Application of Nanocellulose-Based Aerogels in BTE

Subjects: Cell & Tissue Engineering

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Based on the principles of biology and engineering, bone tissue engineering (BTE) has been widely used to construct substitutes for repairing and improving bone function. The skeletal system is a highly mineralized, vascularized, and connective tissue, which provides significant mechanical strength, fracture toughness, and weight-bearing capacity to protect internal organs. An ideal bone substitute should mimic the microstructure of natural bone tissue and provide a biological environment for bone regeneration and tissue repair. Furthermore, the design and preparation of hybrid nanocellulose hydrogels should fully understand the structure and composition of natural bone tissue.

Keywords: nanocellulose ; aerogel ; bone tissue engineering ; bone defect

1. Microstructure of Bone ECM

The main inorganic component of bone is hydroxyapatite (HA) crystals, which are embedded in the extracellular matrix (ECM) of bone. As the organic component of bone tissue, bone ECM is mainly composed of type I collagen fibers and serves as an inductive template for bone repair [1][2]. The mineral hydroxyapatite crystals deposit along the long axis of collagen type I fibers and present a hierarchical deposition within zones between collagen fibrils at the nanoscale [3][4][5].

The minerals of bone tissue are hierarchically assembled from the nanoscale [3]. Before mineralization, the organic phase of bone has been assembled, which can finely regulate crystal nucleation and growth. Needle-like mineral particles coalesce horizontally into platelets, neither inside nor outside the fibers, but form fractal-like hierarchical bone architecture with continuous intersecting fibers [3]. The mineralized collagen fibers on the microscopic scale are arranged in a complex hierarchical structure. At the macro level, most bones contain helical patterns in their anatomical shapes to increase adaptation to force. At the micro level, the spiral secondary bone itself is formed by concentric slices of mineralized collagen fibers. In terms of scaffold designing, biomimetic approaches, which can simulate molecular structural and biocompatibility with complex natural bone tissue [6][7][8][9], have gained increasing attention. By exploiting the unique properties of the pure or composite nanocellulose scaffolds, it is possible to improve the properties of the biomimetic materials with controlled and layered structures in nanostructures [10].

Electrospinning offers clear advantages for the preparation of scaffolds based on nanocellulose, including control over composition, structural design, and functional expansion [11][12]. It is a promising method for producing 3D aerogels in BTE and for mimicking the extracellular matrix (ECM) [13][14][15][16]. The core–shell structure of electrospinning is composed of PHB/G and PHB/G/Fe_3O_4 compositions, which result in lower melting points compared to pure PHB scaffolds. The resulting hybrid scaffolds have a lower crystallinity and are non-toxic, with the added benefit of high saturation magnetization in the magnetite composite scaffolds, which makes them well-suited for biomedical applications [17]. In addition, gas foaming is a process that involves introducing inert gas-foaming agents into the polymer phase, generating gas bubbles inside the 2D scaffolds via subsequent chemical reactions to expand the interconnected pores within the scaffolds [18]. Aerogels can also be prepared using gas foaming technology, which involves reassembling tightly packed 2D electrospinning nanofibers into fluffy 3D scaffolds with high porosity and large pores [13]. While 3D aerogels produced by gas foaming show great promise in BTE applications, there have been very few studies on fabricating nano cellulose-based aerogels using this technology. Therefore, future research should focus on this area.

2. Nanocellulose Aerogel Alone

Since 1971, when the first generation of a cellulose-based aerogel with a large specific surface area was fabricated, various studies have been performed to evaluate the toxicity, antibacterial properties, and mechanical properties of nanocellulose aerogels and provide a theoretical basis for their application in BTE [19][20][21][22]. Li et al. prepared a CNC-based aerogel by direct ink writing and freeze-drying and proved that the resulting aerogel exhibited dual porous and controllable structures [23]. The main disadvantage of CNC-based aerogels is obviously their brittleness, which would lead to structural damage during cell incorporation and growth. Optimizing the crosslinking method might improve mechanical performance by adding 2.5 wt% polyamide-epichlorohydrin (kymene) into the nanocellulose polymer dispersion before cross-linking. This increased the Young's modulus of the composite aerogel from 7 MPa to 8.94 MPa [23]. Epoxypropane exhibits significant cytotoxicity, and its linear structure with bulky side chains limits its degradation compared to other types of chemical crosslinking agents, which restricts its usage in tissue engineering [24][25]. In another study, Osorio et al. grafted a hydrazide group onto a carboxylic acid group to form a hydrazone linkage on the surface of a CNC-based aerogel and proved that the prepared cellulose aerogel presented an excellent flexibility, high porosity, and osteoconductive properties after chemical crosslinking [26].

3. Nanocellulose-Based Composite Aerogels

Due to the existence of hydrogen bonds, nanocellulose can not only be self-assembled itself, but also assembled with other polymer materials. The aerogels with cellulose alone presented the disadvantages of hydrophilicity and poor osteoconduction [27][28]. In order to overcome these drawbacks and preserve their inherent superiorities, the composite fabrication methods of nanocellulose aerogels have received more and more attention, as the mechanical properties, biodegradability, bioactivity, and superior biological properties of bone scaffolds are adjusted by combining cellulose with different organic and inorganic compounds [29][30]. The contents regarding the combination of nanocellulose with other materials are summarized as follows (**Table 1**).

Table 1. Cellulose aerogel in bone tissue engineering.

Composite	Preparation Method	Porosity or Pore Size	Mechanical Properties	Seeding cell	Results	Year	Ref.
HA–CNC	Esterification reaction and freeze-drying	91%	Compressive strength: 41.8 MPa	-	Biodegradable, non-toxic, low immunogenicity, biocompatibility and flexible-shaped ability	2018	[31]
CNC	Hydrazone crosslinking and CO_2 supercritical drying	98.8–99.3%	Young's modulus: 25–65 KPa	Osteoblast-like Saos-2 cells	High porosity and effective boe growth promotion osteoconduction	2019	[26]
HA–BC	Freeze-drying(cryogels)and $scCO_2$drying(aerogels)HMDA crosslinking	30–80 nm	Modulus of elasticity: 10.91±3.26 gPa, hardness 0.37±0.18 gPa.	-	Excellent mechanical strength	2019	[32]
Gelatin–CNF	Freeze-drying	94–95% 300 μm	35.2–54.7 km	L929 fibroblasts	Suitable for cell adhesion and growth	2019	[33]
CS–CMC	GA crosslinking and freeze drying	82 ± 5% Mesoporous: > 100 μm Microwell: <50 μm	Intensity: 2.51 GPa Modulus: 139 MPa	MG63	Cell viability increased significantly	2019	[34]
PEGDA–CNF	SLA and freeze drying	Average pore size: 46–69 μm	The elastic deformation was 35KPa under 30% stress	BMSC	Suitable for cell adhesion and growth	2019	[35]
PCL–CS-cellulose acetate	Electrospinning and freeze-drying	-	Compression modulus up to 0.31 MPa Compression modulus: 45 ± 6 Kpa	MC3T3-E1	Improves cell adhesion, infiltration, and osteogenic differentiation	2020	[16]

Composite	Preparation Method	Porosity or Pore Size	Mechanical Properties	Seeding cell	Results	Year	Ref.
SF-cellulose	Chemical crosslinking and freeze drying	-	Tensile strength: 7.73 MPa Bending strength: 25.91 MPa	HEK-293 T cells	Excellent mechanical strength	2021	[36]
CS–CNF	freeze drying	97.20%	Young's modulus: 0.28 MPa	-	Excellent mechanical properties	2021	[37]
SF–n–HA–cellulose	Chemical crosslinking and freeze drying	99.20%	Young's modulus: 12.7–22.4 MPa	HEK-293T cells	Controllable degradation rate; Good mineralization capacity;	2021	[38]
PEGDA–CNF	Stereolithography and freeze-dried	Mesopora: 400–800 um Micropore size: 20~100 μm	Young's modulus: 2.94 MPa	Mouse BMSC	Controlled pore structure Adjustable Poisson's ratio	2021	[39]
CS–CNC	Chemical crosslinking and carbon monoxide$_2$Supercritical drying	20–60 nm	At 0% strain, the compressive strength is 13.3 MPa	-	Reduce gel shrinkage Excellent osteoconduction	2021	[40]
BC	Freeze-dried and inoculated with BMP2	Large bore: > 100 μm, micropores: <100 μm, nanopores: <100 nm	-	BMSC	osteoconduction	2021	[41]
COL–n–HA–CNF	Thermal crosslinking and freeze-dried	90% 75 ± 18 μm	The elastic modulus is (12.95 ± 4.77) MPa and the compression ratio is (0.4067 ± 0.084) MPa.	Rabbit BMSC and human vascular endothelial cells	Control releasing ability osteogenesis and vascularization abilities.	2022	[42]
ε-poly-l-lysine-TEMPO CNF	Esterification, crosslinking with CA and freeze drying	≥85.05%	Tensile strength: 22 MPa	-	Antimicrobial properties and degradability	2022	[43]
PEGDA/cellulose	SLA and freeze drying	20–50 microns	0.58 ± 0.0222 MPa	BMSC	Dynamic Poisson's ratio promotes differentiation at different stages of BMSC	2022	[44]

3.1. HA–Nanocellulose Aerogels

Scaffolds with organic and inorganic components can mimic the microstructure of natural bone, which not only promotes the proliferation of osteoblast lineage cells, but also provides an optimal microenvironment for the formation of blood vessels. Traditionally, the inorganic phase of biomimetic bone tissue has been mainly focused on inorganic materials such as nanosilicate particles, calcium phosphate, and bioactive glass.

As an environmentally friendly biomaterial, hydroxyapatite (HA) has excellent biocompatibility and constitutes the inorganic phase of bone, which can release various bone conduction ions to the surrounding environment [9][45][46]. However, the disadvantages of HA, such as low in vivo absorption, low crack resistance, and poor bone irritation, limit its clinical application [47]. The addition of HA to cellulose-based aerogels can enhance the mechanical properties of building organic-inorganic aerogels. Huang et al. attached an in-situ HA coating of about 10 nm to the CNC surface and then crosslinked it with polymethyl vinyl ether malonic acid (PMVEMA) and polyethylene glycol (PEG) to enhance the mechanical properties of the composite. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) show esterification between CNC, HAP, PMVEMA and PEG. The results show that the attachment of HA increases the compressive strength of the obtained stent to 41.8 MPa, which provides broad potential for the development of BTE stent [31]. Cheng et al. also fabricated an HA-BC aerogel and mineralized it in situ by embedding the aerogel in $CaCl_2$and

K_2High crude oil$_4$Solution. The results show that composite aerogels with excellent biocompatibility enhance the mechanical properties and mimic the structure of natural bone [32]. The above studies prove that the incorporation of HA into nanocellulose aerogels can not only improve its mechanical properties, but also serve as a template for biomimetic mineralization. In addition, due to the urgent need for biomimetic theory, advanced preparation techniques such as 3D printing can be used to orderly deposit HA layers onto collagen fibers to simulate the microstructure of natural bone tissue.

3.2. Bioactive Glass-Nanocellulose Aerogels

Bioactive glass (BG) can release calcium and phosphate into the surrounding environment, resulting in HA deposition on the surface of biomaterial after in vivo transplantation. Bone substitutes based on bioactive glasses have been widely used in BTE. Kamel et al. prepared a nanofibrillated cellulose aerogel loaded with strontiumborate-based bioactive ceramic particles and rosuvastatin to process the extraction socket [48]. The results showed that composite aerogels exhibited excellent mechanical properties, promoted the proliferation of MG-63 cells, and were promising materials for preserving dental sockets.

3.3. Collagen-Nanocellulose Aerogels

The organic phase of natural bone tissue, as a layered skeleton, plays a vital role in biomineralization. Commonly used biopolymers include chitosan (CS), collagen, cellulose, etc. All of these have proven to be suitable platforms for mimicking the inorganic phase of bone and as an alternative to manufacturing composite scaffolds similar in structure and composition to natural bone [49]. As the main component of the organic phase of bone tissue, collagen (Col) in the natural bone can serve as a template for biomineralization, controlling the orientation and shape of HA crystals by providing nucleation sites. Then, in vivo, biomineralization processes occur and lead to the nucleation and growth of HA nanocrystals along the Colfiber axial direction [50]. In recent years, many studies have focused on the preparation of cellulose biomimetic scaffolds. For example, He et al. prepared biomimetic collagen-carboxymethylcellulose/hydroxyapatite scaffolds from a biomolecular template of collagen-carboxymethylcellulose, and the scaffolds had good biocompatibility. By controlling the ratio of collagen to carboxymethyl cellulose in the template, the bone inductance, bone conductivity, and mechanical strength of the composite can be changed and adjusted according to the requirements of BTE [51]. Xu et al. prepared nano cellulose-collagen (COL)-nanohydroxyapatite (n-HA) organic-inorganic hybrid aerogels by adding collagen and HA to cellulose aerogels and found that composite aerogels exhibit porous 3D structures with high compressive strength, excellent osteogenic and angiogenic abilities in vitro and in vivo [42]. Based on the above studies, it can be concluded with certainty, Organic-inorganic hybrid materials based on Col and nanocellulose combined can construct multi-level biomimetic scaffolds from macro to micro, which have great potential for bone defect repair.

3.4. Chitosan–Nanocellulose Aerogel

Chitosan (CS), with a structural similarity with glycosaminoglycan, has excellent osteoconduction ability [52][53]. In a study, high-pressure homogenization and freeze-drying technologies were utilized to fabricate CNF-based and chitosan-based composite aerogels. The results showed the CNF aerogels exhibited the highest porosity, lowest density, and worst mechanical properties. However, adding chitosan into CNF can not only significantly improve the mechanical properties but also reduce the water absorption of the composite aerogels [37]. In another study, Matinfar et al. prepared biphasic and triphasic calcium phosphate fiber-reinforced CS- carboxymethyl cellulose (CMC) porous scaffolds by a freeze-drying method [34]. The broad band observed in the chitosan spectrum between 3367–3449 cm^{-1} corresponds to the stretching vibration of N–H and O–H groups. In addition, the CMC powder spectrum exhibited distinctive bands at 1602 cm^{-1}, 1424 cm^{-1}, and 1330 cm^{-1}, which are characteristic of carboxyl, methyl, and hydroxyl groups, respectively. Furthermore, a band at 1057 cm^{-1}, attributed to the stretching vibrations of -CH2OH, was also observed. The biphasic fiber was composed of HA and triclinic apatite, and the triphasic fiber was composed of HA, β-tricalcium phosphate, and calcium pyrophosphate. Thus, the incorporation of the organic phase into the CS–CMC aerogels further enhanced their mechanical properties and effectively solved the above-mentioned problem.

3.5. PVA–Nanocellulose Aerogel

Polyvinyl alcohol (PVA) is also a favorable biopolymer. With insufficient mechanical strength, which is significantly lower than natural bone, PVA alone is not suitable to be fabricated into BTE substitutes. Incorporation of PVA into the nanocrystalline cellulose scaffolds could also solve this problem and tailor their biological performance. Zhou et al. synthesized a PVA/CNFs/gelatin hybrid aerogel by the utilization of gelatin as the crosslinking agent. The modulus of the PVA/CNFs/gelatin aerogels is 1.65 MPa, significantly higher than those of the pure CNF and PVA/CNF aerogels [27]. Cataldi et al. combined nanocrystalline cellulose with PVA to fabricate a composite scaffold with enhanced tensile stress, contributed by the involvement of the nanocrystalline cellulose. However, the incorporation of an excessive amount of nanocrystalline cellulose also led to the agglomeration of nanoparticles and decreased the tensile stress of the composite scaffold [54]. Liu et al. prepared CNFs/PVA/montmorillonite aerogels and investigated the effects of crosslinkers (borax and glutaraldehyde) on the formation of the interface bonding and porous network. The results proved that glutaraldehyde crosslinking resulted in larger and looser pores of the composite aerogels as compared with those prepared by the borax crosslinking method [55]. Therefore, adding nanocellulose would increase the mechanical performance of the composite scaffolds, whereas incorporation of PVA enhances their biocompatibility.

3.6. SF–Nanocellulose Aerogel

Silk fibroin (SF), with favorable biocompatibility and noncarcinogenic ability, is extracted from silkworm cocoons and has the ability to promote preosteoblasts proliferation and MSCs osteogenic differentiation, demonstrating favorable advantages in bone regeneration [56][57]. However, its short absorption times and low mechanical properties limited the application of SF in the BTE field due to the high requirements for bone substitutes and the relatively long healing process of bone tissues. After the combination of SF and nanocellulose materials with relatively longer absorption periods and higher mechanical properties than SF, SF/nanocellulose composites exhibit the advantages of both SF (good biocompatibility, easy degradation, and excellent osteoinductive ability) and nanocellulose (remarkable mechanical strengths and long absorption time), making them great prospects for functional applications in BTE. Chen et al. prepared mineralized self-assembled silk fibroin (SF) –cellulose composite aerogels with an interpenetrating network by freeze-drying. In situ mineralization was then performed to control the nucleation and growth of n–HA crystals onto the surface of the composite aerogels [36]. After the mineralization of HA, the zeta potentials of the cellulose aerogel and SF/nanocellulose composite decreased from −11.1 mV and −26.3 mV to −6.3 mV and −4.1 mV, respectively. These zeta potentials are close to the −5.8 mV of n–HA. The results show that mineralized SF–cellulose composite aerogels have a good microstructure such as ideal cancellous bone, moderately adjusted compressive strength, and high degradative rate in vitro. In addition, it can also promote the proliferation of human embryonic kidney cells (HEK293T) which has potential in BTE [36]. Although only a few studies have focused on SF–cellulose-based aerogels and their application in BTE fields, there is still an attractive potential for nanocellulose-based aerogels in repairing bone defects.

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