Seema Garg
Amrish Chandra
Suresh Sagadevan *Editors*

Emerging Sustainable Nanomaterials for Biomedical Applications

Springer

Emerging Sustainable Nanomaterials for Biomedical Applications

Seema Garg · Amrish Chandra · Suresh Sagadevan
Editors

Emerging Sustainable Nanomaterials for Biomedical Applications

Springer

Editors
Seema Garg
Amity Institute of Applied Sciences
Amity University
Noida, Uttar Pradesh, India

Amrish Chandra
School of Pharmacy
Sharda University
Greater Noida, Uttar Pradesh, India

Suresh Sagadevan
Nanotech and Catalysis Research Centre
University of Malaya
Kuala Lumpur, Malaysia

ISBN 978-3-031-63960-9 ISBN 978-3-031-63961-6 (eBook)
https://doi.org/10.1007/978-3-031-63961-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Nanomaterials are materials at the nanoscale (1–100 nm) that exhibit unique physical, chemical, and biological properties due to their small size and high surface area. These properties have opened up new opportunities for nanomaterials in various scientific and industrial fields including medicine.

In the realm of biomedicine, nanomaterials offer a wide range of potential applications, including targeted drug delivery, medical imaging, diagnostic tools, and tissue engineering. They can interact with biological systems at the cellular and molecular levels, enabling precision and efficacy in medical treatments. The emerging nanomaterials, such as carbon-based nanomaterials (e.g., carbon nanotubes), metal nanoparticles (e.g., gold and silver), Photocatalytic material (e.g., TiO_2), quantum dots (e.g., carbon and silicon quantum dots), and drug functionalized nanoparticles are showing promising applications in biomedicine.

This book encompasses a detailed prominence on emerging nanomaterials for their medical applications and public health. Various nanomaterials and quantum dots, e.g., carbon nanotubes, carbon Quantum Dots, silicon quantum dots, silica nanoparticle, endo-fullerenols with lanthanides, magnetically functionalized nanoparticles, photocatalytic TiO_2 nanomaterial, and drug-functionalized nanoparticles have been covered in this book. Biomedical applications of emerging nanomaterials viz., clinical applications, bone and cartilage tissue engineering, bioimaging applications, bacterial infections treatment, reduction of greenhouse gases, evaluation of cytotoxicity, and implant dentistry have been encapsulated in this book. This book also reveals the application of nanotechnology in the mitigation of air pollution exposure for better public health, and elaborates challenges and opportunities associated with the development of functionalized electrospun fibers.

This book focusses much towards emerging nanomaterials for biomedical applications and covers the following chapters:

Chapter "Carbon Nanotubes for Cartilage and Bone Tissue Engineering" elaborates on carbon Nanotubes for bone and cartilage tissue engineering. Cartilage and bone are crucial components of the human body that impact the health of millions of people worldwide. The degradation or loss of cartilage and bone severely affects mobility, decreasing the quality of life. It deliberates on the detailed pathway of CNT

use in cartilage and bone tissue engineering, as well as its importance for future research. This chapter covers various factors of CNT, including fabrication methods, long-term biocompatibility, degradability, and the impact of hybrid scaffolds on cell activity.

Chapter "Synthesis of Silicon Quantum Dots for Bioimaging Applications and Their Impact on Public Health" summarizes the synthesis of silicon quantum Dots (QDs) for bioimaging applications and their impact on public health. QDs are nanoscale semiconductor particles with distinct optical and catalytic properties because of quantum confinement. They have several uses in photonics, biology, and other areas. To overcome difficulties in producing high-quality QDs with stable qualities has led to a focus on researching microwave-assisted synthesis and scalable production methods.

Chapter "Silica Nanoparticle for the Treatment of Bacterial Infections" covers silica nanoparticle for the treatment of bacterial infection. The emergence of antibiotic tolerance in numerous bacteria species, resulting in ineffective therapy, is a major challenge in the therapy of bacterial illnesses. One approach to overcoming this limitation is to utilize nanoparticles which can effectively react with the bacterial surface, destroying their cell wall and causing death of cells.

Chapter "Structuring of Endofullerenols with Lanthanides in Aqueous Solutions" elaborately discusses the structuring of endo-fullerenols with lanthanides in aqueous solutions. Endo-fullerenols with lanthanides (Ln) being effective molecular magnets are attractive in biomedicine as spin labels and especially as magnetic contrast agents to increase the diagnostic capabilities of Magnetic Resonance Imaging.

Chapter "Biomedical Applications of Magnetically Functionalized Nanoparticles" covers biomedical Applications of magnetically functionalized nanoparticles. Incorporating magnetically functionalized nanoparticles into biomedical contexts has emerged as a revolutionary avenue within nanomedicine. The discussion extends to consider toxicological aspects and biocompatibility, drawing insights from in vitro and in vivo studies that address potential immunological responses and long-term toxicity.

Chapter "Bioimaging Probes Using Functionalized Inorganic Nanoparticles" discusses bioimaging probes using functionalized inorganic nanoparticles. The remarkable versatility of functionalized inorganic nanoparticles (NPs) as bioimaging probes is undeniable. Their vast surface area, customizable size, and easy surface functionalization, inorganic NPs have vastly improved the competences of conventional imaging techniques. The dynamic and evolving landscape of functionalized inorganic nanoparticles as bioimaging probes presents a promising avenue for continued research and innovation in the field of medical imaging.

Chapter "Gene Transformation Using Nano-technology and Delivery Methods of the Bio-modifier-Conjugated Complex to Plant Cells" focusses on gene transformation using nanotechnology and delivery methods of the bio-modifier-conjugated complex to plant cells. Many methods of genetic engineering of plant cells such as *Agrobacterium*-mediated plant transformation, electroporation, biolistics/gene gun, or polyethylene glycol (PEG) mediated have been in use since long. The present

review comprehensively analyzes the various delivery methods of the bio-modifier-conjugated complex to plant cells and highlights the importance of the development of new efficient strategies for plant genetic transformation with greater simplicity.

Chapter "Metal and Metal Oxide Enabled Sensors for Biomedical Applications" deliberates metal and metal oxide enabled sensors for biomedical applications. Sensors have become a boon to many user-friendly device applications and cover almost all fields like hospitals, industries, buildings, environment, surgical operations, human healthcare, and so on. The key highlights of the sensors are: non-toxic, readily available, synthesized easily and cost-effective. The biomedical applications of these metal and metal oxide-based sensors are detailed more in this chapter.

Chapter "Biogenic Metal Nanoparticles for Antibacterial and Antifungal Applications and Their Challenges" covers biogenic metal nanoparticles for antibacterial and antifungal applications and their challenges. Conventional methods for the synthesis of nanoparticles often rely on toxic chemicals and extreme conditions, raising concerns about their environmental impact and safety. This chapter presents a promising alternative to biogenic metal nanoparticles.

Chapter "Nanobiotechnology: an Applicable Approach for Sustainable Future" elaborates nano-biotechnology, an applicable approach for a sustainable future. The process of creating a sustainable future entails technique that simultaneously meet the needs of the present and future generations. In this chapter, authors investigated the current developments and constraints of biotechnology as well as the nano-based substitutes that nanotechnology offers. Biotechnology is likely to make many more advancements in the future, and when combined with nanotechnology, it brings humanity one step closer to a sustainable future.

Chapter "Application of Nanotechnology in the Mitigation of Air Pollution Exposure for Better Public Health" discusses the application of nanotechnology in the mitigation of air pollution exposure for better public health. Till today, air pollution is one of the leading causes of global burden of diseases. This chapter discusses about the public health and economic burden due to air pollution followed by the implications of science and technology in the form of nanotechnology to mitigate air pollution for better public health.

Chapter "Challenges and Opportunities Associated with the Development of Functionalized Electrospun Fibers" shows the challenges and opportunities associated with the development of functionalized electrospun fibers. Electrospinning is a cost-effective and versatile technique for producing highly porous and functional fiber mats from micro/nanofibers as building blocks. The subsequent sections delve into various methods for preparing functionalized electrospun nanofibers, addressing associated challenges and opportunities. This exploration aims to provide insights into the landscape of electrospinning, highlighting its potential for innovation in various domains, from advanced technologies to medical applications.

Chapter "Photocatalytic TiO_2 Nanomaterial to Reduce Greenhouse Gases" reveals photocatalytic TiO_2 nanomaterial to reduce greenhouse gases. Titanium dioxide (TiO_2) is gaining popularity as a potential contender for photocatalytic carbon dioxide (CO_2) reduction to transform industrial CO_2 gas into fuels. After that, the newly developing advancements and improvements in TiO_2 nanostructures by

making hybrid nanostructures and heterostructures for conquering the aforementioned barriers to obtain good photocatalytic activity, as well as the capacity to prevent electron-hole pair recombination, are explored.

Chapter "Carbon Quantum Dots, Its Synthesis and Evaluation of Its Cytotoxicity" exhibits carbon quantum Dots, its synthesis and evaluation of its cytotoxicity. Currently preferred over semiconductor quantum dots (QDs) due to their solubility, low toxicity, environmental friendliness, and easy and inexpensive production that yields desired optical features, carbon quantum dots (CQDs) are a novel class of nano-carbons. With an emphasis on their synthetic pathways, chemical and optical properties, biomedical applications, evaluation of cytotoxicity, and strategies to overcome cytotoxicity—as well as fresh perspectives in this fascinating and exciting field—this chapter emphasizes the current progress and advancement of CQDs.

Chapter "Role of Nanomaterials in Implant Dentistry" displays the role of nanomaterials in implant dentistry. Implant dentistry has revolutionized the field of oral healthcare, providing durable solutions for tooth loss and structural support. Through controlled release mechanisms, nanomaterial-based coatings exhibit sustained antimicrobial activity, inhibiting bacterial colonization while preserving the surrounding oral microflora.

Chapter "Drug-Functionalized Nanoparticles for Clinical Applications" summarizes drug-functionalized nanoparticles for clinical applications. In this chapter, the types of drug-functionalized nanoparticles (NPs) and their clinical applications have been extensively reported. This comprehensive overview underscores the potential of drug-functionalized NPs to transform therapeutic modalities and offers insights into future advancements in this interdisciplinary field.

Noida, India — Seema Garg
Greater Noida, India — Amrish Chandra
Kuala Lumpur, Malaysia — Suresh Sagadevan

Contents

Carbon Nanotubes for Cartilage and Bone Tissue Engineering

Sivaraj Durairaj, Himadri Shekhar Roy, Malika Arora, Keshav Kant Ahuja, and Deepa Ghosh

Abstract Cartilage and bone are crucial components of the human body that impact the health of millions of people worldwide. The degradation or loss of cartilage and bone severely affects mobility, decreasing the quality of life. Conventional methods for replacing or treating cartilage and bone-related defects have often failed to replicate the functions of natural bone and cartilage. Herein, we explore the potential of carbon nanotubes (CNTs) as promising materials for cartilage and bone treatment. This exploration discusses their important characteristics, along with the synthesis and characterization of CNTs. Additionally, it examines the transformative impact of CNTs at the cellular level for repairing injured tissues and revitalizing life. Firstly, it explores the influence of CNTs on tissue recovery or regeneration. Secondly, it reveals the development of secure and efficient implementation strategies for CNTs and their composites at the affected site. Finally, it deliberates on the detailed pathway of CNT use in cartilage and bone tissue engineering, as well as its importance for future research.

Keywords CNT · Tissue engineering · Bone · Cartilage · Osteochondral defects

1 Introduction

Globally, over 1.7 billion people are affected by musculoskeletal disorders, including osteoarthritis and fractures, which have a profound impact on global health. Osteoarthritis (OA) alone affects over 300 million people, which is projected to

S. Durairaj · H. S. Roy · M. Arora · K. K. Ahuja · D. Ghosh (✉)
Chemical Biology Unit, Institute of Nano Science and Technology, Sector-81, Mohali 140306, Punjab, India
e-mail: deepa.ghosh@inst.ac.in

S. Durairaj
e-mail: siva191091@gmail.com

M. Arora
e-mail: malika.ph21241@inst.ac.in

S. Garg et al. (eds.), *Emerging Sustainable Nanomaterials for Biomedical Applications*,
https://doi.org/10.1007/978-3-031-63961-6_1

escalate due to the aging population and lifestyle factors. Millions of individuals endure severe fractures and injuries leading to multiple hospital visits, discomfort, risk of infections, and cost. To overcome these limitations, there is an urgent need for proper regeneration therapies to ensure recovery and functionality. Injuries affecting the articular cartilage and its underlying bone are classified as osteochondral defects (OD) [1, 2] predominantly occurring in the ankle and knee due to factors such as overweight, degenerative disease, aging, iatrogenic intervention, irregular nutrition and metabolism, genetic predisposition, haemophilia, and post-traumatic deformation [3]. Damage to the AC causes subchondral bone damage, which leads to full joint destruction. Due to the physical relationship between articular cartilage and bone, biochemical and molecular crosstalk that occurs across this region during OD has been postulated [4, 5].

The soft connective tissue of cartilage, particularly articular cartilage (AC), is essential for smooth, painless movement and structural support. Among the three main types, AC is vital for the function of synovial joints by reducing friction during articulation. It provides mechanical support to the knee, hip and trachea, emphasizing its significance in facilitating seamless joint motion and overall joint health [6]. The limited regenerative potential of AC is attributed to its avascular, aneural, and alymphatic nature [7, 8]. AC has a highly organized structure comprising of chondrocytes embedded in an extracellular matrix (ECM). The balanced synthetic and catabolic actions of chondrocytes maintain cartilage integrity. The ECM's composition consists of proteoglycans, water, collagen II, IX and XI, chondroitin sulphate, and non-collagenous proteins are crucial for its function [9, 10]. Aggrecan is an essential proteoglycan for hydration and mechanical strength in AC [11]. AC is organized into four zones: superficial zone, middle zone, deep zone, and calcified zone. The superficial zone has high tensile properties, the middle zone has fewer chondrocytes, the deep zone has low cell density and high aggrecan content, and the calcified zone exhibits different forms of collagen [12, 10]. Notably, articular cartilage minimizes impact forces through the absorption of vibration and ensures low-friction joint movement by transferring loads to the subchondral bone. Despite its crucial role, AC faces challenges in self-repair due to trauma, high mechanical loads, and degradation, compounded by the absence of nerves, blood vessels, lymphatic vessels, and low chondrocyte activity [7]. Similarly, bone is a dense and active vascularized tissue that undergoes constant remodelling processes of ossification, mineralization, and resorption throughout life. It is made up of mineralized ECM (extracellular matrix), consisting mainly of hydroxyapatite (calcium and phosphate in the ratio of 1.67), collagen, various cells (osteoclasts, osteoblasts, and osteocytes), and non-collagenous proteins [13–15]. Under normal circumstances, bone resorption and growth occur simultaneously, establishing a dynamic equilibrium. In case of any damage or defects formed, it falters the bone's dynamic equilibrium [16]. Minor trauma, like small fractures may allow the bone to return to its original state, but severe defects may require external support due to its insufficient regenerative capacity. Bone repair may be delayed, stopped or even misaligned if the defect is large enough, as is the case with critical-sized bone defects. This is because the body's regeneration ability becomes inadequate in such circumstances [17, 18].

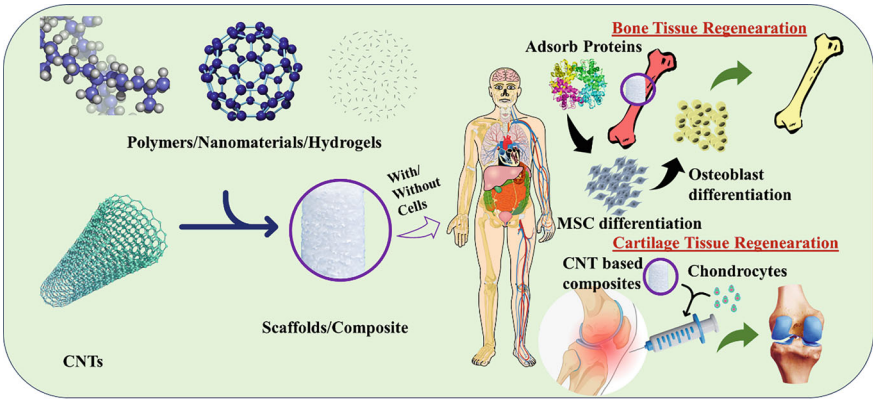
Current strategies for cartilage regeneration include autologous chondrocyte implantation (ACI), mosaicplasty and microfracture, which are currently employed for cartilage regeneration [19–22]. ACI and mosaicplasty are used to repair the cartilage through stimulating bone marrow activity, which in turn promotes the migration and differentiation of endogenous BMSCs (mesenchymal stem cells produced from bone marrow). Ossification and cartilage enlargement during treatment limit its use in cartilage regeneration [23, 24]. In addition to this, there are some downsides to these therapies, such as fibrocartilage development, less-than-ideal effects on tissue integration, infection, rejection, and a lack of autografts [25, 26]. Therefore, highlighting the urgent need for novel tissue regenerative (tissue engineering) therapies to overcome these constraints and enhance the outcomes of the patient. The procedures for addressing bone injuries include autografts, allografts, and synthetic bone graft substitutes. Whereas bone graft supply is limited along with its associated risks, synthetic bone substitutes have several limitations [2, 27]. Therefore, research in this field holds immense potential for improving patient outcomes and alleviating the burden of OD.

Tissue engineering (TE) is a field of biomedical engineering that utilizes cells, materials, and the right molecular and physical parameters to enhance or replace the damaged biological tissues [28]. Tissue engineering is a promising approach to address the limitations of the traditional approaches such as pharmaceuticals, prosthetic devices, or organ transplants by aiming to create tissues and organs artificially to mimic natural functions, even in cases of extensive defects or injuries exceeding a critical size. It replicates the complexity of natural tissues through advancements in biology, materials science, chemistry, and engineering. In TE, apart from cells and growth factors, biomaterials play a crucial role as scaffolds for cell growth and differentiation [29, 30].

Carbon nanotubes (CNTs) have promising applications in the fields of energy, the environment, and tissue engineering. Carbon nanotubes (CNTs) are carbon allotropes made entirely of carbon molecules joined by sp^2 bonds [31]. CNTs can be conceptualized as a single sheet of graphene that has been rolled into cylindrical nanoparticles. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are two types of CNTs, both have cylindrical shapes composed of carbon atoms with exceptional mechanical strength, conductivity, and biocompatibility. Single-walled carbon nanotubes are hexagonal layers of carbon atoms that have a diameter of 0.4 nm and a length of several micrometres. They are ideal for use in biosensors and imaging agents because of their special mechanical and electrical properties. MWCNTs consist of multiple concentric layers of SWCNTs with diameters ranging from 1 to 10 nm, providing high mechanical strength and thermal conductivity suitable for tissue engineering and regenerative medicine applications [31, 32]. Therefore, they have been widely studied for their potential use in TE and regenerative medicine due to their exclusive properties [33]. Particularly in constructing scaffolds, CNTs have been widely explored over conventional materials utilized in tissue engineering applications owing to their exceptional mechanical, electrical, biological, and thermal properties compared to other biomaterials. CNTs

have been particularly employed for damaged or diseased bone and cartilage regenerations, creating functional replacements with sufficient mechanical strength and electrical conductivity, unlike traditional tissue engineering strategies [33]. Especially, the electrical conductivity of the CNTs supports the growth and functions of cells at the treatment site. The nanostructured dimensions of CNTs mimic the extracellular matrix proteins that support integration and interaction with the surrounding tissues, resulting in enhanced biocompatibility. Therefore, CNT can serve as a potential candidate to construct scaffolds in tissue engineering. The scaffolds direct the growth of new tissue and provide support for cell migration, proliferation, and differentiation. CNTs can be functionalized with specific molecules that promote differentiation to specific cell types to favour the formation of specialized tissues such as bone or neurons, by loading with growth factors or small molecules thereby allowing for targeted delivery to the site of interest. CNTs can potentially be utilized to transport drugs or genetic material to specific tissues. It can also be employed to develop sensors that track the healing of injured tissue. The biocompatible and biodegradable nature of CNTs helps to recover and heal defects sites without causing any adverse effect to the surroundings/normal tissues. Despite its significance, two crucial points such as dispersion and binding must be addressed to ensure the successful implementation in bone and cartilage tissue engineering. Surface modification, functionalization, and optimization of fabrication methods are the ways to ensure the uniform distribution of CNTs and strong binding with the matrices. Overall, CNTs hold great potential in bone and cartilage tissue engineering applications.

This book chapter specifically explores the application of CNTs in bone and cartilage tissue engineering as well as drug delivery system. The goal is to contribute to the understanding and advancement of CNTs in the field of bone and cartilage tissue engineering. It also discusses CNTs' application in scaffolds, polymers, composites, and hydrogels for bone and cartilage tissue engineering as represented in Scheme 1.



Scheme 1 Carbon nanotubes for bone and cartilage regeneration

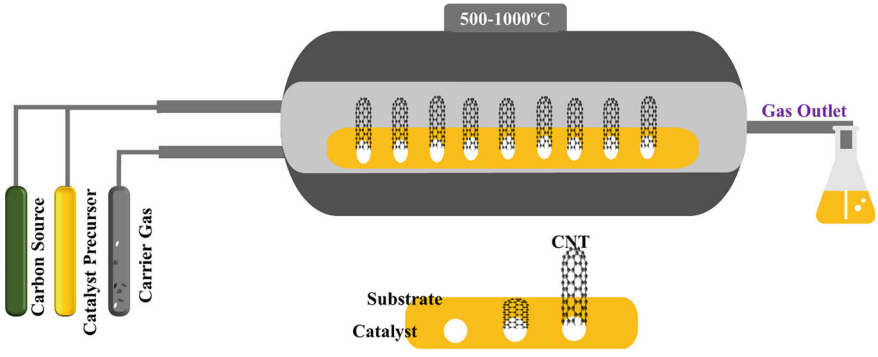
2 Synthesis and Functionalization of CNTs

2.1 Synthesis of CNTs

A variety of techniques for the synthesis of CNTs have been reported; however, the three methods that are most frequently used are arc discharge, laser ablation, and chemical vapour deposition (CVD) [34, 35]. These approaches enable the synthesis of high-quality CNTs with multiple structures and morphologies that can be explored in both fundamental and practical applications. Other techniques to prepare nanotubes include gas phase catalytic process (HiPCO), fluidized bed method, core shell polymer microsphere technique, aerosol precursor approach, low temperature route, arc water process, plasma method, and nebulous spray method, etc. However, these techniques are employed far less frequently than the ones mentioned above.

In comparison to the other ways of CNT synthesis, CVD technique uses hydrocarbons or other carbon-containing precursors, and it is classified as a thin-film deposition method. CVD approach requires a temperature of less than 1200 °C to produce nanotubes with high yield (Scheme 2). Also, this method is effective and more easily accessible [36, 37]. In addition, the CVD process enables to control the length, wall number, diameter, and alignment of nanotubes as well as structure. However, the CNTs produced by CVD have higher percentages of structural flaws and longer amount of reaction time (minutes to hours) as compared to the CNTs prepared by laser evaporation or the arc discharge method. Arc discharge and laser vaporization are high-temperature (higher than 3000 K) processes, but these methods produce nanotubes in short reaction time in the range of micro to milliseconds. In most cases, chemical vapour deposition is considered to be the most cost-effective method for the preparation of carbon nanotubes [38, 39].

The CVD process is further subdivided into three types: floating catalyst CVD (FCCVD), substrate-supported catalyst CVD (SVD), and high-pressure CVD (HiPco). In FVD method, a precursor catalyst and a carrier gas are delivered into a



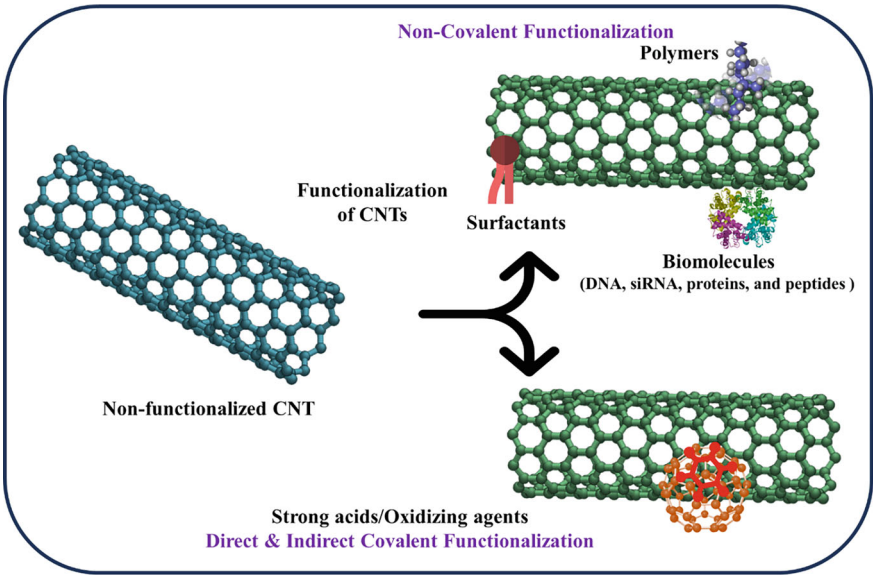
Scheme 2 Chemical vapour deposition schematic diagram

reactor's high-temperature zone and decomposed to generate free nanoparticles that promote the formation of CNTs [40]. The catalyst particle formation, the nucleation and growth of carbon nanotubes, and the direct capture of CNTs take place continuously. Additionally, by merely altering the experimental conditions and collecting methods, the CNTs generated by FVD may self-assemble into macro-structures with different morphologies such as bucky-books, films, and fibres [34, 40, 41]. As a result, the synthesis of carbon nanotubes by FCCVD differs significantly from other methods such as high-pressure carbon monoxide and substrate-supported catalyst CVD procedures in several ways, including continuous production, structural controllability, bulk morphological variety, and associated applications [42]. Significant growth has been achieved in the regulated preparation of CNTs using FCCVD in recent years. Thus, several obstacles persist, such as growth repeatability, homogeneity, controllability, and scalability, which prevent the produced CNTs from being used on a big scale [43]. In this book chapter, we highlight the benefits and features of the FCCVD approach, as well as provide a thorough evaluation of its use for the preparation/fabrication of CNTs with customizable morphologies and microstructures, with their attractive attributes and possible applications. The main challenges in the preparation and usage of CNTs produced via FCCVD are explored, as are potential solutions and emerging trends [39].

FCCVD is a well-recognized approach for creating high-yielding CNT films and fibres. Generally, CNTs prepared using FCCVD have shorter lengths, random alignment, and disordered entanglements. The conventional ultralong CNTs and CNTs prepared by FCCVD method growth processes- have a number of common properties [44]. Firstly, after a CNT begins to develop on a catalyst nanoparticle in an FCCVD process, it continues to float in the gas flow, analogous to the flying-kite-like formation of ultralong CNTs [45]. Secondly, both ultralong CNTs and CNTs prepared by FCCVD method follow a free growing mode. As a result, they both have tiny diameters, few layers, excellent structures with few faults, and quick development rates [46].

2.2 Functionalization of CNTs

The insolubility in aqueous and organic fluids has prevented the widespread use of carbon nanotubes (CNTs) despite their outstanding distinctive features. Apart from their stable structure, weak van der Waals and interfacial interactions between the host and CNT limit their use in various applications. Additionally, substantial long-range van der Waals forces cause CNT aggregation easily, resulting in poor dispersion [47]. The insoluble and intractable properties have restricted CNT usage in many applications. This can be resolved by functionalization techniques that alter the surface of CNTs (Scheme 3). Functionalization decreases long-range van der Waals forces and promotes CNTs-matrix/solvent interaction, resulting in homogeneous dispersion or CNT solubilization [48]. Thus, functionalization increases CNT



Scheme 3 Covalent and non-covalent functionalization of CNTs

reactivity, solubility, and chemical modification via ion adsorption, metal deposition, grafting processes, etc. [47].

Carbon nanotubes become easily dispersible in water once they have been functionalized. The solubility and dispersibility of functionalized CNTs change with respect to the type of functionalization, influencing their solubilization and binding ability to target cells or tissues. Consequently, CNT biocompatibility and stability in biological fluids depend on effective and stable surface functionalization [48]. Functionalization of CNTs can be classified into covalent and non-covalent functionalization. Non-covalent functionalization, mediated through various forces such as electrostatic force, van der Waals force, hydrogen bonds, and π-stacking interactions, allows for surface modification without affecting the physical properties or structure of CNTs. While covalent functionalization is more complicated and may induce changes in physical properties and structural damage, it offers greater control over the type of functionalization, strong linkage, functional group orientation, and specific site functionalization on the side wall of CNTs [49]. However, it is generally more expensive than non-covalent functionalization. Schematic diagram of CNT functionalize is given in Scheme 3.

3 Carbon Nanotubes in Cartilage Tissue Engineering

CNTs emulate extracellular matrix constituents owing to their intrinsic graphene structure, which harbours internal cavities that are capable of encapsulating or adsorbing diverse compounds such as metal ions [50–52]. This attribute is pivotal for the functional efficacy of CNT-based scaffolds, as CNTs have the ability to engage with various biomolecules such as nucleic acids, collagens, growth factors, proteins, adhesion molecules, and other extracellular matrix components [53, 54].

These nanotubes hold promise for their unique properties. Incorporating CNTs into biomimetic constructs could offer enhanced mechanical properties, conductivity, and structural support, aligning with the intricate hierarchical structure of cartilage. Furthermore, the tunable properties of CNTs, dictated by their configurations, may be harnessed to tailor specific characteristics desirable for cartilage regeneration and repair in tissue engineering applications. This intersection of nanotechnology and cartilage tissue engineering presents exciting opportunities for advancing the field with innovative materials and strategies.

3.1 CNT Integration in Cartilage Tissue Engineering

3.1.1 CNTs as Substrates for Cell Growth

Carbon nanotubes exhibit sizeable potential as a platform to facilitate cell growth, particularly for chondrocytes. Multi-walled nanotubes (MWCNTs) and bundles of single-walled nanotubes (SWCNTs) share a size range comparable to extracellular matrix molecules like collagens. The fundamental structural unit of collagen, approximately 300 nm in length and 1.5 nm in diameter, aligns with the dimensions of these CNTs [55]. Consequently, their utilization enables biomimicry of the natural nanotopography found in cartilage.

Furthermore, the manipulation of CNT arrangement through varying production methods offers the ability to create substrates with diverse characteristics. This includes surfaces that are either anisotropic or isotropic, rough or smooth, thereby mimicking the structures inherent at micro and nano-level in specific tissues. As shown in Fig. 1, yarns and fibres of CNT can be intricately woven into 2D and 3D textiles, enabling the control of porosity at various length scales [56, 57]. The capacity to modify the substrate topography is crucial, as the emulation of the fibrous, nanoscale structure of the extracellular matrix maintains the ability of chondrocytes to synthesize cartilage components. This capacity is particularly pertinent given that the loss of this synthetic capability represents a significant challenge in conventional chondrocyte culture methods [58].

The surface of CNTs has demonstrated its ability to promote cell adhesion [54, 53]. In a specific context, carbon nanotubes can be synthetically cultivated in a vertical orientation on a substrate, generating a “CNT forest” characterized by densely packed

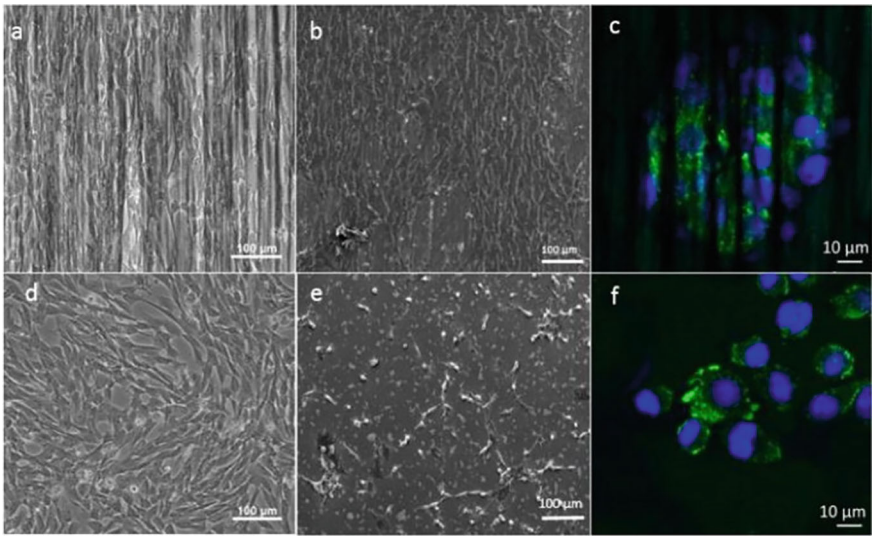


Fig. 1 Chondrocyte growth on flat, aligned CNT arrays (**a**, **b** and **c**) and on TC-plate (**d**, **e** and **f**). (**a** and **d**) Optical micrographs on day 4 (**b** and **e**) confocal fluorescence images after 6 days (**c** and **f**) cells are stained with collagen type II antibodies and visualized using Alexa488-conjugated antirabbit secondary antibodies (green). Nucleus was stained with DAPI (blue). Reproduced with permission from Journal of material Chemistry B from Ref. [56]

nanotubes arranged in parallel. Cellular cultures, encompassing human chondrocytes and Chinese hamster ovary cells (CHO), on such surfaces, revealed cell alignment along MWCNT bundles. This alignment corresponded with diminished cell clustering and an augmented proliferation rate. Moreover, a notable percentage of cells exhibited 3:1 aspect ratio or greater than 3:1, indicative of CNTs serving as platforms for cell adhesion and growth [55]. Miyanji et al. observed that the incorporation of carboxyl-functionalized single-walled carbon nanotubes (SWCNTs-COOH) had led to a reduction in fibre diameter, degradation rate, and water contact angle. Simultaneously, there was an enhancement in hydrophilicity, tensile strength, cell viability, and stability. The observed effects were ascribed to the outstanding intrinsic electrical conductivity and mechanical properties of SWCNTs, coupled with the existence of carboxyl functional groups embedded within their structure [59].

Likewise, Allahyari et al. investigated human osteosarcoma derived MG-63 cells (exhibiting fibroblast-like morphology) proliferation. The researchers generated different concentrations of MWCNTs incorporated chitosan films, specifically 0%, 0.2% (w/v), and 0.4% (w/v). Their findings indicated that augmenting the nanotopographical features led to a corresponding elevation in the cell population, suggesting a positive correlation between nanotopography and cell proliferation [60]. Chanine et al., cultured chondrocyte utilizing composite formulations comprising 2% agarose combined with two distinct types of carbon nanotubes:

carboxyl-functionalized single-walled carbon nanotubes and covalently polyethylene glycol (PEG)-modified SWCNTs. The SWCNT-COOH variant demonstrated heightened cell viability in comparison to a control, while the SWCNT-PEG scaffold proliferation was suppressed. Notably, an elevated concentration of SWCNT-PEG resulted in a significant production of extracellular matrix, despite registering the lowest cell viability [61].

Tissue engineering is limited by chondrocyte specific orientation, making cartilage architecture replication difficult. Janssen et al. reported the unidirectional orientation of chondrocytes through employing VA-MWCNT micropillars, with the Young's modulus similar to the natural ECM of articular cartilage. Controllable chondrocyte proliferation for achieving unidirectional orientation was achieved by adjusting the VA-MWCNT micropillar spacing and size. The presence of micropillars reportedly enhanced cell attachment, proliferation, and ECM production compared to control groups [62].

Another similar study involved the development of cross-linked composite scaffolds based on carbon nanotubes, demonstrating their effective compatibility with proteins. The incorporation of 0.5 wt% SWCNT-COOHs in the scaffolds resulted in enhanced mechanical properties, increased bone marrow-derived mesenchymal stem cells (BMSCs) proliferation, and demonstrated its non-cytotoxic behaviour. Animal experiments corroborated the superior cartilage repair outcomes in the 0.5 wt% group after 12 weeks, underscoring the pivotal role of appropriately incorporating SWCNT-COOHs in promoting the repair of articular cartilage [63].

3.1.2 CNTs Improve Mechanical Properties of Scaffolds

Organic biological polymers (chitosan, hyaluronic acid, cellulose, and fibrin) have been employed in jaw support development and implantable devices owing to their notable bioactivity and heightened conductivity. Nevertheless, the application of these polymers is beset by challenges, notably in the form of unpredictable thermal degradation and inadequate mechanical stability, particularly in endeavours to replicate the biological characteristics of organic matrices within bone cells. In addressing these challenges, the incorporation of carbon nanotubes into a polymer matrix has proven beneficial, enhancing the overall properties of the materials by establishing stable hydrogen bonds. This strategic addition contributes to overcoming the limitations associated with thermal stability and mechanical strength. Substantial advancements have occurred in creating composite materials for skeletal tissue engineering, incorporating carbon-based biological polymers. This represents a promising direction in the development of sophisticated biomimetic materials [33–65].

CNTs with Natural Polymer

Chitosan, derived from the deacetylation process of chitin, is a polysaccharide known for its exceptional biocompatibility, antimicrobial attributes, and degradation characteristics, making it a highly promising nanomaterial with diverse applications [66]. Its versatility enables seamless integration into various structures and compositions conducive to osteogenesis and cellular proliferation. Because of chitosan's aqueous solubility, different negatively charged proteoglycans deposit more readily and aid in the skeletal matrix's mineralization following implantation [67, 68]. The incorporation of carbon nanotubes into the chitosan matrix is pivotal for attaining a homogeneous dispersion of chitosan molecules. The surface modification of both chitosan and carbon nanotubes enhances interactions between the organic and inorganic phases, as a result, it needs higher energy levels to overcome biochemical bonding forces. Consequently, this enhances the mechanical properties of the substrates. In a recent study, the uniform distribution of 1 wt% MWCNTs across the chitosan medium demonstrated a corresponding distribution in compressive strength and elastic modulus. In addition, chitosan containing only 0.8 wt% MWCNTs showed significant changes in the primary tensile strength and elastic modulus, which improved from 1.08 to 2.15 GPa and from 37.7 MPa to 2.15 GPa, respectively [69]. Additionally, the effectiveness of utilizing chitosan-CNT composites as a sophisticated strategy to augment the material properties of hyaluronic acid (HA) in the context of bone regeneration was validated from the corroborative studies. These results highlight the versatile possibilities of these composite materials in expanding tissue engineering applications [70].

CNTs with Synthetic Polymer

Biodegradable polymers are essential for the preparation of scaffolds for cartilage tissue. Natural polymers have low mechanical characteristics despite having significant rates of degradation, which limits its use in cartilage tissue engineering [71]. Hence, synthetic polymers with increased mechanical strength have been employed in order to overcome these constraints of natural polymers in tissue engineering and regenerative medicine. PLGA, polyglycolic acid (PGA), polycaprolactone (PCL), and polylactic acid (PLA) are a few notable examples. The biocompatible CNT incorporation into a polymeric matrix has the ability to enhance the biomechanical characteristics of the matrix. For cartilage tissue engineering, an injectable thermogel known as stereocomplex cholesterol-modified 4-arm PEG-PLA (scPLA-Cholgel) was developed in a particular study [72]. In comparison to scPLAgel, the generated scaffold showed improved chondrocyte adhesion, greater mechanical strength, and a bigger pore size. Mirmusavi et al. prepared 1 wt% of carboxyl-functionalized MWCNTs incorporated poly 3-hydroxybutyrate (P3HB)-nano-micro scaffolds for cartilage tissue engineering. It revealed the decreased fibre diameter, high specific surface area, increased tensile strength, and bioactivity with the inclusion of MWCNTs. MWCNTs and saturation conditions both contributed to the

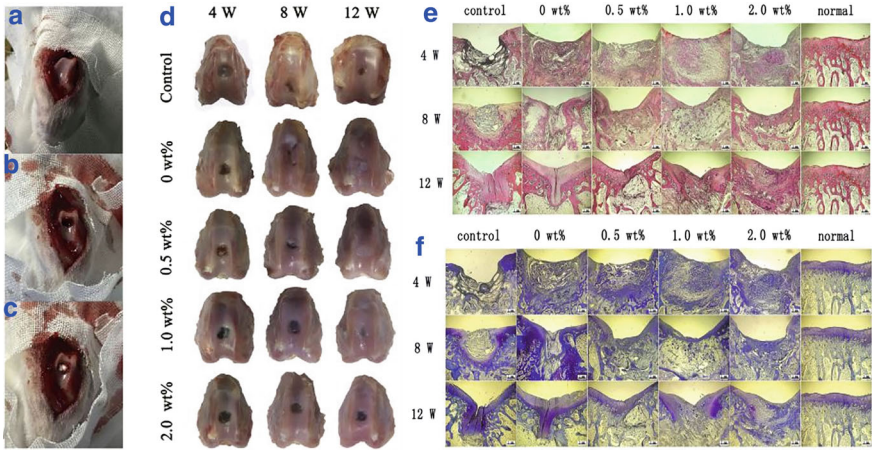


Fig. 2 **a**–**d** Rabbit model of articular cartilage defect and gross observation of the defects with time. **e** H and E staining result of the defect recovery 4, 8, and 12 weeks after injury (bar = 1 mm). **f** Toluidine blue staining result of the defect recovery at 4, 8, and 12 weeks after surgery (bar = 1 mm). Reproduced with permission from Colloids and Surfaces A from Ref. [63]

enhancement of hydrophilicity, which greatly enhanced the tensile strength compared to control. Furthermore, the scaffold containing MWCNTs exhibited a slow degradation rate. In addition to chitosan and silk, MWCNTs created an environment that was conducive to chondrocyte adhesion and development. For long-term cartilage tissue engineering applications, the P3HB-chitosan-MWCNTs/silk (S) nano-micro scaffold stands out as a potential option [73]. Zadehnajar et al. prepared PCL-gelatin (70/30) electrospun scaffold with 0.5 wt% of COOH-functionalized MWCNTs, assessing its impact on physiochemical properties as well as cellular response. It decreased average fibre diameter, heightened hydrophilicity, and increased tensile strength, while maintaining porosity percentage and mechanical strength. Importantly, this reinforcement exhibited no adverse effects on chondrocyte behaviour, as indicated by the study's findings [74]. In a study on cartilage repair in rabbits, researchers developed a CNT-based composite scaffold with COOH-functionalized SWCNTs, revealing enhanced mechanical properties and cell proliferation without cytotoxic effects on BMSCs. Implantation results indicated that a 0.5% SWCNT addition yielded the most effective cartilage defect repair compared to 1.0 and 2.0% groups (Fig. 2) [63].

Stocco et al. used contemporary methods to create porous PDLLA/VA-CNTs/nano-hydroxyapatite (PDLLA/CNT/nHA) scaffolds for the treatment of osteochondral lesions. The addition of CNT/nHA resulted in the formation of a porous scaffold, providing favourable conditions for cell growth. The nanocomposite showed decreased expression of type I collagen mRNA and superior chondrocyte viability [75]. Moreover, to increase the CNT application in cell adhesion and proliferation, several reports have shown that the cell adhesion characteristics of CNT can be improved by varying their surface roughness level. In a particular investigation,

Elídóttir et al. observed a significant augmentation in nanoscale roughness (approximately a 270% increase through the incorporation of CNT coating on polydimethylsiloxane (PDMS)-based scaffolds), which proved conducive to enhancing cell adhesion. Moreover, the manipulation of chondrocyte growth direction was achievable by introducing micro-sized anisotropic features, even in the absence of CNTs. However, in the presence of unevenly arranged CNTs, cellular adherence to microscale features was disrupted [76]. A study by Brunner et al. looked into how different levels of nano-roughness affected the development of human embryonic stem cells (hESCs). By varying the ultrasonic energy levels (80, 1000, and 5000 kJ) applied to the dispersion of amine-functionalized multi-walled carbon nanotubes (MWCNT-NH2) in chloroform, the nano-roughness was modulated. Their findings showed a clear relationship between the final cell count and the degree of roughness. Specifically, after 120 h of culture, the substrate with the highest nanoscale roughness, measuring 467 ± 56 nm (when the lowest sonication was applied), exhibited a higher cell population. However, excessive sonication energy led to significant debundling of the carbon nanotubes (CNTs) in solution. Consequently, the scaffold produced had a smoother topography, resulting in fewer cells adhering to these substrates [77].

CNTs in Composites

CNTs serve as versatile fillers in polymer matrices, yielding nanocomposites with adjustable mechanical and electrical characteristics. Notably, the integration of CNTs into polydimethylsiloxane (PDMS) has demonstrated intriguing properties [78–81]. For instance, the electrical percolation threshold was remarkably low at 0.003 wt% when utilizing multi-walled nanotubes, resulting in a conductivity of 0.003 S/m [81]. In thin films of CNT/PDMS prepared by varying the CNT from 1 to 12% revealed the tensile strength ~8 MPa to 4 MPa, respectively. Remarkably, sheet resistances diminished from 500 to 2 Ω/ϒ with increasing CNT content [79]. Moreover, CNT incorporation extends beyond PDMS to various polymers, enabling the modulation of scaffold conductivity and stiffness to meet specific tissue requirements. Examples include polycarbonate urethane (PCU) [82], polymethylmethacrylate (PMMA) [83], and even decellularized articular cartilage. These endeavours aim to enhance the mechanical properties and structural integrity of constructs, showcasing the adaptability and promise of CNT-polymer composites in diverse scientific applications [84, 85]. In an alternative investigation, Mirmusavi et al., synthesized an electrospun composite utilizing PCL, chitosan, and carboxyl-functionalized-MWCNTs at varying concentrations, specifically 0.5 and 1 wt%. Chondrocytes exhibited favourable growth on scaffolds containing MWCNTs, demonstrating heightened cell viability in comparison to samples devoid of MWCNTs. Furthermore, the inclusion of nanotubes led to an increase in the crystallinity of the scaffold, thereby enhancing its bioactivity and stability. This augmentation correlated with an improved healing rate of natural cartilage [86].

Utilizing the electrospinning technique, Karbasi et al., fabricated a composite consisting of poly(ε-caprolactone) and gelatin in a 70:30 ratio, supplemented with

1 wt% multi-walled carbon nanotubes for its potential applications in cartilage tissue engineering. The incorporation of MWCNTs resulted in enhancement of the hydrophilicity and bioactivity of PCL/gelatin blend. This finding was corroborated by water contact angle measurements, wherein PCL-gelatin/MWCNTs exhibited a measurement of 26.32 ± 4.66, while PCL/gel displayed a markedly higher value at 74.59 ± 10.08 [87].

3.1.3 CNT in Hydrogels

3D polymeric networks Hydrogels saturated with aqueous solution bear a structural resemblance to various tissues of the human body microenvironment [88]. Consequently, these hydrogels have been extensively explored in biomedical applications as scaffolds [89]. Specifically, degradable hydrogels have garnered attention in tissue engineering due to its capacity to break down over time, making them suitable for integration with cells and serving as effective substitutes for tissues in biomedical applications. By using carbon nanotubes to reinforce gelatin methacrylate (GelMA), Shin et al. developed a biocompatible hydrogel platform that can be used to create cell-responsive materials for three-dimensional (3D) structures [90]. GelMA is improved by CNT addition without sacrificing porosity or preventing cell development. CNT-GelMA hybrids with photopatterning enable the creation of microscale structures. In encapsulated CNT-GelMA microgels, NIH-3T3 cells and human mesenchymal stem cells demonstrate favourable growth and proliferation. The creation of complex three-dimensional biomimetic structures and In-vitro cell research are facilitated by the great pattern fidelity of CNT-GelMA.

In a recent investigation conducted by Jiayi Lv et al., a novel hydrogel incorporating carbon nanotube (FEK/C) and a composite scaffold (FEK/C3-S) based on polycaprolactone were successfully synthesized to repair cartilage and subchondral bone. Through varying the concentration of carbon nanotubes, the composite scaffold exhibited controlled microstructure, mechanical attributes, and conductivity. Remarkably, this scaffold demonstrated the ability to enhance osteogenesis and chondrogenesis. Furthermore, the composite scaffold exhibited a notable capacity to accelerate the regeneration of rabbit knee joint defect model cartilage and subchondral bone, which is clearly depicted in histological images as shown in Fig. 3 [65].

3.1.4 Functionalized CNTs in Cartilage Tissue Engineering

Notwithstanding their commendable characteristics and extensive utility, carbon nanotubes face a prominent obstacle arising from their intrinsic insolubility in both aqueous and organic. This is attributed to their constant structure composed solely of carbon elements. Furthermore, CNTs manifest exceptionally strong long-range van der Waals forces, predisposing them to spontaneous aggregation. This aggregation makes dispersion challenging, significantly hindering their processability due

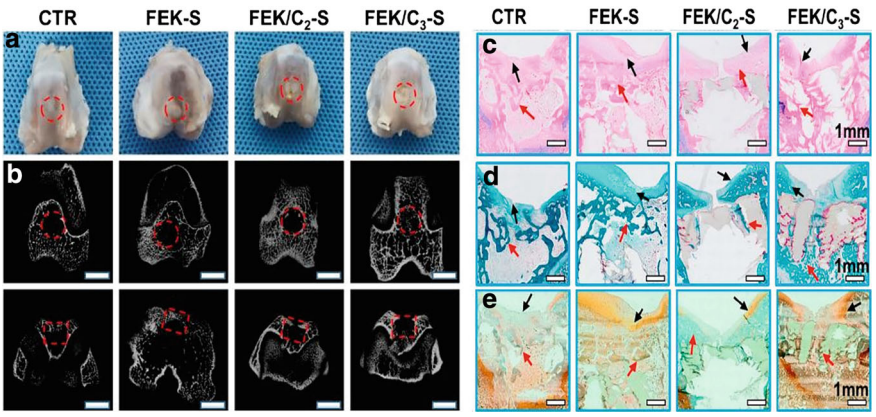


Fig. 3 Effects of composite scaffolds on articular cartilage and subchondral bone defect repair in rabbits **a** images of rabbit knee joints after articular defect and scaffold 8 weeks post-surgery; **b** micro-CT images of repaired knee joint from coronal central plane (upper panel), transverse central plane (lower panel). The defect area is shown in a red circle (top view) or a red square (side view); **c**–**e** Histological analysis of knee joints. **c** H and E staining of the knee joint (first row) **d** Masson staining and **e** Safranin-O/fast green staining of the defect joint after being treated with different scaffold for 8 weeks. Black and red arrows indicated cartilage and subchondral bone, respectively. Reproduced with permission from Pharmaceutics from Ref. [65]

to their insoluble and intractable nature. Consequently, this limitation has impeded the utilization of CNTs in numerous applications [34]. CNTs exhibit high chemical inertness, through either rigorous acid treatment or bioconjugation processes enhance its biocompatibility and dispersibility by adding functional groups on its surface. This approach has gained significant attention and serves to greatly broaden the potential application areas of CNTs. These functional groups play a crucial role in reducing the attraction of long-range van der Waals forces, enhancing the interaction between carbon nanotubes and the matrix/solvent, and promoting a uniform CNT dispersion or solubilization [91–94]. Consequently, functionalization enhances reactivity by enhancing additional chemical modification, and improves solubility, biocompatibility, adsorption, cell adhesion, drug delivery, and other biomedical and chemical applications [34]. According to a study by Liu et al., there was very little toxicity to human mesenchymal stem cells when carboxyl-functionalized single-wall carbon nanotubes (COOH-SWCNTs) were dispensed in the medium for the cells. Importantly, the presence of COOH-SWCNTs did not adversely affect the adipogenic, osteogenic, and chondrogenic potentials of the hMSCs [95]. However, the migration of single-walled carbon nanotubes through the cell wall to the nucleus was revealed using fluorescence-labeled carbon nanotubes. According to these findings, chondrocyte viability had no adverse effects by prolonged exposure to carboxyl-(COOH-) and polyethylene glycol- (PEG-) functionalized SWCNTs in 2D cultures, 3D pellet cultures or nanocomposite scaffolds. Surprisingly, this exposure also increased the expression of extracellular matrix proteins that are unique to articular cartilage. Furthermore, in comparison to the control group, the functionalized

SWCNTs enhanced the biomechanical characteristics in cell-laden nanocomposite structures [61].

Although the use of carbon nanotubes as fillers in conjunction with other substances has shown promising results for cartilage TE, questions about CNT toxicity still exist. These ambiguities are often attributed to variations in dosage, nanotube size, purity, and routes of administration. However, addressing these concerns requires extensive long-term in-vivo research to determine whether potential inflammatory responses observed by various groups outweigh the benefits of utilizing nanotubes in cartilage Tissue Engineering [96].

4 Carbon Nanotubes in Bone Tissue Engineering

CNTs are integral to bone tissue engineering, leveraging their strength and biocompatibility. Functioning as scaffolds, they facilitate cellular growth and contribute substantially to bone tissue regeneration. Notably, their unique characteristics, including electrical conductivity, hold promise for advancing bone repair. Ongoing research is dedicated to optimizing CNTs in the realm of regenerative medicine, highlighting their use in bone tissue engineering. The inherent rod-like design and nanoscale dimensions of CNTs play an essential role in bone tissue engineering. These structures act as potent reinforcement materials, effectively emulating the arrangement of collagen fibres within the extracellular matrix. Beyond their structural mimicry, CNTs have a tubular nature which provides a distinctive advantage—a substantial surface-area-to-volume ratio. This characteristic proves advantageous, facilitating efficient protein adhesion and elevating the drug-loading capacity significantly. The ultralight carbon nanotubes have remarkable mechanical properties. They have a density of 1.3–2.0 g/cm^3, a tensile strength of 11–52 GPa, a bending strength of 14.2 $\pm$ 8 GPa, and a Young's modulus that ranges from 32 to 1470 GPa. Their natural chemical stability and robust adsorption capability towards the majority of organic compounds. Make them particularly attractive in biomedical applications. Surface modifications, commonly achieved through the introduction of functional groups such as hydroxyl, carboxyl, and alcohol groups, further enhance their versatility. Studies have demonstrated the robust adsorption capability of essential biomolecules, including bone morphogenetic protein (BMP), amino acids, and type I collagen, onto CNT surfaces. CNTs have electrochemical and electron-conductive qualities that aid in promoting osteoblast proliferation and bone formation. Additionally, CNTs perform a dual purpose by maintaining the electrical function of cell membranes and enhancing the functionality of calcium ion channels in addition to triggering an increase in cellular and osteogenic expression indicators for plate-like crystal formation. CNTs can increase the efficiency of cellular communication, through enzyme-binding proteins. Furthermore, because of its very high cell constants, the addition of CNTs promotes stem cell development and helps to exert control over cell physiology. This, in turn, may promote osteogenic differentiation

and stimulate apatite mineralization, facilitating the rapid process of bone regeneration. This enhanced bone-forming potential might be attributed to CNTs' ability to concentrate bone-inducing proteins, effectively instructing local cells to form bone tissue. Several studies have demonstrated that MWCNTs possess the ability to oxidize and aggregate enzymes such as rhBMP-2. This process stimulates the activation of alkaline phosphatase (ALP), as well as the expression of genomes Cbfa1 and COL-1A. Consequently, these occurrences promote the rapid osteogenic development of adipose-derived mesenchymal stem cells [97–99]. Notably, multi-walled carbon nanotubes exhibit a remarkable ability to replicate the band/gap structure found in collagen, resembling the composition of bone-like hydroxyapatite (HAp). In a comparison investigation between the primary mineral constituent of naturally occurring bone HA and MWCNTs, MWCNTs demonstrated a remarkable capacity to induce osteogenic differentiation in human adipose-derived mesenchymal stem cells (HASCs), surpassing HAp in this crucial aspect. The underlying mechanism appears to involve MWCNTs' ability to activate Notch signalling pathways by concentrating specific bone-inducing proteins. MWCNTs have been observed to promote *in-vivo* ectopic bone regeneration in the dorsal muscles of mice. This suggests their capacity to influence downstream gene therapy reactions, offering a potential avenue for bone tissue regeneration without requiring additional support for cultivating renewable bone tissue. This multifaceted performance suggests the role CNTs can play as promising materials in advancing the field of tissue engineering, particularly in bone regeneration applications [70].

4.1 CNTs with Natural Polymers

Natural biopolymers, including chitosan, collagen, fibrin, and hyaluronic acid have become promising biomaterials for bone scaffolds or implants, mainly because of the unique biocompatibility and osteo-conductivity properties. However, their uncontrolled degradation rates pose a challenge, leading to low mechanical stability when attempting to emulate the mechanical and biological properties of the bone tissue matrix. The introduction of CNTs into the polymer matrix holds the potential to enhance material properties through the formation of robust hydrogen bonds. Notably, recent advancements have demonstrated progress in the realm of bone tissue engineering using CNT-biopolymer composites. This signifies a promising direction in utilizing the unique properties of CNTs to enhance the overall performance of biopolymers for bone-related applications [33].

4.1.1 Chitosan

Chitosan (CS) is a polysaccharide that is produced when chitin is deacetylated. It is a desirable biomaterial with a wide range of uses because of its improved biocompatibility, degradation, and antibacterial qualities. Chitosan readily integrates into diverse

shapes and formulations to promote cellular growth and osteogenesis. Its water-soluble characteristics enable the accumulation of various negatively charged proteoglycans, facilitating the skeletal matrix mineralization, post-implantation. CNTs play a role in uniformly distributing individual chitosan molecules inside the chitosan matrix, modifying the surfaces of both CS and CNTs can increase interactions between the natural and inorganic components. This higher energy need is exploited in order to transcend biological bonding energy and improve the mechanical properties of the substrates. The combination of chitosan and CNTs holds promise for creating advanced materials with enhanced properties for various biomedical applications. A recent study explored the potential of MWCNTs to enhance CS films for bone regeneration applications. By incorporating MWCNTs at various concentrations (0.1–1%) into the CS matrix using a simple solvent casting method, researchers investigated the resulting nanocomposite films' structural, mechanical, and biological properties. Optical microscopy, X-ray diffraction, and Raman spectroscopy revealed homogeneous MWCNT dispersion within the CS matrix. Notably, incorporating even small amounts of MWCNTs significantly improved the mechanical properties of the films. With only 1% MWCNTs, the tensile modulus and strength rose by 47% and 33%, respectively. Additionally, viscosity increased by 15% with MWCNT inclusion, potentially facilitating the injection of low-viscosity CS pastes. To assess biocompatibility, murine osteoblasts were cultured on the nanocomposite films. No significant toxicity effects were observed at day 3 or 7, suggesting good cellular interaction with the materials [100]. Likewise, several studies showed that a uniform distribution of 1 wt% MWCNTs throughout the chitosan medium resulted in a significant increase in the tensile modulus and strength from 1.08 to 2.15 GPa and 37.7 MPa to 2.15 GPa respectively, and a noticeable improvement in the elastic modulus and compressive strength. This highlights the positive impact of incorporating MWCNTs into the composites [69]. Another study explored the use of MWCNT/CS scaffolds fabricated with a well-defined microchannel porous structure, demonstrating promising biocompatibility and biodegradability. *In-vitro* studies using C2C12 murine myoblast cells revealed excellent cell proliferation, viability, and adhesion on the scaffolds. Notably, enhanced alkaline phosphatase activity indicated that the presence of recombinant human bone morphogenetic protein-2 (rhBMP-2) encouraged the development of C2C12 cells towards an osteoblastic lineage. *In-vivo* studies further validated the biocompatibility of the MWCNT/CS scaffolds. Their adsorption properties assist in rhBMP-2 incorporation, leading to ectopic bone formation within implanted muscle tissue as shown in Fig. 4 [101].

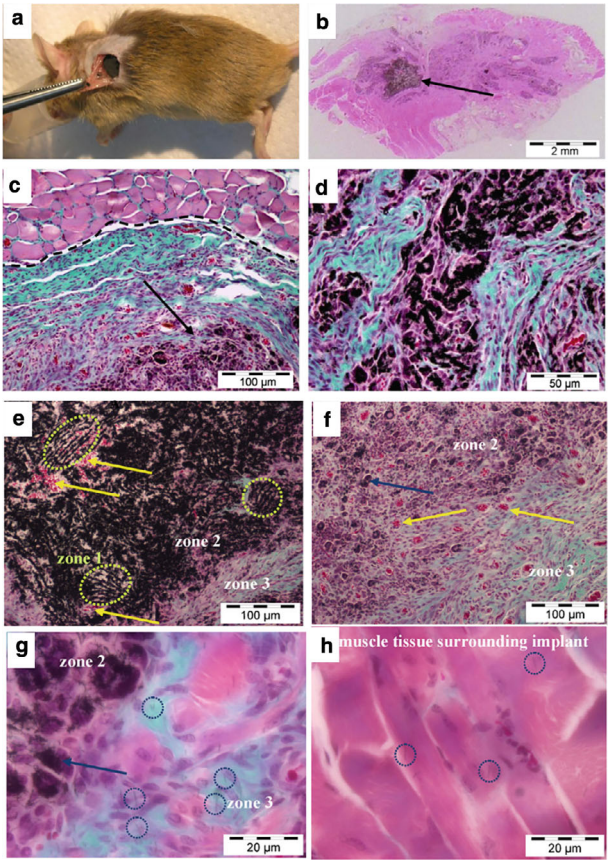


Fig. 4 **a** Shows the implantation of rhBMP-2 adsorbed MWCNT/CS scaffolds in the subcutaneous muscular pocket of mice. Optical microscope micrograph **b** shows regenerated bone tissue and a minor fraction of the remaining MWCNT/CS scaffold. Optical micrograph **c** shows a detail of regenerated bone tissue (collagen expressing cells, blue–green) after major disassembly of the MWCNT/CS scaffold, surrounded by muscle tissue (pink). It is remarkable the well-limited interface between adjacent tissues (black dash line). The remaining MWCNT/CS scaffold (black) is pointed by black arrow. Optical micrograph **d** shows a detail of the remaining scaffold plenty of fibroblasts (purple), prior to its disassembly and colonization by collagen expressing cells (blue–green). Optical micrographs **e**–**g** show the three main distinguishable zones observed after 3 weeks of scaffold implantation; zone 1 where the MWCNT/CS scaffold structure remains basically intact (marked by green circles), zone 2 where non-differentiated fibroblasts (purple) are colonizing the partially disassembled structure of MWCNT/CS scaffold (representative clusters of MWCNT/CS aggregates are pointed by white arrows), and zone 3 where MWCNT/CS scaffold is fully disassembled and single MWCNT/CS (some representative MWCNT/CS are marked by blue circles) are dispersed within regenerated bone tissue. Note the abundance of blood vessels (erythrocytes, some representative pointed by yellow arrows) at zone 2. Optical micrograph **h** shows some few single MWCNT/CS (also marked by blue circles) dispersed within muscle tissue surrounding the implant. Reproduced with permission from Biomaterials from Ref. [101]

In order to increase the stability and strength and promote bone regeneration, several researchers used a combination of chitosan-MWCNTs-HAp. Venkatesan et al. constructed an innovative bone scaffold using freeze-dried functionalized multi-walled carbon nanotubes (f-MWCNTs), chitosan, and HAp, with chitosan connected to both f-MWCNTs and HAp through ionic bonding (NH_3^+-COO^-) and metal interactions. This scaffold showcases impressive properties, including interconnected porosity, thermal stability, controlled degradation, and heightened *in-vitro* cell proliferation. The scaffold's matrix, replicating the extracellular matrix, not only mirrors its structure but also significantly enhances osteoblast proliferation, alkaline phosphatase (ALP) activity, protein content, and mineralization in MG-63 cells [102]. Zhang et al., reported the development of a three-dimensional porous chitosan composite, integrating two types of synthesized single-walled carbon nanotubes through arc discharge: with a magnetic field (B-SWCNT) and without a magnetic field (N-SWCNT). In comparison to pure chitosan, the composite—which included 20 wt% nanocrystalline hydroxyapatites—showed better compressive moduli and tensile strength. According to the study, the addition of magnetically synthesized SWCNT and nanocrystalline hydroxyapatite created a favourable cellular environment that improved human foetal osteoblast cell adhesion and proliferation (CRL11372). This resulted in an improved osteoblast density within a chitosan nanocomposite containing both B-SWCNT and 20% nanocrystalline hydroxyapatite [103].

4.1.2 Collagen

Collagen serves as the predominant organic element in the composition of bone, playing a crucial role in imparting the robust properties of bone, including its viscoelasticity and toughness. Collagen's biodegradability, low antigenicity, and cytocompatibility have made it a promising biomaterial for bone tissue regeneration in clinical settings [104]. However, pure collagen possesses a relatively soft nature (elastic modulus: 14.6 ± 2.8 kPa) and is not directly suitable as a bone replacement material [105]. By combining collagen with CNTs, the resulting composite materials are expected to exhibit enhanced mechanical properties, addressing the limitations associated with the softness of pure collagen. Hirata et al. conducted a study demonstrating that a multi-walled carbon nanotube-coated 3D collagen. The scaffold, upon implantation in the femur, exhibited prompt osteoblast differentiation compared to an uncoated collagen scaffold. Furthermore, the MWCNT-coated scaffold induced the formation of new bone within the pores after a 28-day period [106]. S. Türk et al., present the fabrication of a highly porous collagen composite scaffold functionalized with carbon nanotubes, chitosan, and hydroxyapatite (Col/f-MWCNT/CS/HAp) using the freeze-drying method. The reported Col/f-MWCNT/CS/HAp composite, with a Ca/P ratio of 1.52 akin to natural bone (1.6), exhibited hydrogen bonds between collagen's –OH, $–NH_2$, and CO groups and the $–NH_2$ and OH groups of chitosan, as well as the –CO and –OH groups of f-MWCNT. Employing a biomimetic method