Review Article

Investigation of Carbon-Based Materials for Tissue Engineering Applications

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Abstract

Today, carbon materials are among the most widely used materials in the field of scientific-technological leaps. The biochemical properties of these materials have led to their widespread use in medical and biomechanical fields, and their different and special morphology has led to their suitable replacement for body tissues and solving joint problems and osteochondral problems. However, more systematic approaches to the engineering design of carbon-based cells and scaffolds are needed, and the related challenges still need to be addressed through extensive research. In this research, a comprehensive study of carbon materials and their benefits in medicine is done, focusing on increasing the effect of these materials in the area of osteochondral and joint repair and regeneration. In this regard, a review of all types of carbon allotropes including diamond, graphene compounds, fullerene, carbon nanotubes, amorphous carbon, and carbon dots has been done and the Biocompatibility properties of scaffold carbon base materials have been investigated.

Keywords: Bones, Carbon-Based Scaffolds, Biocompatible Materials, Tissue Engineering.

1. Introduction

Tissue engineering is the medicine of regeneration and production of cells with a micro or macro bio-imitation environment, as well as the regeneration of damaged body tissues and the replacement of traditional implants with active biological structures and biomaterials. In this regard, a combination of materials in different dimensions and methods has been investigated in the last three decades from a kinetic point of view, extracellular matrices, and tissue engineering scaffolds [1, 2]. Despite these advances, most compounds are still being tested in laboratory settings and are therefore not available to patients, especially those that deal with the simultaneous repair of different interconnected tissues. One of these interconnected areas is the joints and their problems. The prevalence of joint pain is the result of general inactivity and unprincipled exercise. For example, 25% of the world's population may suffer from hip osteoarthritis during their lifetime [3, 4]. Today, joint repairs are still performed using metal, ceramic, and polymer implants or autografts, allografts, and xenografts. While conventional implants are less than optimal in terms of biomechanical performance, tissue engineering is considered a suitable choice. However, new material families and fabrication technology techniques should be studied to overcome existing biomechanical mismatches, dimensional limitations, biocompatibility issues, and spatiotemporal design adjustability with detailed requirements [5, 6].

Carbon and carbon-based materials are often referred to as future materials are mentioned. Since the beginning of tissue engineering, carbon materials have been used to restore musculoskeletal tissues. Due to having suitable mechanical properties, carbon-based materials include a very diverse set of biomaterials, which have the possibility of being replaced by joint tissues of the human body. Compared to other biomaterials synthesized to replace joint tissues, carbon-based materials minimize mechanical incompatibility with other body organs and establish proper biocompatibility with them. The potential of carbon-based materials for the repair and regeneration of human tissues, especially for the treatment of bone and joint injuries, is very high. However, extensive research is needed for the engineering design of carbon-based cells and scaffolds. The current research focuses on the use of carbon-based materials for osteochondral regeneration and joint tissue engineering, and builds on previous studies that have treated bone, [7,8] cartilage, [9] and ligament [10] independently, or simply focused on a single type of carbon material, [11-13] is established. In this research, all carbon materials are examined to meet the needs of tissue engineering in the production of living engineering structures with different dimensions, such as osteochondral and joint repair and reconstruction.

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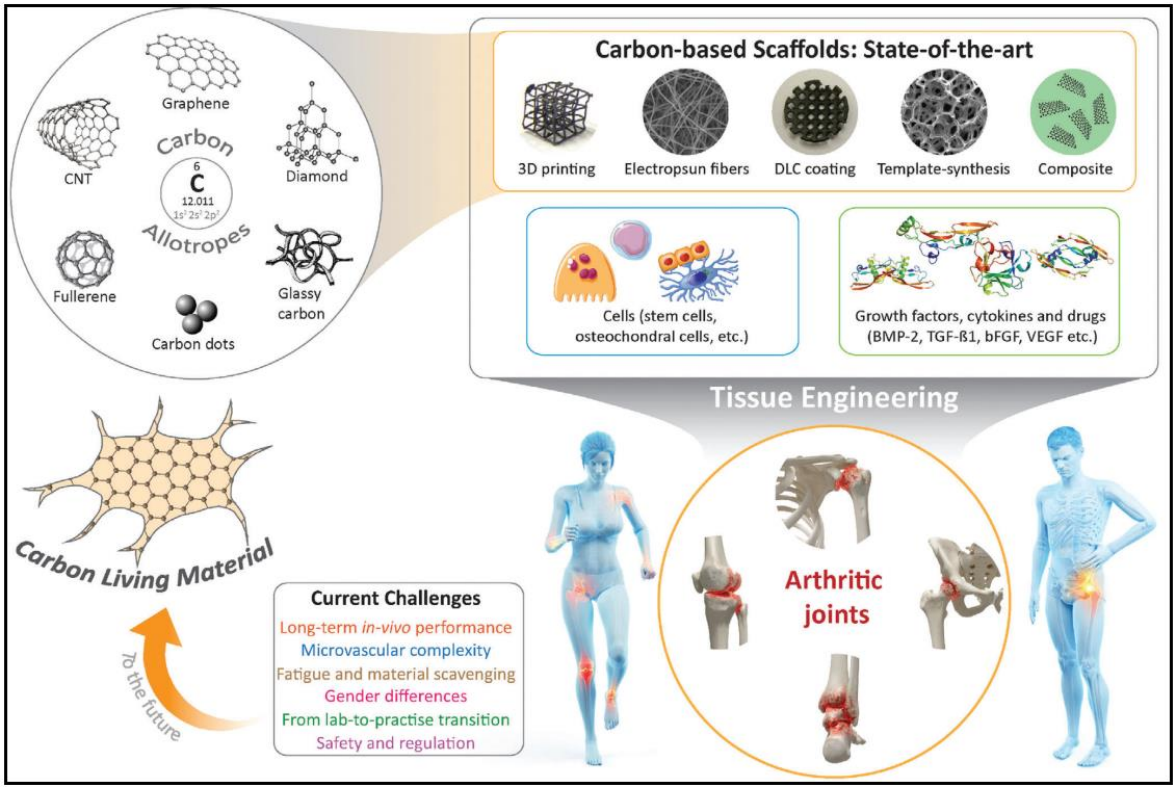

Fig. 1. Graphical illustration of carbon allotropes, carbon-based scaffolds for tissue engineering [7-10].

Based on this, a comprehensive description of the most advanced carbon-based materials for tissue engineering in medicine, such as carbon nanotubes, graphene and graphene oxide, carbon fibers, glassy carbons, and nanodiamonds, are reviewed. Fig. 1. shows a graphical illustration of the types of carbon materials available and the scaffolds produced from them, used in tissue engineering arthritic joints, and their use as carbon living materials.

2. Carbon Types for Tissue Engineering and Regenerative Medicine

In its ground state, carbon has an electronic structure of 1s22s22p2 with four electron vacancies in its outer electron shell. Such an electronic arrangement allows carbon atoms to participate in the formation of strong covalent bonds with other carbon atoms in different hybridization states (sp, sp^2, sp^3) and enables the existence of several allotropes of carbon in the solid state [14,15] among All carbons. Allotropes diamond and graphite are the only natural allotropes [16]. Although, diamond and graphite are composed of only carbon atoms, their properties are very different. For example, diamond is the hardest material known and is electrically an insulator. In comparison, graphite is soft and has significant electrical conductivity [15]. The reason for such contrasting properties between the two allotropes lies in their atomic arrangement.

2.1. Diamond

Diamond has a cubic lattice (fcc) structure in which the sp^3 hybridized carbon atoms form a tetrahedral space branch. A carbon atom at the center of the tetrahedral forms covalent carbon-carbon bonds with four other carbon atoms at the four tetrahedral vertices. In each bond, the participating electron pairs from the two carbon atoms occupy different spin states, which satisfy the Pauli exclusion principle and make the carbon-carbon bonds very strong [17]. Such strong carbon-carbon bonds account for the excellent mechanical stiffness and conductivity. Diamonds are very weak electric.

2.2. Graphene, Graphene Oxide, and Reduced Graphene Oxide

The unit cell of graphite is called graphene, which was first isolated from bulk graphite by Andre Gaim and Konstantin Novoselov in 2004 [18]. Graphene is a two-dimensional sheet of sp^2-hybridized carbon atoms, in which each carbon atom forms covalent bonds with three other carbon atoms in the σ plane, forming planar arrays of two-dimensional hexagonal lattice units with a lattice constant of 2.46 Å (the allotrope section carbons in Fig. 1). The in-plane σ covalent bond results in a short inter-atomic distance of ≈ 1.4 Å, which is significantly stronger than sp^3 bonds in diamond [19]. Graphene can be classified according to the number of layers: monolayer graphene, bi-layer graphene, and multilayer graphene ($2 \leqslant$ layers $\leqslant 10$) [20] For bi-layer and multilayer graphene, the $2p_z$ orbitals of

carbon atoms, which are perpendicular to the structure are planar, overlapping with $2p_z$ orbitals of carbon atoms from adjacent parallel graphene sheets and forming out-of-plane bonds.

The distance between the layers of graphene sheets in graphite is 3.35 Å. Graphene layers can be placed on top of each other in two arrangements, AA and AB [21]. In the AA arrangement, all carbon atoms are vertically stacked on top of each other, while the AB arrangement is characterized by two interconnected layers that move at a distance of half the lattice vector in the layer plane (Fig. 2a). AB arrangement is energetically the most stable bi-layer and multilayer graphene arrangement [22]. Multilayer graphene, as well as graphite, has a rhombohedral ABC arrangement (Fig. 2a), where half of the carbon atoms in the third layer C are vertically aligned with the carbon atoms in the A layer and the other half with the carbon atoms in the B layer [22, 23]. It should be noted here that multilayer graphene behaves very differently from bulk graphite. When graphene layers are produced by oxidizing agents, polar groups are formed on the surface of graphene by increasing the distance between graphene layers. When graphene layers are treated with oxidizing agents, polar groups are introduced to the graphene surface by increasing the distance between graphene layers.

Such modified graphene is called graphene oxide (GO). According to the L-K model, hydroxyl groups and other groups are randomly distributed on the surface of graphene, while the edge of the graphene layer has carboxyl and carbonyl groups (Fig. 2b). GO includes aromatic sp^2 domains and aliphatic sp^3 domains that facilitate surface interactions [27].

The degree of aromatic and aliphatic carbon atoms depends on the degree of oxidation and the distribution of oxide groups on the graphene layer. Such modifications in graphene layers lead to different surface properties for GO [27, 28]. For example, GO is hydrophilic, while raw graphene is hydrophobic. However, oxygen-containing groups lead to a significant decrease in the electrical conductivity of GO [29]. To restore electrical conductivity, GO is usually converted to reduced graphene oxide (rGO) through various chemical, thermal, and electrochemical methods, to minimize the amount of oxygen-containing groups [29, 30]. In addition, rGO has better thermal and mechanical properties compared to GO, but it is still less interesting than graphene due to higher surface defects.

2.3. Fullerene

Fullerene is one of the first allotropes of carbon that was discovered before graphite and diamond. Fullerenes are found as spherical structures of carbon atoms in the Buckminster-Fuller spatial arrangement. The atoms forming the spherical structure are sp^2 hybridized and each atom forms covalent bonds with three adjacent carbon atoms, resulting in hexagonal and pentagonal arrangements. These form a stable spherical shape. Compound C60 (also known as Buckminsterfullerene) is the most commonly studied fullerene, in which 60 carbon atoms form a sphere with 12 pentagons and 20 hexagons [16, 31]. The radius of fullerene C60 is 0.35 nm. The smallest fullerene structure reported to date is C20, and theoretically, the formation of all fullerenes with C20 + 2F structures (where $F \neq 1$ and $F \geqslant 0$) is possible [32, 33].

2.4. Carbon Nanotube

Carbon nanotubes (CNTs) have been widely investigated in various research fields since 1991 [24]. Carbon nanotubes are composed of sp^2 hybridized carbon atoms and are formed by rolling graphene sheets into a seamless cylindrical structure. The hollow cylindrical structure of carbon nanotubes can be opened or closed by fullerene caps. In addition, CNTs can be single-walled or multi-walled. The single-walled CNT (SWCNT) structure is formed by rolling or rolling a single-layer graphene sheet. The structures of SWCNTs usually have a diameter of ≈1 nm [34] and their properties strongly depend on the orientation of the graphene sheets during rolling. There are three types of CNT configurations based on the orientation of graphene sheets, namely Armchor, Zigzag, and Chiral, which are responsible for the metallic, semi-conductive, and semi-metallic properties of CNTs, respectively [35, 36]. As shown in Fig. 2c., when the multilayer graphene sheets are rolled, they form multi-walled CNTs (MWCNT), and the distance between the concentric layers of the cylindrical graphene rolls is ≈ 3.4 Å. The structures of MWCNTs have a conventional diameter from 10 to 50 nm and a length ranging from 1 to 20 μm [35].

2.5. Amorphous Carbon, Glassy Carbon, and Carbon Dots

Amorphous carbon, according to Robertson's definition, is a highly disordered form of carbon [37]. This structure has both sp^2 and sp^3 hybridized carbon, and it is determined by the concentration ratio of sp^2 and sp^3 sites that this ratio depends on the manufacturing process in many cases. Fig. 2d. shows an example of a transmission electron microscope (TEM) image of an amorphous carbon obtained from the pyrolysis of a biopolymer, where the short-range order due to the sp^2 carbons can be observed. Typically, an evaporated or sputtered amorphous carbon film has 85% or more sp^3 carbon bonds [38, 39].

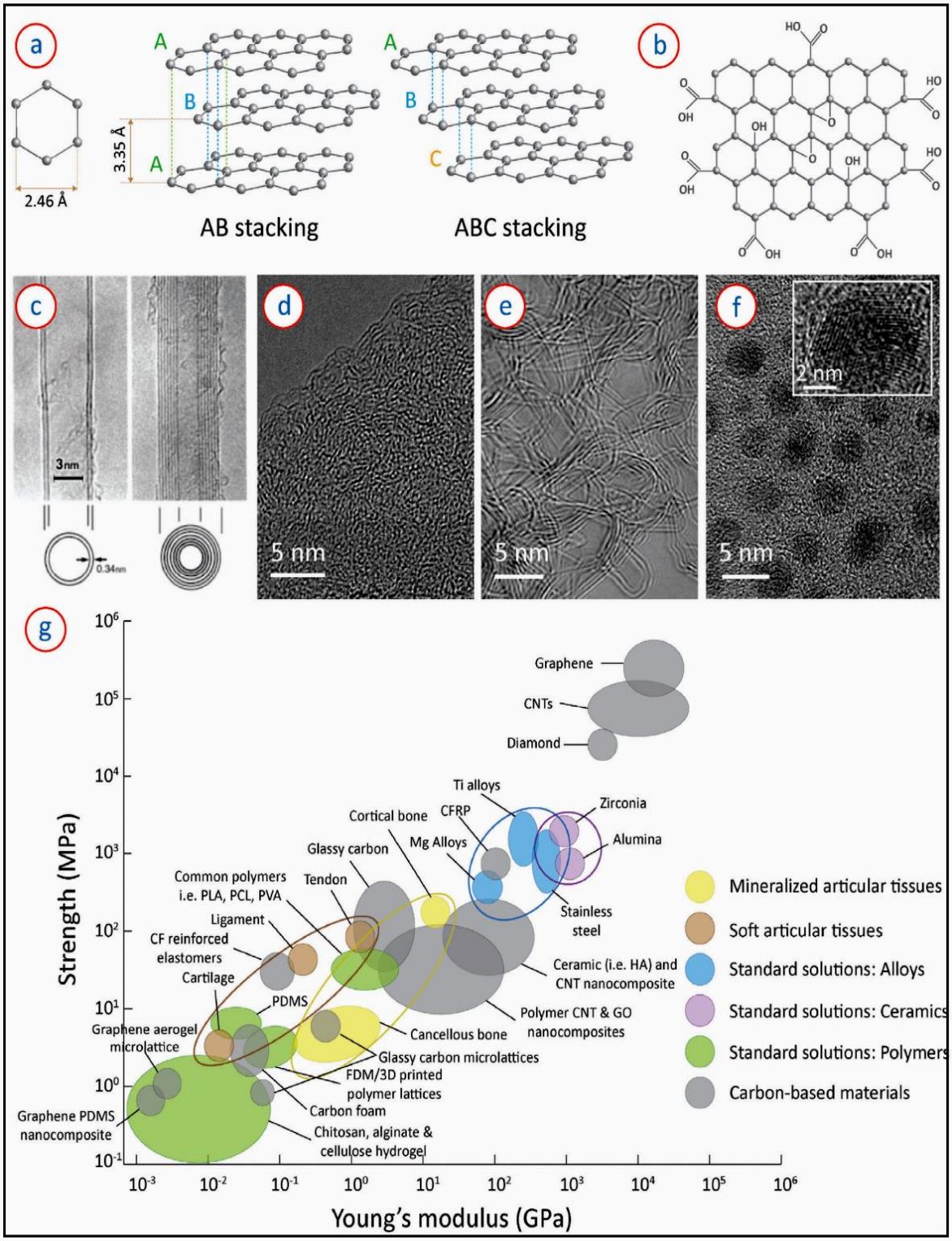

Fig. 2. a) Lattice parameters and arrangement of graphene stacking in multi-layer graphene. b) Surface groups attached to the graphene sheets in graphene oxide. c) TEM image of CNTs, showing the tubular structure of MWCNTs [24]. d) TEM image of amorphous carbon, showing disordered and short-range order in the microstructure. e) TEM micrograph of glassy carbon, showing the turbostratic arrangement of long-range graphitic planes [25]. f) TEM image of carbon quantum dots, showing nanometric particle size. Inset shows the crystalline arrangement of multiple graphene planes in an individual CQD [26]. g) Comparison of strength and Young's modulus of carbon materials, illustrating the bio-mechanical versatility of the carbon-based solutions in comparison to other standard solutions.

Carbon films that have a very high concentration (almost exclusively) of sp^3 carbon are called diamond-like carbon (DLC) because of their high hardness, which originates from diamond-like sp^3 hybridized carbons. DLC films are usually hydrogenated to 50% hydrogen for better stabilization of the layers [38]. Non-hydrogenated DLC films are designated as tetrahedral amorphous carbon (taC). Glassy carbon, often referred to as glassy carbon, is a non-graphitizable carbon that is usually obtained through high-temperature pyrolysis of an organic precursor [40, 41]. This type of carbon has sp^2 hybridized carbon atoms that form long-range graphene sheets. However, unlike graphite, these graphene layers are randomly oriented and form a turbostratic arrangement (asymmetric basal planes or angular planes), as shown in Fig. 2e. Along with turbostratic graphene sheets, there is also a significant amount of fluorine or fullerene-like curved graphene sheets [25]. Turbostratic graphene layers and fullerene-shaped structures form numerous cavities and closed pores in the microstructure, making glassy carbon lightweight but impermeable to gases. Carbon dots, which are also known as carbon quantum dots (CQD), are almost zero-dimensional carbon nanoparticles with an average size of less than 20 nm [42]. An example of an electron microscope image of CQDs is shown in Fig. 2.f [26]. Graphene quantum dots are mainly composed of sp^2-hybridized carbon atoms and have multilayer graphene sheets (Fig. 2.f), which are attached with chemical groups at their edges or in interlayer graphene sheets. CQDs exhibit excellent fluorescence properties.

2.2. Properties of Carbon-Based Scaffolding Materials

The most important feature of a scaffold material is that it should be compatible with the environment

without inhibiting the immune system. All carbon allotropes show bioactive properties without the need for surface treatment. Especially in joint tissue engineering, most carbon materials do not show any or minimal toxic chemical interactions when faced with osteoblast cells. However, the size, shape, concentration, surface function, and collection speed of carbon nanomaterials significantly affect the biocompatibility of carbon materials, especially CNTs and graphene. When these materials are used as scaffolds, they exhibit toxic activities [43, 44]. Graphene and CNTs when used as scaffolds can penetrate the cell membrane and cause serious damage to it. Further transfer of these substances to cells and cell nuclei can cause inflammation and even genotoxicity [45]. Fig. 3. shows different mechanisms of cytotoxicity caused by graphene nanomaterials. However, most of these responses or cytotoxic behaviors are observed when graphene or CNTs are used as nanomaterials and these nanomaterials can freely react with cells. When these materials are used in composites and their free movement is limited, cytotoxic effects are minimized or not found at all [45-47]. Another method to reduce cytotoxicity is surface coating or surface preparation. For example, preparing the surface of graphene nanomaterials with polyethylene glycol or bovine serum albumin significantly reduces the toxicity in macrophage cells [48].

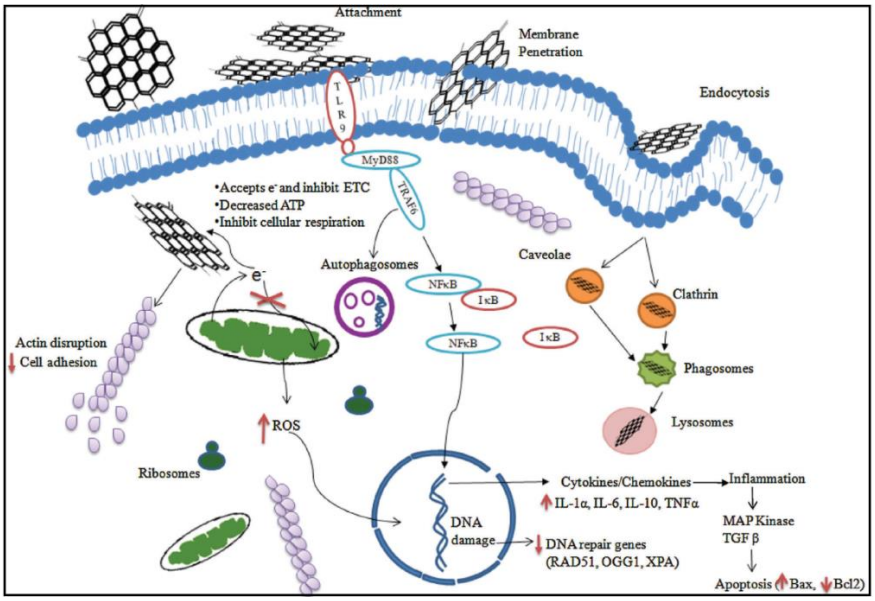

Fig. 3. Mechanisms of cytotoxicity induced by graphene nanomaterials. Graphene interacts actively with the cell membranes and penetrates it due to its small size. Upon entry, it can result in an increase in the production of reactive oxygen species (ROS) by inducing oxidative stress. It can further inhibit electron transport chain, causing a depletion in cellular adenosine triphosphate (ATP) level. These phenomena can result in DNA damage and inflammation, which can further trigger cell apoptosis [43].

Carbon has a very good interaction with the human body. In tissue engineering, it is necessary to develop synthetic scaffolds, whose mechanical properties are regulated by the characteristics of the tissues being repaired.

Cells can feel the strength of their small environment and reproduce, grow, and migrate based on this [49-51]. Often, soft substrates that simulate the biomechanical properties of the brain are neurogenic. Medium-strength substrates that simulate muscles are myogenic, and high-strength substrates that simulate collagen bones can differentiate bones from mesenchymal stem cells [47, 52]. Depending on their morphology, carbon allotropes cover a wide range of tensile strength and Young's modulus. For example, graphene nanomaterials promote stem cells to bone cells (osteoblastic lineage) due to their high strength [47, 53]. High tensile strength (more than 50 GPa), high Young's modulus (more than 1 TPa) of CNTs. It causes their use in collagen fibers [54-56].

Apart from the repair of joint tissues, the mechanical versatility of carbon-based materials allows the use of an as in other parts of the body such as the nervous system [57], skin [58], heart [59], skeletal and muscular [60, 61] and cartilage joints [62]. The surfaces of carbon materials can not only be used to stabilize tissue cells, but these materials can also be used in drug delivery systems.

For example, graphene can absorb dexamethasone and β-glycerol phosphate, which can accelerate the separation of bone from MSCs [47]. Also, the appropriate surface interaction of carbon nanomaterials leads to the use of these materials as a strengthening component in several ceramic and polymer composite scaffold materials. For example, graft can be converted into GO through oxidation, which shows good hydrophilic properties due to proper surface interaction. Also, GO provides the possibility of being dispersed in several organic solvents and polymer matrices and facilitates the construction of stable and high-strength composite scaffolds [8].The good electrical conductivity of carbon materials (except diamond, which is electrically insulating) is an added advantage in tissue engineering applications. These substances can facilitate the electrical stimulation of cultured cells and as a result, improve the possibility of cell proliferation and osteogenic activity [63, 64].Due to the excellent electrochemical properties of carbon materials, carbon scaffolds can show self-sensing activities during cell culture. In addition, carbon-based materials exhibit excellent photothermal activity under light irradiation, which leads to a local increase in temperature. The photothermal properties of carbon materials make it possible to achieve antimicrobial effects by hyperthermic killing of bacteria [65, 66]. The synergistic effect of photothermal and photodynamic activities of carbon materials can facilitate wound healing, muscle repair, and cancer treatment [66, 67]. In addition, in the field of joint tissue engineering, various structures of carbon-based materials including fibers - compressed and non-compressed - for ligaments, tendons, and muscle nerves, spongy and interwoven meshes for cartilaginous and sub cartilaginous defects; [68, 69] and plates and foam for spinal bone [70, 71] can be bio-engineered (biomimetic), produced [72, 73]. The use of different carbon-based materials for the repair of different joint tissues is summarized in Table. 1.

3. Carbon-Based Materials in Tissue Engineering

3.1. Carbon Fibers

Carbon materials are the main candidates for the regeneration of damaged tissues. Therefore, preliminary studies have been conducted on the advantages and disadvantages of common carbon fibers with graphite. The mechanical capabilities of carbon fibers as well as their ability to weave to produce chords and three-dimensional structures lead to the wide application of these materials in ligaments and tendons [74, 75], cartilage [68, 69], and bone repair [70, 76-78]. Woven carbon fibers can be used in surgery using simple planting methods. The biocompatibility of these fibers is average. Therefore, polymer coatings are applied to them [75], which is a common method in medical implants such as metal stents with polymer coating. Although the initial stability after fiber implantation was remarkable, the long-term in vivo performance was not satisfactory.

This issue was influenced by biomechanical incompatibility with the tissues being repaired, fragility, final fragmentation, or increased osteoarthritis [10, 74]. To improve the long-term performance of fibers, in addition to the use of polymer coatings as biointerfaces to minimize mechanical inconsistencies, the use of carbon fiber-polymer composite structures has become common [76].

3.2. Carbon Nanotubes

Nanocomposites and carbon nanotubes (CNTs) have been proposed as a suitable alternative to carbon fibers in tissue engineering. Superior mechanical properties, lightweight structure, and tunable electromechanical behavior have led to the wide application of CNTs. SWCNT and MWCNT materials and their composites are used to design and manufacture cartilage, bone, (Fig. 4.)[55, 79-81] tendons, and ligaments [55, 56]. Their biomechanical similarity to collagen fibers and their 3D fabrication capability through various techniques such as electrospinning, solvent casting, freeze drying, phase separation, and several rapid prototyping tools such as bioprinting, digital light processing, laser stereolithographic, or deposition

Melted resin provides the possibility of using these materials in the repair of different tissues [80]. In addition, these materials can be easily hybridized with conventional carbon fibers and increase their properties and versatility as biomedical materials [94]. CNT materials can cross cytoplasmic and nuclear membranes. This capability can be used for anti-cancer treatments, genetic treatments, or DNA transfer to the nucleus [95]. However, concerns about their toxicity related to cell membrane disruption and possible carcinogenic effects in the respiratory system have also been reported [96, 97]. Especially for joint tissue engineering, the cytotoxic effects of carbon nanotubes have been reported to impair the viability of primary osteoblast cells and prevent osteoblast mineralization [98, 99].

The order of cytotoxicity was found as SWCNTs > bilayer CNTs > MWCNTs. However, these cytotoxic effects can be minimized or even eliminated by appropriate surface functionalization or the use of CNT composites as scaffold materials [46, 100].

Table. 1. Summary of carbon materials used in particular tissue engineering.

Type of carbon	Brief description of scaffolding structure	Objective articular tissue	Described biocompatibility issues
Carbon fibers and meshes CNTs	Fiber bundles as innovative prostheses	Ligament and tendon	Adequate tolerance and in vivo performance [74]
	Carbon fibers coated with gelatin	Ligament	Mild inflammation. Brittleness and possible debris [75]
	Carbon fibers with joint capsule or lyophilized dura	Ligament	Unacceptable long-term increase of osteoarthrosis [10]
	Carbon fiber pads	Cartilage	Successful results in knee condyle after 5.8 years [68]
	Woven carbon fiber pads	Cartilage	Successful healing and mechanical endurance [69]
	Carbon fiber composite bone plates	Bone	All fractures united, 67% showed remodeling within 6 months, 5 showed an unexpected reaction[70]
	Carbon fiber/flax/epoxy sandwich	Bone	Potential candidate with adequate fatigue properties [76]
	Carbon fiber web with bone morphogenicprotein	Bone	High bone-tissue compatibility [77]
	Electrospun carbon nanofibers [78]	Bone	-
	Block structure made of carbon nanotubes	Bone	Bone formation is verified[79]
	Bioprinted CNT-reinforced scaffolds	Bone and cartilage	Toxicity and means for its limitation [80]
	2D CNT sheets and 3D CNT textiles	Cartilage	Cartilage growth is verified [81]
	CNT-based biomaterials	Varied tissue	Biocompatibility and carcinogenicity[55]
Graphene and its derivatives	3D printed graphene composites	Soft tissues	Promising biocompatibility along 30 days [82]
	Graphene combined with biomaterials	Bone	Enhanced osteogenic responses [83]
	Graphene based biomaterials	Bone	Biocompatibility [47]
	Graphene oxide flakes in hydrogels	Cartilage	Efficient delivery of biofactors and tissue growth [84]
	Polymer scaffolds with graphene and CNTs	Bone and cartilage	Biomimetic performance, acceptable biocompatible [85]
	Graphene-polymer composites	Ligament and tendon	Promising biocompatibility of grapheme surfaces [86]
Carbon dots	Carbon dots in electrospun nanofiber mats	Varied tissues	Low cytotoxicity, enhanced cell proliferation [87]
	Carbon dots in biomedical applications	Varied tissues	Low toxicity compared to other quantum dots[88]
Glassy carbon	Reticulated vitreous carbon scaffold	Bone	Successful repairs, minor concerns, extremely detailed discussion about biocompatibility [89]
	Reticulated vitreous carbon foams	Bone	Suggest appropriate results for biomed applications[90]
	Bioglass coated glassy carbon scaffolds	Bone	Potential for bone formation [91]
	Design-controlled glassy carbon scaffolds	Varied tissues	Remarkable cell adhesion in vitro [92]
	Multi-scale glassy carbon scaffolds	Varied tissues	Remarkable cell adhesion in vitro [93]

3.3. Graphene and Graphene-Based Materials

Graphene, GO, and rGO are used in the field of neural tissue engineering due to their electrical conductivity. Their two-dimensional structures are biomimetic (biomimetic or biocompatible) due to the presence of surface biomolecules, and due to their suitable mechanical properties, they can be used for the synthesis of composites based on It was used on graphene with significant mechanical strength [101]. In the field of joint tissue engineering, graphene, and graphene-based materials are considered suitable materials for bone, cartilage, tendons, and ligaments repair and regeneration [102]. The osteogenic behavior of graphene is shown in Fig. 5. [47, 82, 83]. To use graphene, challenges such as its toxicity in high concentrations and its non-biodegradable nature should be considered [83].

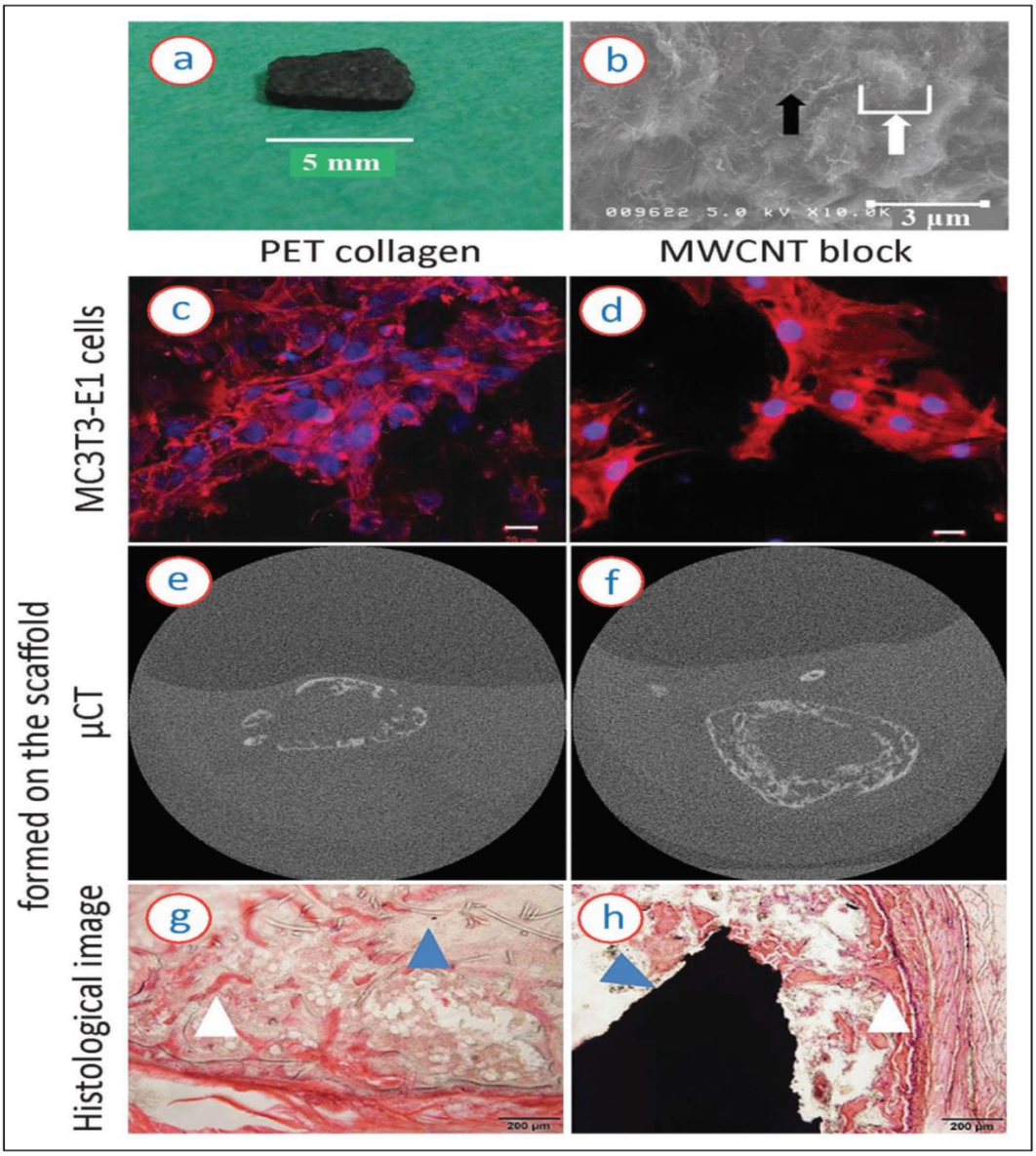

Fig. 4.a) Photograph and b) scanning electron microscopy (SEM) image of a MWCNT block. The black arrow and white arrow in the SEM image represent nan sized irregularity formed by MWCNT fibers and micro sized irregularity formed by an aggregate of MWCNT fibers, respectively.
c–h) Comparison of the MWCNT block scaffold with a PET-reinforced collagen scaffold. c,e,g) Results on the PET-reinforced collagen scaffold and (d,f,h) on the MWCNT scaffolds.
c,d) Cell culturing results on the scaffolds at 3 weeks after subcutaneous implantation of rhBMP-2 loaded scaffolds in mice; e,f)μCT images of ectopic bone formed on the scaffolds; and
g,h) histopathological image of subcutaneous ectopic bone formation in mouse. The white and blue arrow in (g) represent remnant PET fibers and newly formed bone, respectively. The white and blue arrow in (h) represent an MWCNT block and newly formed bone, respectively. The MWCNT showed better areal coverage of cells, yielding to more rigid and complete bone formation, without any debris within the newly formed bone [79].

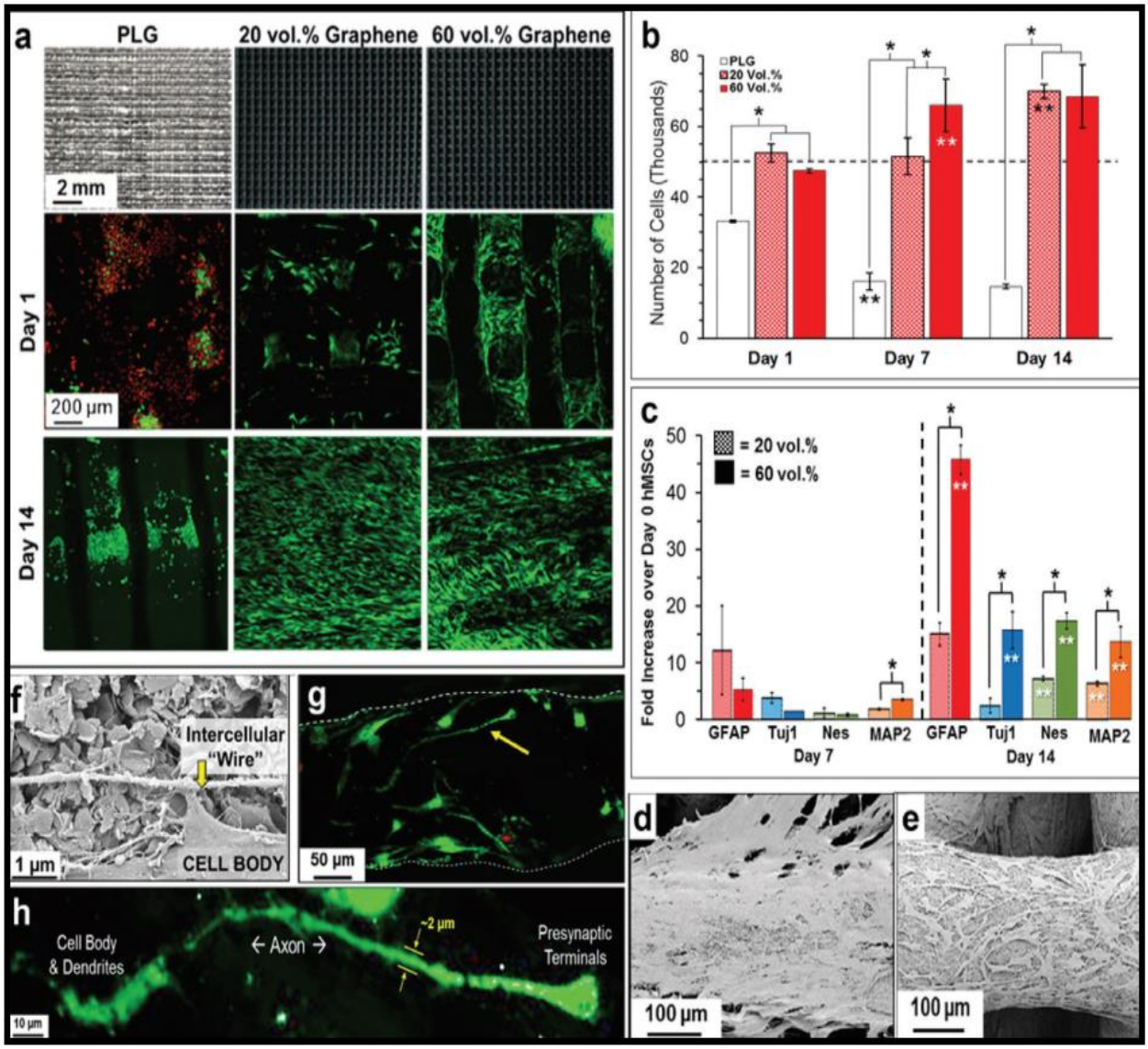

Fig. 5. Example of osteogenic behavior of graphene scaffolds.
a) Top row: Photographs of 3D printed scaffolds of PLG materials with 0%, 20%, and 60% graphene (volume %); Scanning laser confocal 3D reconstruction projection of live (green) and dead (red) human mesenchymal stem cells (hMSCs) cultured at day 1 (middle row) and day 14 (bottom row) on the scaffolds.
b) Number of hMSCs present on the scaffolds at day 1, 7, and 14, suggesting superior cell proliferation on the scaffolds with higher concentration of graphene.
c) Neurogenic relevant gene expression of the cells on the graphene scaffolds at day 7 and 14, showing better gene expression with higher content of graphene.
d,e) SEM of hMSCs on the 20% and 60% graphene loaded scaffolds, respectively.
f) High magnification SEM of hMSCs cell on 60% graphene scaffold at day 7, showing hMSCs connecting via along "intercellular" wire.
g) Scanning laser confocal 3D reconstruction of live (green) and dead (red) hMSCs cells on day 14 for 60% graphene scaffold.
h) High magnification image of the cell indicated by yellow arrowing (f) showing the detailed features [82].

Considering that cartilage is more challenging to regenerate (compared to bone), graphene seems to provide suitable cues for stem cells. Graphene oxide pieces have been used as cartilage cell growth factors in 3D hydrocells [84]. Also, nanocomposites (rGO and HAp) help to form new bone cells and produce calcium and phosphate minerals without any limitations [103].

Graphene and materials based on carbon nanotubes for joint repairs where both bone and cartilage are needed. They are used for repair [85].
The performance of graphene-based nanomaterials as reinforcement in polymer-carbon nanocomposites for ligament and tendon repair has been investigated [86].

To produce three-dimensional graphene-based scaffolds, apart from the use of graphene-based nanocomposites, the possibility of synthesizing graphene foams for use in cardiac [104] and nerve [105] tissue engineering has been investigated.
In both cases, increased cell proliferation has been reported for 3D graphene compared to 2D graphene. These foams have been synthesized by the vapor phase deposition process using nickel templates [106].
Hydrographene foams or other carbon materials are suitable substitutes for bone, cartilage, tendon, ligament, or joint restorations.

3.4. Carbon Dots

The nearly zero-dimensional nature of carbon dots (C-dots) with sizes below 10 nm [107] precludes their use for repairing large defects in joint injuries and tissue engineering in general.
However, some unique features of these materials lead to their use in innovative diagnostic methods and treatments.
These materials have various applications in the energy, medicine, and ecology industries and are suitable alternatives to CNTs and graphene with lower costs [42, 107, 108].
The medical applications of these materials as drug delivery materials and theranostics have received more attention, although their biocompatibility and toxic effects in the body (in vivo) and laboratory conditions (in vitro) are major challenges in using these materials [109].
Carbon nanoparticles can also be used in tissue engineering scaffolds. For example, it has recently been shown that C-dots can track and enhance osteogenic differentiation of MSCs [110].
The role of these materials as electrically conductive nano biomaterials for tissue engineering and regenerative medicine has also been proposed [88].

3.5. Glassy Carbon

Glassy carbons are used to fabricate biomimetic porous mesh structures, especially for trabecular bone tissue engineering.
For example, reticulated glassy carbons, which are available in the market as RVC foam, have been tested both inside and outside the body and show very good adhesion of MSCs and bone regeneration properties.
But the incompatibility with cartilage regeneration [89] is probably due to its excessive stiffness. morphology, compressive strength, and cell compatibility with human osteoblasts have been investigated [90].
Nowadays, using sucrose molds, reticulated glassy carbons have been made at low cost, and their The creation of hydroxyapatite scaffolds, using a multistep process including thermal decomposition of raw materials to produce glassy carbon molds with natural and complex anisotropic pore structures, followed by carbonation and phosphating, is another successful strategy for repairing bone structures [111].
Nowadays, the controlled production of glassy carbon scaffolds for tissue engineering is done through pyrolysis of complex biomimetic patterns (Fig. 6a.) [92].
The possibility of achieving 3D multi-scale glassy carbon, with a structural lattice filled by softer microfibers, has been also demonstrated (Fig. 6b.) [93].
These functionally graded structures show potentials toward biomimetic designs, in which a structural lattice may help to regenerate bone, while fiber meshes and micrometric carbon threads may support the reconstruction of cartilage and fibrillar tissues.

3.6. Nanodiamonds and Diamond-Like Carbon

The beauty and perfection of diamond is also starting to have an impact on tissue engineering and biotechnology in general [112].
For advanced biomedical applications, diamond is especially promising in nano-structured arrangements, basically as nanoparticles, nanostructured diamond films, and composite scaffolds with a matrix containing diamond nanoparticles as fillers, also known as nanodiamond-loaded nanofibrous scaffolds.
Their effects have been tested with bone-derived cells and shown potentials for bioimaging, biosensing, and drug and gene delivery, as a complement to tissue engineering structures.
However, the number of reports on nanodiamond cytotoxicity is increasing, with reviewed studies including in vitro and in vivo studies [112].
Regarding diamond-like carbon (DLC) coatings, their benefits for improving the in vitro biological response of polymeric and metallic tissue engineering scaffolds and implants have been investigated (Fig. 6c.) [113, 114].
These coatings tend to enhance cell adhesion and provide a remarkable surface hardness, thanks to their high content of sp^3 hybridization, as well as an adequate corrosion resistance against chemicals, abrasion endurance, good biocompatibility, and uniform flat surface [115].

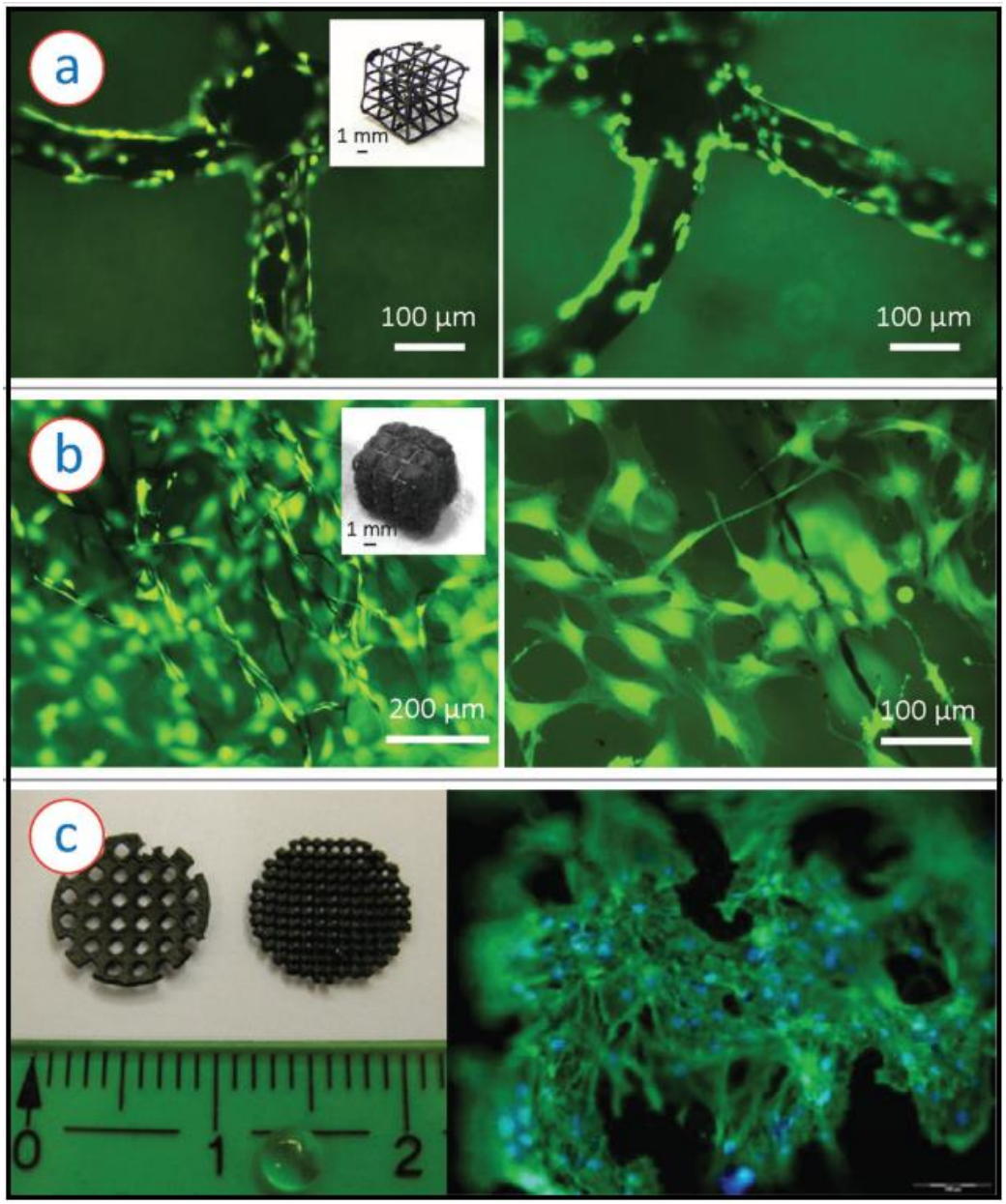

Fig. 6.a) Osteoblast like murine MC3T3-E1 cells cultured on additively manufactured glassy carbon micro lattice structures on day 3 after cell seeding, showing good cytocompatibility of the cells. The inset shows an example of a glassy carbon architecture [92].
b) The murine MC3T3-E1 cells cultured within a carbon fiber filled glassy carbon micro lattice architecture (inset showing a photograph of the hybrid structure). The cells were proliferated not only over the micro lattices and along the carbon fibers (left), the cells also established intercellular connections within the voids between adjacent fibers (right), yielding a 3D cell colonization [93],
c) Left: Example of DLC coated rapid prototyped scaffolds. Right: Cell growth validation of the DLC coated scaffolds by culturing h MSCs cells on the scaffolds [113].

4. Conclusion

Tissue engineering is the regeneration and production of cells with a micro or macro imitation environment, as well as the regeneration of damaged body tissues and the replacement of traditional implants with active biological structures and biological materials. In this regard, tissue engineering materials and scaffolds have been investigated. The prevalence of joint pain is the result of general inactivity and unprincipled exercise. Today, joint repairs are still performed using metal, ceramic, and polymer implants or auto graft, allograft, and xenograft.

While conventional implants are suboptimal in terms of biomechanical performance, tissue engineering is considered a viable option.

Since the beginning of tissue engineering, carbon materials have been used to regenerate musculoskeletal tissues.

Due to having suitable mechanical properties, carbon-based materials include a very diverse set of biomaterials that have the possibility of replacing the joint tissues of the human body. Carbon-based materials minimize mechanical incompatibility with other body organs and create proper biocompatibility with them.

The potential of carbon-based materials for the repair and regeneration of human tissues is very high, especially for the treatment of bone and joint injuries. All carbon materials are suitable to meet the needs of tissue engineering in the production of living engineering structures such as bone, cartilage, ligament, and joint repair and regeneration. Accordingly, the most advanced carbon-based materials can be used for tissue engineering in medicine, such as carbon nanotubes, graphene and graphene oxide, carbon fibers, glassy carbons, and nano-diamonds with individual characteristics.

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