are present in the surrounding environment, which enables the fabrication of 3D scaffolds [**172**]. The features like molecular weight, structural composition, and amount of alginate employed in

scaffolds have a major impact on providing cell adhesion, mechanical strength, proliferation, biocompatibility, and osteogenic differentiation [**170**]. The physical characteristics of the alginates are governed by the characteristics and dimensions of the sequences as well as their molecular weight. Alginates formed from algae possess a broad range of molecular weights, but those obtained from bacteria have large molar masses and a high degree of polymerization (DP) [**173**].

Alginates are widely used because of their solubility in neutral and alkaline settings due to the carboxyl groups in alginate that are charged at pH values higher than 3–4 [**174**]. Due to its hydrophilic nature, it can keep the surroundings physiologically wet by absorbing and losing water. Depending on the application, alginate's molecular weights typically range from 60,000 to 700,000 Daltons [**175**]. Unlike other polysaccharides, alginate can produce gels regardless of temperature [**176**]. In BTE, the concentration of alginate directly impacts the calcium content; as the concentration of alginate increases, the calcium level in cell culture also increases [**177**]. It contains groups that are simple to protonate, and in the pH range above its pKa, polyanionic chains are generated with negatively charged carboxylic groups [**178**]. Alginate can produce stable hydrogels in the presence of certain polyvalent cations (such as Ca2+, Sr2+, Ba2+, and Al3+) by ionically interacting their carboxyl functional group with the cation [**179**]. The affinity of interaction with ions depends on the G and M blocks in the alginate structure. Alginate with more G content than M or alternating M and G blocks has more affinity for divalent cations [**180**]. The presentation of G and M depends on the source from which alginate has been isolated. The ionic crosslinking offers a suitable environment for cell loading, which gives them an edge over other materials used as solid scaffolds [**181**]. Erol M. *et. al.* demonstrated in their work that coating alginate on boron-containing bioactive glass-based scaffolds enhanced the mechanical and bioactivity characteristics of the scaffold [**182**]. Ghosh *et. al.* also investigated the influence of alginate by incorporating it into the fluorenylmethoxycarbonyl-diphenylalanine (FmocFF) peptide composite hydrogel. SEM analysis revealed a fibrous nanostructure like bone ECM, and rheological experiments show thixotropic behaviour and a high storage modulus of the scaffold, suggesting this hydrogel can create a temporary, three-dimensional cellular environment to encourage bone healing [**183**].

Alginate is biocompatible, promotes osteogenic cell growth, and shows low toxicity towards the physiological environment. The biocompatibility of alginate depends on the G/M residues present in it, and it increases with a low content of G units. Tam *et. al.* compared the biocompatibility of two industrially available alginates having different G contents (IntG = 44%) and (HiG = 71%), respectively. The observation clearly proved that gel beads made from IntG showed higher biocompatibility than HiG [**184**]. To study osteogenic differentiation on an alginate matrix, Westhrin *et. al.* cultured MSCs with and without alkaline phosphate modification in alginate beads. In both cases, MSCs expressed higher levels of osteoblast-specific mRNA compared to MSCs in conventional cell cultures, proving that alginate beads offer an environment that enhances osteogenic differentiation [**185**]. Similarly, Zhou *et. al.* developed oxidised alginate-fibrin microbeads containing human umbilical cord mesenchymal stem cells (hUCMSCs) and looked over their degradation, release of cells, and differentiation of the osteoblasts. The results revealed that after the 4th day, oxidised alginate-fibrin microbeads started degradation along with the release of cells, and the released hUCMSCs showed outstanding bone mineral synthesis, osteodifferentiation, and proliferation, proving that alginate-fibrin microbeads have the potential to encourage tissue regeneration [**186**] Moreover, alginate microparticle and microfiber aggregated scaffolds also proved to be a great matrix for BTE [**187-189**].

Other polysaccharides

Hyaluronic acid (HA) is a glycosaminoglycan that is present in almost all mammalian species. It was first extracted from the vitreous corpus of the cow's eye by Karl Meyer and John Palmer in 1934 [**190**]. It is particularly common in the tissues of developing embryos and in the extracellular matrix (ECM) of adult soft connective tissues [**191**]. Microbial organisms can also synthesise hyaluronic acid. N-acetyl-D-glucosamine and D-glucuronic unbranched repeating disaccharides make up the structure of HA. It is produced naturally by a class of integral membrane proteins known as hyaluronan synthases and digested by a family of enzymes known as hyaluronidases [**192**]. In addition to its numerous functions in healthy tissues, it has also been linked to malignancies, angiogenesis, drug resistance, inflammation, water homeostasis, and altered extracellular matrix viscoelasticity [**193**]. HA and its derivatives are widely employed in tissue engineering because they biocompatible, biodegradable, nonimmunogenic, and can provide the necessary viscoelasticity. For the purpose to generate a hybrid hydrogel with a combination of properties, HA and its derivatives are frequently mixed with other materials. Bone substitutes based on HA and its derivatives offer the versatility to be modified into any shapes or sizes, including conversion to porous scaffolds, nanofibers, films, nanoparticles, and microspheres, for bone tissue regeneration, when combined with different tissue-engineered processing techniques [**194,195**]. Its physical and biological features in solution or hydrogel form make it ideal for a variety of body repair technologies.

HA is commonly employed in orthopaedics since it occurs naturally in the articular cartilage, fluid present between the joints, and joint capsule. High viscosity, flexibility, and a strong negative charge are some of the physicochemical characteristics of HA. For the development application involving tissue engineering, the viscosity of HA is of crucial importance. Along with these

characteristics, HA has specific functional groups (acetamido, carboxyl, and hydroxyl) in its structure that facilitate its crosslinking to form hydrogel, which is why HA based scaffolds are very commonly used for various biomedical applications [**196-198**]. The crosslinking modification of HA improves its mechanical strength, thereby rendering it a better choice for tissue engineering applications. In his study, Janarthanan et al. crosslinked alginate with HA using acyl-hydrazone, hydrazide interactions, and calcium ion methods to synthesise hydrogel bioink. They observed that modified Alg-HA gels had remarkable biocompatibility, tunable mechanical characteristics, and were extremely dynamic and shear-thinning [**199**]. Similarly, in another research work, Cui and coworker created hyaluronic acid (HA) hydrogels with a triple degradation behaviour by modifying HA with 3,3'-dithiodipropionate hydrazide (DTPH) and crosslinking it with polyethylene glycol dilevulinate (LEV–PEG–LEV) by reacting the ketone carbonyl groups of polyethylene glycol dilevulinate (LEV-PEG-LEV) with the hydrazide groups of 3,3-dithiodipropionate hydrazide-modified HA (DTPH-HA). Authors reported that after alteration, the morphology of hydrogels was very porous, with pore sizes varying from 20 to 200 m, and showed biocompatibility for osteoblast-like MC3T3-E1 cells [**200**]. **Fig. 7** demonstrate a decrease in pore size when the AFnSi crosslinker concentration increases, which could be result of an increased degree of crosslinking in the hydrogels [**201**]. Other findings also revealed that molecular weight of HA can affect the osteoblast proliferation and differentiation [**202**].

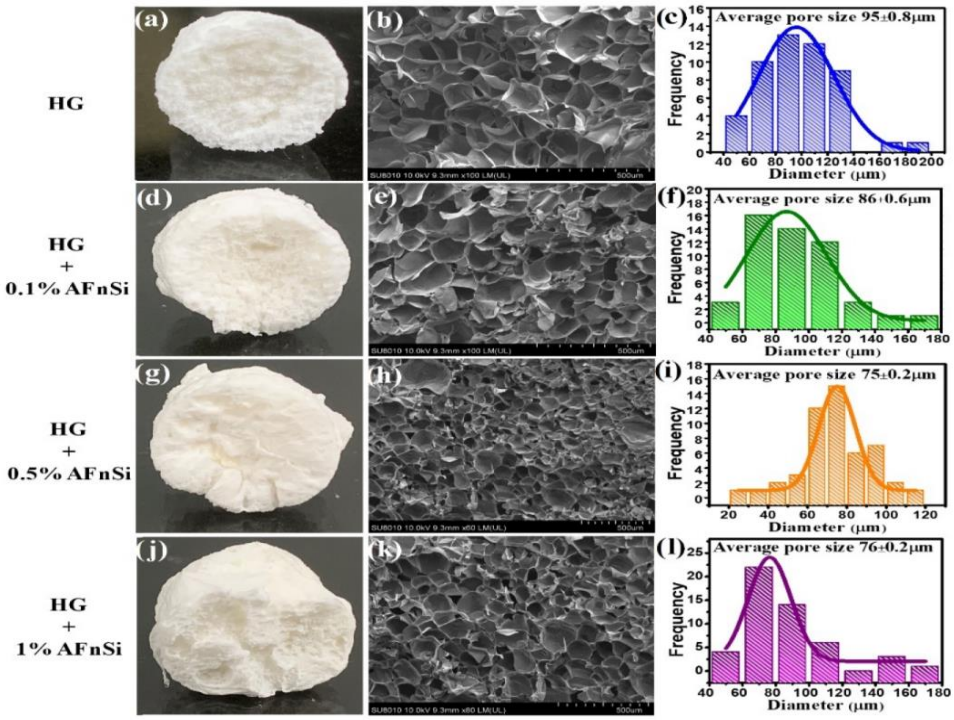

Fig. 7. Cross-sectional SEM picture of hybrid hydrogel scaffolds with various AFnSi concentrations along with its size distribution histograms [**201**].

Hyaluronic acid, being an endogen, has reduced immunogenic characteristics [**203**] and can mimic the biological matrix for regenerative surgery. To maintain tissue homeostasis and organise the ECM, HA interacts with a variety of proteins or proteoglycans. It helps keep tissues hydrated and lubricate some tissues in addition to mediating solute transport through the extracellular space [**204**]. HA is also non-toxic and has antibacterial activity [**205**]. It also interacts with CD44 antigens which can control cellular activities like cell migration and adhesion [**206**]. Injectable hydrogel made from HA has emerged with an enormous scope for bone and cartilage tissue engineering. Makvandi and team biosynthesised injectable hydrogel nanoparticles including hyaluronic acid, β-tricalcium phosphate, and corn silk extract to determine their possible utilisation in bone tissue regeneration. Ag NP-containing samples showed antibacterial activity for both gram-negative and gram-positive bacteria without showing toxicity to the cells [**195**]. HA can also facilitate osseointegration of metallic and hydroxyapatite implants by controlling cell adhesion, migration, and differentiation. Layer-by-layer self-assembly technology was used by Song et al. to create a HA/chitosan multilayer loaded with icariin over the titanium implant's surface. The findings demonstrated that the coating accelerated early osseointegration in vivo and improved osteoblast proliferation, viability, and adhesion [**207,208**].

Starch is a polysaccharide that present in higher plants, bacteria, algae, and in protozoa [**209**]. It is a crucial source of carbs for humans and is frequently present in meals like

bread, rice, and potatoes. Structurally, starch is made from two primary polymers of glucose named amylose (20%–30%) and amylopectin (70%–80%). Unlike amylose, which is made up of a linear chain of glucose molecules, amylopectin is made up of a branching chain. Both polymer contains D-glucose residues which are α-(1,4)-linked and attached by β-(1,6)-glycosidic connections to produce branch structure [**210**].

The source from which starch is separated affects the starch's characteristics. For instance, as compared to starch isolated from microbes, starch obtained from plants has a distinct chemical composition. To enhance it properties and use it as scaffold, starch is usually blend with other ceramics, natural and synthetic polymers. Wu et al. through electrospinning technique prepared starch- based nanofiber combined nano graphene oxide (nGO) scaffold to test its potential in bone regeneration application. Scaffold fibre fused with nGO showed greater electro spinnability and thermal stability [**211**]. Starch can also use as enhancer with other material to improves their properties. Numerous hydroxyl groups in starch increase the hydrophilicity of bone scaffold surfaces. Asl A et al. studied the impact of starch on polyhydroxybutyrate (PHB) scaffolds. Scaffold with 10 wt% starch showed elevated tensile strength. Incorporation of starch also enhances scaffold's hydrophilicity and PHB degradation rate [**212**]. Amylose content in starch also affects mechanical strength of overall scaffold [**213**].

Like another natural polysaccharide, starch is also biocompatible, renewable, non-toxic. In scaffolds starch concentration can modifies the biological properties of tissue scaffolds. To determine this, You B.C *et. al.* developed nano-hydroxyapatite/starch bone scaffold with different proportion of starch. The high starch content (80wt.%) in the nano-hydroxyapatite/starch bone scaffold results in nano-hydroxyapatite/starch interfaces with potent intermolecular interactions that can control biomineralization and degradation [**214**].

APPLICATION OF POLYSACCHARIDE-BASED AEROGEL SCAFFOLDS IN BTE

Polysaccharide-based aerogel scaffolds have promisable applications in bone tissue engineering (BTE) because of their biodegradability, biocompatibility, and ability to mimic the extracellular matrix of bone tissue. In vitro studies have shown that polysaccharide-based aerogel scaffolds support the differentiation and growth of osteoblasts, the bone-forming cells, and have the potential to induce bone regeneration. In vivo experiments on animal models have also revealed the effectiveness of these scaffolds in promoting bone regeneration.

Chitosan based aerogel

Chitosan-based aerogel scaffolds gained significant attention for its potential applications in bone tissue engineering (BTE) due to their unique properties mentioned in the previous section. Chitosan-based aerogel scaffolds were also examined for their ability to release drugs or growth factors around the bone defect site in a controlled and sustained manner, thereby enhancing therapeutic efficacy. In recent work, Rayes Peces *et. al.* prepared homogeneous chitosan (CS)-silica hybrid aerogels with 3-glycidoxypropyl trimethoxysilane (GPTMS) employing the sol-gel technique and CO2 supercritical drying for bone tissue engineering. The authors reported that the in vitro experiment showed the production of a hydroxyapatite (HAp) layer with no cytotoxic effect on human osteoblasts (HOB). Also, actin stress fibres and mature focal adhesion complexes were observed in the osteoblast cells grown on hybrid samples [**215**] **(Fig. 8)**. Furthermore, another group of researchers, fabricated a silica (SiO2)/chitosan (CS) composite aerogel for bone tissue regeneration. The osteoblast in vitro experiment showed vastly enhanced attachment, cell proliferation, and phenotypic alterations in cells cultured on the scaffold [**216**].

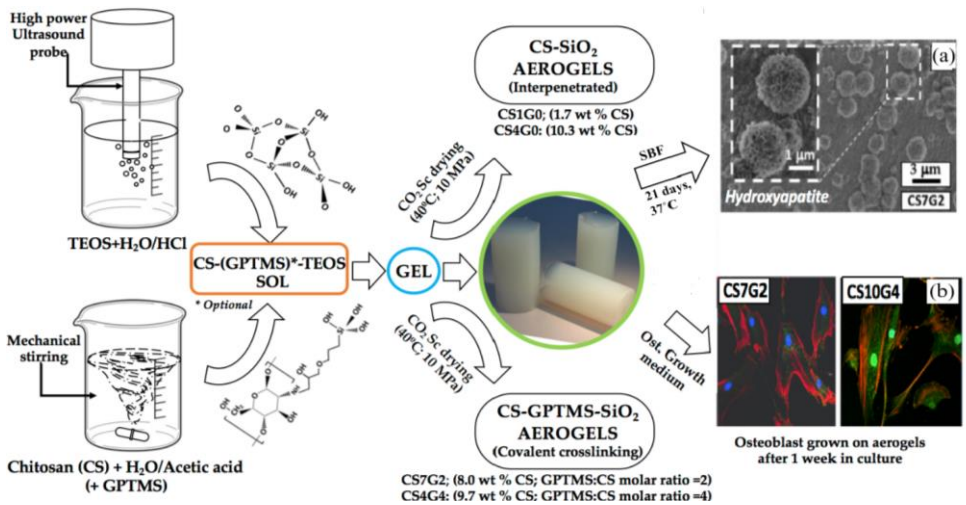

Fig. 8. Illustration and preparation of novel synthesis route for obtaining homogeneous chitosan (CS)-silica hybrid aerogels showing SEM micrograph and Live/Dead staining of osteoblasts culture to evaluate the viability and compatibility of the aerogels [**215**].

Furthermore, chitosan-based scaffolds doped with metallic nanoparticles are also widely used for BTE. The chitosan-based 3D hybrid scaffold (CS-PLA-HA) embedded with nanoparticles could promote MG-63 cells growth. Scaffolds dobbed with TiO2 had a positive influence on HA production [**217**]. In another study on bone tissue engineering, Sharifi *et. al.* synthesised scaffolds from polycaprolactone/chitosan (PCL/CS) and PCL/ carboxymethyl chitosan (PCL/CM) by electrospinning technique and cultured them with human osteoblast cells (MG63). The study reveals that scaffolds made of PCL or CMC can be a great choice over PCL or CTC for BTE applications [**218**]. In accordance with specific research, directly applying N-carboxybutyl chitosan to the wound promotes faster wound healing and prevents the production of scars following cosmetic surgery. According to other studies, periodontitis can be treated more effectively by simply administering chitosan ascorbate to the gums [**107**].

Cellulose-based aerogel

Cellulose-based aerogels are a revolutionary third-generation of aerogels which recently received huge interest as it has high adsorption efficacy, green prospects, and cost efficiency. In the literature, cellulose nanocrystal (CNC) is proven to be non-cytotoxic [**219**] and supports cell proliferation [**220**]. Aerogels made of chemically cross-linked cellulose nanocrystals (CNCs) provide a variety of advantages when used as scaffolds for bone repair. A group of researchers designed two different kinds of CNC aerogels by isolating CNCs from phosphoric acid or sulfuric acid to yield CNCs containing sulphate and phosphate half-ester surface groups, respectively. Morphology characterization of aerogel demonstrates that both kinds of aerogel are made up of condensed CNC sheets that are spaced apart by macropores, which are more than 100 mm in diameter. In vitro testing of aerogels with osteoblast-like Saos-2 cells for bone tissue scaffolds revealed an increase in cell metabolism. Following the 14 days of immersion in a simulated body fluid, all aerogels showed hydroxyapatite development. After being implanted in bone defects, sulphated cellulose nanocrystal aerogels significantly increased the bone volume percentage and demonstrated osteoconductivity, confirming the aerogel's capacity to promote bone formation and cell proliferation [**221**]. Bacterial cellulose (BC) is regarded as having flexible potential for use in bone regeneration because of its non-toxicity, high purity along with its biocompatibility. In one of the studies, authors examined the potential effect of a 3D porous microsphere of collagen (COL)/BC/bone morphogenetic protein 2 (BMP-2)-based scaffold in BTE application on the osteogenic differentiation of mouse MC3T3-E1 cells. The prepared porous scaffold was effective at promoting proliferation, adhesion, osteogenic differentiation and exhibited good biocompatibility. Additionally, osteoblast indicators include calcium nodules were produced by osteoblast development [**222**]. In another study, Xiao *et. al.* synthesised a bioactive mesoporous glass and bacterial cellulose (MBG/BC) nanocomposite-based scaffold and seeded it with human bone marrow stromal cells (hMBSCs). The prepared MBG-doped scaffolds were able to accelerate differentiation by stimulating the expression of bone-linked genes through the release of Ca_2^+ and PO_4^{3-} which can promote osteogenic differentiation [**223**]. Scaffolds based on plants are frequently utilized for tissue engineering because of the distinctive features they have. Sharmila *et. al.* produced a scaffold using Cissus quadrangularis (CQ) and Spinacia oleracea (SO) extracts along with carboxymethylcellulose (CMC) and alginate by lyophilization. The liquid displacement method for porosity measurement displayed, 78% and 62% porosity increase for Alg/CMC/SO and Alg/CMC/SO-CQ respectively. Alg/CMC/SO scaffold also showed 94.55% cell viability, which was higher than Alg/CMC/SO-CQ (77.62%), concluding the potential use of *Spinacia oleracea (SO)* for tissue regeneration [**224**]. **Fig. 9** highlights the manufacturing process of cellulose-based aerogels.

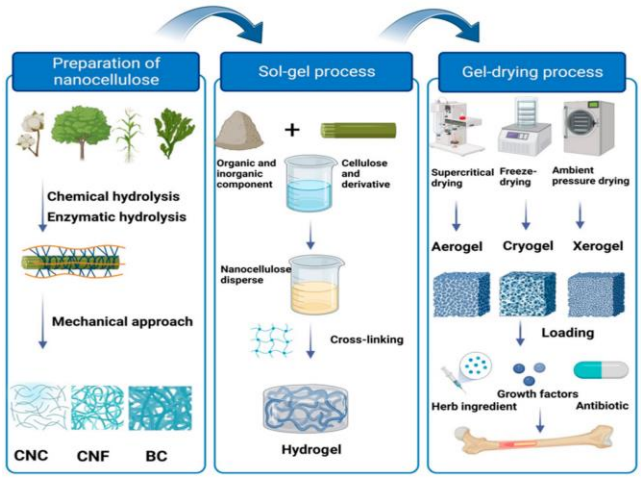

Fig. 9. Fabrication process of cellulose-based aerogels using the sol-gel method involves three main steps. First nanocellulose is prepared by purifying cellulose through chemical and enzymatic hydrolysis, followed by separation to form nanoscale cellulose materials using mechanical techniques. Second a sol-gel process takes place, where the polymer is dispersed in a solvent and undergoes a crosslinking stage. Third the gel-drying process is carried out [**225**].

Alginate-based aerogel

Alginate-based aerogels are getting used as scaffolds in bone tissue engineering because they can support bone cells proliferate and differentiate. In addition to their use as scaffolds, alginate-based aerogels are also used as vehicles for drug delivery in the branch of bone tissue engineering. In their study, Mejuto and team prepared aerogel by combining 3D printing and supercritical drying methods using alginate and hydroxyapatite. The cell viability studies on BALB cells showed no toxicity effect or adverse influence on the normal cell environment. Mesenchymal stem cells (MSCs) had seeded with scaffolds, and nuclei were stained after a couple of days, indicating favourable

adhesion and proliferation of the seeded cells **(Fig. 10)**. The authors performed a scratch test to analyse cell migration with BALB cells and observed an increase in fibroblast migration on alginate-based aerogels [**226**]. Yashaswini *et. al.* fabricated three types of microspheres using alginate (Alg), graphene oxide-dexamethasone (Alg-GO-Dex) and graphene oxide (Alg-GO) through air-dry and freeze-drying methods to evaluate their potential as bone graft substitutes. The author reported increased cell proliferation after the addition of dexamethasone and remarkable apatite production after 30 days of submersion of the Alg-GO microsphere using SBF solution, suggesting the Alg-GO-Dex microsphere as a feasible and viable substitute for bone transplant [**227**]. Another recent study intended to create a hydroxyapatite-reinforced nanohybrid formulation based on thiolated sodium alginate that can enhance the architecture of bones in bone diseases. The result indicated that thiolated sodium alginate/polyethylene glycol/ hydroxyapatite-based nanocomposite was 100-200nm in size, non-cytotoxic for MG63 cell line and supported the nanocomposite's ability to target and heal bone [**228**]. Martin *et. al.* designed hybrid alginate-based aerogels with starch as a second biomaterial using solvent exchange and the supercritical drying method for tissue engineering and regenerative medicine (TERM) applications, particularly for BTE. The SEM analysis of fabricated aerogel scaffolds revealed both mesoporous and microporous textures. The result of in vitro analysis showed that cells were migrating on the scaffolds surface, which were biologically active and non-cytotoxic [**229**]. Alginate-based composite aerogel also proved to have great antibacterial potential against bone-related pathogenic diseases. For example, Xiao *et. al.* prepared a composite aerogel that was created out of an alginate aerogel and a (Cu/TGC@PDA). Investigation of the swelling and retention properties of fabricated aerogel showed great hygroscopic properties with a good percentage of overall water absorption. To test antibacterial activity, the authors used S. aureus and E. coli as model microorganisms. Cu/TGC@PDA aerogel showed greater antimicrobial ability, which proves that they are appropriate for the use of infected bone tissue [**230**].

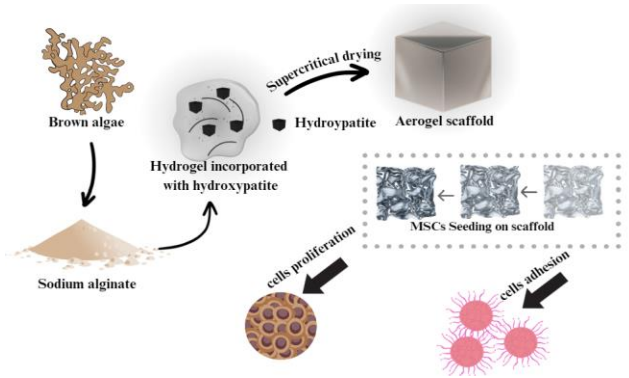

Fig. 10. A schematic illustration demonstrates the preparation and application of an alginate-based aerogel scaffold fabricated through 3D-printing and supercritical drying. This scaffold exhibits exceptional porosity, biocompatibility, and excellent fidelity to the CAD-pattern design, adapted from [**226**].

Other polysaccharide - based aerogel

Aerogel from another polysaccharide has been also used for BTE. HA is broadly used for not only bone but also cartilage tissue engineering. It is an effective material to deliver growth factors loaded with it without any interruption. Bae *et. al.* synthesized hydrogels made of HA that have been photocured and introduced with growth and differentiation factor 5 (GDF-5). The result of this work revealed that to supply osteogenic differentiation factors like GDF-5, hydrogel based on HA is a useful biomaterial, and GDF-5 can also be helpful in promoting the production of new bones [**231**]. In another experiment, authors designed an HA-based composite scaffold with chitosan using freeze-drying techniques. The prepared scaffold was found as noncytotoxic and capable of encouraging cell adhesion. The study also evaluated extracellular matrix (ECM) formation using staining methods and quantifications of glycosaminoglycan and DNA. The result suggested that incorporation of HA enhances cartilage ECM production, and this composite matrix could be useful for cartilage healing [**232**]. To explore the beneficial characteristics of HA for scaffolding, researchers mostly used it in its modified form. To examine the chondrogenic differentiation Nedunchezian *et. al.* made hydrogel by combining gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA) crosslinked with acrylate-functionalized nano-silica (AFnSi). The SEM result of scaffolds showed that amount of AFnSi crosslinking has an impact on the hybrid hydrogel's structural stability. In vitro study proved that hydrogel with 0.5% (w/v) acrylate-functionalized nano-silica (AFnSi) crosslinker supports human adipose-derived stromal cells (hADSCs) growth [**201**].

Starch, like other polysaccharides (chitosan and cellulose), is cost-effective, which is one of the reasons for its usage in tissue scaffolds. In one of the studies, supercritical drying processes were used to make very porous poly(ε-caprolactone) (PCL) construct with starch aerogel microspheres (1 micron in size) and a bioactive substance (ketoprofen, an NSAID) for bone reconstruction. The result of SEM characterization showed an immensely porous structure, which can encourage cell proliferation [**233**]. In another research work, researchers have developed a bio-nanocomposite by combining porous starch with silk fibroin nanofiber. For attaining bioactivity, they used calcium phosphate and test scaffold potential for bone tissue development. It is demonstrated through cell culture tests using osteoblast-like cells (MG63) on calcium phosphate-coated scaffolds that adding nanofibers of SF to the starch hydrogel enhances cell survival, adhesion, and proliferation [**234**]. Furthermore, Arriaga *et al.* also analysed cell survival of hydrogel made of starch that has been loaded with calcium carbonate or hydroxyapatite. Prepared hydrogel showed higher percentages of cell viability with zero toxicity [**235**]. **Table 5** shows a few recent research that used different polysaccharides in bone and other tissue engineering applications.

Table 5. Other polysaccharides that have been used in recent studies for bone and other types of tissue engineering.

Polysaccharides	Composite and type of scaffold	Fabrication technique	Type of cells	Research outcome	References
Ulvan	PCL-ulvan, chondroitin sulfate and κ-carrageenan aerogels	Freeze-drying	hADMSCs	Integration of ulvan into the polycaprolactone matrix successfully increased cell viability and attachment.	[236]
Xanthan gum	Alginate/xanthan gum/ TEOS crosslinked hydrogels	Freeze-drying	3T3 fibroblast	Production of collagen was proved by SDS-PAGE analysis	[237]
Chondroitin sulfate	gelatin/polyvinyl alcohol/chondroitin sulfate nanofibrous mat	Electrospinning	HDF	The scaffolds exhibited adequate cell adhesion, growth, and proliferation, as well as no toxicity.	[238]
Dextran	Polyacralamide/ dextran mineralized with hydroxyapatite hydrogels	Micellar copolymerization	MC3T3-E1 osteoblasts	Hydrogel exhibits superior mechanical properties, excellent osteoconductivity, and the ability to support bone regeneration	[239]
Heparin	PCL/keratin/ heparin/VEGF mats	NRD	HUVECs & HUASMCs	Mats have the potential to accelerate endothelial cell growth while inhibiting smooth muscle cell growth, which is desirable for vascular tissue engineering	[240]
Xanthan gum	Graphene oxide/xanthan gum/hydroxyapatite aerogels	Lyophilization	MG 63	The scaffold's porosity and polar functional groups contributed to regulating cell-matrix interactions, leading to enhanced osteoconductivity	[241]
Silk fibroin	MBG/SF sponge	3D printing	hBMSCs	Scaffolds exhibited improved compressive strength, approximately 20 MPa, and displayed good biocompatibility.	[84]
Carrageenan	Carrageenan incorporated with whitlockite nanoparticles and DMOG injectable hydrogel	NRD	ADMSCs	Increased protein expressions of RUNX2, COL, and OPN osteogenic markers have been observed in nanocomposite hydrogel.	[242]
Pullulan	PulMA/PEGDA hydrogel	NRD	Rabbit's MSCs	In addition to supporting the production of glycosaminoglycan (GAG) and the chondrogenic phenotype of MSCs, hydrogel showed strong cell adherence and proliferation.	[243]

***** NDR- no data reported, hADMSCs: human adipose-derived mesenchymal stem cells, TEOS: Tetraethyl orthosilicate, HDF: Human dermal fibroblast, MC3T3-E1: Mouse calvaria-derived osteoblast, MBG: Mesoporous bioactive glass, SF: Silk fibroin, hBMSCs: Human bone marrow-derived mesenchymal stem cells, DMOG: dimethyloxalylglycine, ADMSCs: Rat adipose-derived mesenchymal stem cells, PCL: Poly(ε-caprolactone), HUVECs: Human umbilical vein endothelial cells, HUASMCs: Human umbilical arterial smooth muscle cells, PulMA: Methacrylated pullulan, PEGDA: polyethylene (glycol) diacrylate

CHALLENGES AND FUTURE PROSPECTS OF POLYSACCHARIDES IN BONE TISSUE ENGINEERING

Bone defects caused by various reasons impose a negative impact on human lives as they are an important structural part of the body. Every year, millions of people worldwide experience bone diseases that affect both their physical and mental health. The traditional surgical approach to treating bone impairment primarily uses autogenous or allogenic bone transplantation, bone handling, periosteal transplantation, and other therapy techniques, but these techniques have drawbacks such as a delayed treatment cycle, significant surgical stress, immunological rejection, and a high failure rate [**244**]. Furthermore, these procedures cost the socioeconomic system significantly.

Bone tissue engineering has gained tremendous attention from researchers and provides a solution to bone diseases. Tissue engineering is a complicated and challenging process that requires expertise, rational knowledge, and dedication to develop artificial tissue and organs. Since it is a complicated process that deals with scaffolds, cells, and biologically active molecules, it is crucial to use materials that are non-toxic to living tissues and cells. Early bone TEs had some successful experiences that encouraged scientists to prepare scaffolds that can be easily modified to do the needful function of tissue regeneration, more specifically. Natural biomaterial for scaffolds is environmentally and ecologically safe and has no toxic effects on living cells, whereas synthetic material-based scaffolds are poorly compatible and produce toxic by-products after degradation.

Being biodegradable and biocompatible, polysaccharides have been frequently used for tissue scaffolding for the past few decades. These are eco-friendly and non-immunogenic, so the chances of developing antibodies against the implanted scaffolds are very low. They have sugar monomers in them and are essential for sustaining the extracellular matrix. Despite the encouraging outcomes reported in the literature, additional study will be required to investigate a variety of challenges that must be resolved for their successful use in this field.

Polysaccharides have lower mechanical strength compared to synthetic materials like polymers and ceramics. Some polysaccharides like chitosan, cellulose and starch are brittle in nature. Chitosan is ineffective in the aqueous phase. Similarly, cellulose cannot dissolve in some organic solvents, the hydrophilic nature of alginate limits its ability to absorb protein, which inhibits cell attachment and restricts its potential in the field tissue engineering. Most polysaccharides are water-soluble and can oxidise at temperatures higher than their melting point. Moreover, biological scaffolds sometimes degrade when stored for a longer period. The degradation rate of polysaccharides is difficult to control, and this can affect the rate of bone regeneration. Therefore, new techniques need to be developed to control the degradation rate of these materials. The above-mentioned limitations could be overcome if other biomaterials were to be incorporated with polysaccharides to create composite scaffolds. The mechanical characteristics of polysaccharides, which are necessary for tissue scaffolding, were proven to be improved by mixing two or more biomaterials (synthetic or natural) to create a scaffold.

Future prospects for polysaccharides in bone tissue engineering include the development of new processing techniques, such as electrospinning, to improve their mechanical properties. Blending it with other biopolymers to make polysaccharide composites has been reported in the literature to make durable and histocompatibility scaffolds for BTE. Advances in functionalization techniques can also lead to the introduction of bioactive molecules to enhance their bioactivity. Additionally, research on the immunogenicity of polysaccharides can help address concerns related to their use in tissue engineering. Overall, the application of polysaccharides in bone tissue engineering is quite promising, therefore more investigation needs to be done to overcome the current challenges and fully realise their potential.

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CONFLICTS OF INTEREST

There are no conflicts to declare.

SUPPORTING INFORMATION

Supporting informations are available online at journal website.

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GRAPHICAL ABSTRACT

The characteristics of various natural polysaccharides and their applications in aerogel-based scaffolds for bone tissue engineering as an alternative for wound remodeling and organ transplantation are explored. Further, it emphasizes how these properties make them promising in addressing bone-related concerns and discusses the future prospects and challenges in tissue engineering applications.