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#### Biomolecule-directed carbon nanotube self-assembly

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The strategy of combining biomolecules and synthetic components to develop biohybrids is becoming increasingly popular for preparing highly customised and biocompatible functional materials. Carbon nanotubes (CNTs) benefit from bioconjugation, allowing their excellent properties to be applied to biomedical applications. This study reviews the state-of-the-art research in biomolecule–CNT conjugates and discusses strategies for their self-assembly into hierarchical structures. The review focuses on various highly ordered structures and the interesting properties resulting from the structural order. Hence, CNTs conjugated with the most relevant biomolecules, such as nucleic acids, peptides, proteins, saccharides, and lipids are discussed. The resulting well-defined composites allow the nanoscale properties of the CNTs to be exploited at the micro- and macroscale, with potential applications in tissue engineering, sensors, and wearable electronics. This review presents the underlying chemistry behind the CNT-based biohybrid materials and discusses the future directions of the field.

#### 1. Introduction

Biohybrid materials combine biomolecules and synthetic functional materials,<sup>[1-3]</sup> such as nanoparticles,<sup>[4-6]</sup> polymers,<sup>[7,8]</sup> or small molecules.<sup>[9,10]</sup> Interest in biohybrids research has grown rapidly over the past decades, bridging the fields of chemistry, physics, materials science, nanoscience, biology, and medicine.<sup>[11-14]</sup> Biohybrids combine the highly sophisticated functions of biomolecules with the chemical and physical properties of the synthetic component. Among the vast selection of synthetic materials, carbon nanotubes (CNTs) hold a prominent place due to their high conductivity, strength, and elasticity, and relative chemical inertness.<sup>[15-</sup> <sup>18]</sup> Due to the aromatic nature of CNTs, they are hydrophobic and generally have low solubility in solvents, particularly in water. This presents a challenge as water is the required medium for bioconjugation and a prerequisite for maintaining the native structure and function of biomolecules. Bioconjugation of CNTs is a suitable strategy to enhance their biocompatibility and prevent possible health concerns,<sup>[19-22]</sup> although CNT-based biohybrid materials have not yet been proven to be clinically safe.<sup>[21]</sup> Furthermore, CNT bioconjugation enables the assembly of CNTs into highly ordered structures (Figure 1). It is of significant importance to transfer the excellent mechanical and electronic properties of individual CNTs to the microscale structures, which can then be used as active components.<sup>[23]</sup>

This review focuses on ordered CNT-based biohybrids, and their applications resulting from the well-defined microstructure. First, a brief introduction to the properties of CNTs and the chemical strategies available for increasing their water solubility is presented. Then, a thorough overview of the most relevant CNT-based biohybrids is presented, organised by the featured biomolecules (nucleic acids, peptides, proteins, carbohydrates, and lipids). In order to understand the forces directing the biohybrid self-assembly and simplify the heterogeneous field, the reviewed bibliography in this two first sections will focus on single-walled CNTs (SWCNTs). Finally, selected applications in the fields of tissue regeneration, sensors, and 2

wearable electronics are discussed, highlighting the relevance of well-defined CNT structures. A detailed and comprehensive review of all current chemical and biological conjugation strategies is not included as they were thoroughly reviewed elsewhere.<sup>[24–30]</sup>



Figure 1 Schematic representation of the SWCNT biohybrids reviewed in this article, and the self-assembly process directed by the biomolecule.

#### 2. Structure and properties of carbon nanotubes

Since the first discovery of CNTs in 1991,<sup>[31]</sup> their unique properties, such as high conductivity and elasticity, and low weight have been extensively demonstrated.<sup>[17,32]</sup> In addition, CNTs are among the strongest and stiffest materials currently known.<sup>[16]</sup> CNTs can provide their unique properties to composite and hybrid materials, and due to their nanosized core and relatively large surface area, they are ideal for conjugation with biomolecules. Thus, CNTs have attracted considerable interest for many applications in nanoscience and, in particular, nanomedicine.<sup>[33– 36]</sup>

CNTs are hollow rod-shaped carbon allotropes with a typical diameter of 0.7–100 nm, and lengths from several micrometres to millimetres. Depending on the number of concentrically rolled sheets, CNTs can be classified as SWCNTs or multi-walled carbon nanotubes (MWCNTs). These subclasses present well-differentiated properties: SWCNTs have ultra-high aspect ratio and specific surface, forming an excellent platform to display biomolecules. On the

other hand, the high aspect ratio brings disadvantages, such as increased aggregation in biological media and higher toxicity. Several techniques are used to produce CNTs, including laser ablation, gas-phase catalytic process methods, and arc-discharge or chemical vapour deposition.<sup>[37]</sup> These methods typically produce a mixture of CNTs with different diameters and impurities, and therefore suitable purification methods have been developed.<sup>[38]</sup> Various methods for chemically modifying SWCNTs have been reported, yielding soluble derivatives able to undergo assembly and conjugation by hierarchical self-assembly of individual SWCNTs (**Figure 2**).<sup>[30,37,39,40]</sup>

A key prerequisite for bioconjugation is the successful dispersion of individual and structurally well-defined CNTs into aqueous media.<sup>[41]</sup> However, this is challenging due to the inherent hydrophobicity of the aromatic CNT surface,<sup>[37]</sup> which promotes the formation of aggregates or bundles. To address this, two main strategies were explored: (i) covalent and (ii) supramolecular modification (Figure 2a). First, covalent binding generates a stable interaction through a chemical bond disrupting the SWCNT performance. The reaction can occur at the aromatic sidewall (via cycloaddition or radical addition), the end tip, or an existing defect (through oxidation, esterification, or amidation). Oxidation is one of the most popular reactions to introduce hydrophilic moieties and reactive groups (i.e. hydroxyls and carboxylic acids) that undergo further chemical modification. The reaction conditions significantly affect the degree of oxidation: harsh acidic conditions such as hot nitric acid or sulfuric/nitric acid mixtures with or without sonication induce important changes in the SWCNTs.<sup>[42,43]</sup> In contrast, oxidation with piranha solution (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> mixtures) at 22–25 °C leads to slow shortening of SWCNTs by dissolution of existing defect sites, with little sidewall damage.<sup>[44]</sup> In contrast, supramolecular modification creates a labile bond that does not disrupt the aromatic conjugation of the SWCNT, preserving its excellent properties. Pristine SWCNTs are mixed with

amphiphilic molecules (usually under sonication), with the hydrophobic domain stabilising the aromatic surface *via* van der Waals and  $\pi$ - $\pi$  interactions, and the hydrophilic domain facing the solvent. Other interactions such as electrostatic or hydrogen bonds are suitable for the complexation of oxidised SWCNTs. Finally, a large variety of interactions were reported for directing the self-assembly of SWCNT hybrids into composite materials, including (from low to high individual bond stability) external forces, van der Waals, hydrogen bonding, electrostatic, and covalent/ion mediated interactions (**Figure 2**b).



**Figure 2.** a) Schematic of the SWCNT bioconjugation strategies, and b) interactions employed for the hierarchical self-assembly into self-assembled materials.

#### 3. Carbon nanotube biohybrids

The functionalisation of SWCNTs with a toolkit of biomolecules provides many opportunities for the application of these excellent carbon allotropes in biologically relevant environments. Here, the biohybrids are organised according to the biomolecule (nucleic acids, peptides,

proteins, carbohydrates, and lipids), nature of the interaction, morphology of the conjugate, and their properties. The most significant examples are summarised in Table 1.

 Table 1. Summary of the reviewed CNT-biomolecule composites, the interaction driving the

 biomolecule-CNT recognition and self-assembly, along with their morphology and application.

 Only composites showing hierarchical self-assembly are included.

Biomoloculo		Richybrid formation	Solf accombly/	Richybrid morphology (Application)
Biomolecule	CINT SOURCE	Biorrybrid formation	Composite	Bionybrid morphology (Application)
<b>Nucleic acid -</b> Nucleotide	Pristine	Covalent (1,3-dipolar cycloaddition and arylation/amidation)	H-bond	Bundles <sup>[46]</sup>
	Oxidised (HNO <sub>3</sub> )	Covalent (amidation)	H-bond	2D aligned stripes, <sup>[47]</sup> , nanorings, <sup>[48]</sup> bundles, <sup>[49]</sup> 2D-lozenges <sup>[51]</sup>
ssDNA/dsDNA	Pristine	π-π and van der Waals	Electric field, <sup>[58]</sup> H-bond <sup>[59, 60]</sup>	2D aligned stripes, $^{\rm [58, \ 59]}$ 2D hexagonal network $^{\rm [60]}$
	Oxidised (HNO <sub>3</sub> )	Covalent (amidation)	H-bond	Branched aggregates, <sup>[65]</sup> AuNP-mediated branched aggregates <sup>[64]</sup>
	Oxidised	$\pi\text{-}\pi$ and van der Waals	Covalent bond	Bundles (pH-responsive $H_2O_2$ sensor) <sup>[147]</sup>
DNA origami	Pristine	$\pi\text{-}\pi$ and van der Waals	H-bond	Parallel, <sup>[62]</sup> perpendicular, <sup>[61]</sup> and triangular alignment; <sup>[76]</sup> AuNP-mediated angle control <sup>[63]</sup>
Peptides	Pristine	π-π	lon-induced electrostatic	Fibrous structures <sup>[78]</sup>
	Pristine	Π-Π	H-bond, <sup>[80][77]</sup> van der Waals, <sup>[81]</sup> multiple non- covalent forces <sup>[134]</sup>	Tubular, <sup>[80]</sup> fibres and flat ribbons, <sup>[77]</sup> bundles, <sup>[81]</sup> hydrogel ( <i>tissue</i> <i>engineering</i> ) <sup>[134]</sup>
<b>Protein</b> Collagen	Oxidised <sup>[143]</sup> (H <sub>2</sub> SO <sub>4</sub> /HNO <sub>3</sub> ) <sup>[97]</sup> (formic acid) <sup>[135]</sup>	Multiple non-covalent forces	Multiple non- covalent forces	Bundles <sup>[97]</sup> ( <i>tissue engineering</i> ), <sup>[135]</sup> hydrogel ( <i>tissue engineering</i> ) <sup>[141]</sup>
Silk	Oxidised (formic acid) and pristine. <sup>[73]</sup>	Multiple non-covalent forces	Electric field/shear mixing	Fibrous <sup>[99]</sup> ( <i>tissue engineering</i> ) <sup>[98, 135, 136]</sup>
Protein crystals	Pristine	π-π	Electrostatic	Incorporation in cross-linked lysozyme crystals <sup>[100]</sup>
Hydrophobin	Pristine	π-π	Hydrophobic	Branched bundles <sup>[101]</sup>
Gelatin	Pristine	Multiple non-covalent	Multiple non- covalent	Hydrogel ( <i>tissue engineering</i> ) <sup>[140]</sup>
Carbohydrates Cyclodextrin	Aminated, <sup>[113]</sup> pristine <sup>[149]</sup>	Covalent – amidation, <sup>[113]</sup> $\pi$ - $\pi$ and host-guest (adamantane-CD) <sup>[149]</sup>	H-bond	Fibres (water remediation) <sup>[113]</sup>
		π-π interaction (pyrene) and host-guest (adamantane-CD)	Electric field and shear flow	Fibres ( <i>wearable electronics</i> ) <sup>[149]</sup>

Curdlan/ Schizophyllan	Pristine and Oxidised (H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O <sub>2</sub> )	van der Waals and H- bond	H-bond, <sup>[76]</sup> covalent bond, <sup>[115]</sup> electrostatic. <sup>[116, 121]</sup>	Bundles, <sup>[119]</sup> 2D aligned stripes <sup>[115, 116, 121]</sup>
CNC	Pristine, <sup>[124]</sup> unknown, <sup>[123]</sup> a) oxidised. <sup>[117, 150]</sup>	van der Waals, <sup>[124]</sup> unknown, <sup>[123] a)</sup> H-bond <sup>[117, 150]</sup>	Shear blade, <sup>[124]</sup> dip coating, <sup>[123]</sup> van der Waals, <sup>[117]</sup> shear flow (3D printed) <sup>[150]</sup>	2D aligned stripes, <sup>[123, 124]</sup> lyotropic liquid crystal phase, <sup>[117]</sup> fibre ( <i>wearable</i> <i>electronics</i> ) <sup>[150]</sup>
	Oxidised (HNO <sub>3</sub> )	H-bond	Shear flow (3D printed)	Fibre ( <i>wearable electronics</i> ) <sup>[150]</sup>
Agarose	Oxidised	Multiple non-covalent	Shear flow	Hydrogel ( <i>tissue engineering</i> ) <sup>[146]</sup>
Lipids	Pristine	van der Waals	Electrostatic, <sup>[126]</sup> entropy driven, <sup>[127]</sup> van der Waals. <sup>[159]</sup>	Multilamellar and inverted centred rectangular columnar packing, <sup>[126]</sup> intercalated hexagonal binary superlattice and lamellar, <sup>[127]</sup> fibre ( <i>sensor</i> ) <sup>[159]</sup>

<sup>a)</sup> Authors only state a commercially available aqueous SWCNT dispersion.

#### 3.1. Nucleic-acid-based biohybrids

Nucleic acids are fundamentally amphiphilic molecules consisting of hydrophobic nucleobases and a hydrophilic sugar–phosphate backbone. Thus, single- or double-stranded DNA (ssDNA and dsDNA, respectively) have been widely used solubilising agents. The present section is organised by increasing complexity: from nucleotides to ssDNA, dsDNA, and DNA origami.

#### 3.1.1. Nucleotides

The solubility of CNTs in aqueous solutions can be increased by non-covalent surface functionalisation with nucleotides using a high-speed vibration milling technique. The solubility was shown to increase with increasing number of nucleotide phosphate groups.<sup>[45]</sup> The nucleotides interact with SWCNTs through the aromatic nitrogenous base and the hydrophobic surface of the SWCNT *via* van der Waals and  $\pi$ - $\pi$  interactions. Diphosphate nucleotides showed the highest aqueous solubility, followed by triphosphate and, lastly, monophosphate. Covalently-modified SWCNTs with thymine displayed strong self-recognition when dissolved in dichloromethane due to the thymine-thymine hydrogen bonding, in contrast to its dispersion to monomeric SWCNTs in DMF.<sup>[46]</sup> Adenine-modified SWCNTs assembled into highly ordered stripe-like structures when deposited onto HOPG surfaces.<sup>[47]</sup> In

a similar fashion, oxidised SWCNTs were modified with uracyl derivatives through amidation, resulting in toroidal structures of *ca.* 50 nm in diameter when deposited onto HOPG surfaces.<sup>[48]</sup> It was hypothesised that short curved bundles of SWCNTs with uracyl moieties at the ends interact head-to-tail and form discrete toroids or s-shaped bundles that evolve into rows of rings. Double covalent functionalisation via two reaction sites (*i.e.* amidation of the carboxylic acids in the open ends of oxidised SWCNTs, and 1,3-dipolar cycloaddition of azomethine ylides to the aromatic surface) allowed the introduction of adenine-uracyl, adenine-adenine, or guanidine-adenine nucleotide pairs, respectively.<sup>[49]</sup> The presence of the nucleobases supported SWCNT aggregation in both protic and aprotic solvents. In addition to the previously discussed geometries, guanine can self-assemble into other type of structures, such as circular G-quadruplexes.<sup>[50]</sup> This tetrameric architecture, templated by small cations, was used to direct the assembly of covalently-modified guanine SWCNTs, producing lozenges on HOPG and mica surface.<sup>[51]</sup> The optimal guanine–SWCNT:K<sup>+</sup> ratio was 4:1, while a 1:1 ratio showed only bundles of nanotubes, without any specific orientation.

#### 3.1.2. Single- and double-stranded DNA

A powerful tool for solubilising bundled SWCNTs is sonication of the aqueous solution of SWCNTs and ssDNA, dsDNA, and RNA.<sup>[52–54]</sup> In the dispersion process, ssDNA wraps helically around the SWCNT by  $\pi$ - $\pi$  stacking of the aromatic nucleotide bases of the ssDNA (**Figure 3**a). Removing the excess DNA from the solution will neither affect the dispersion of the SWCNTs nor cause flocculation, indicating that the DNA-SWCNT interaction is relatively strong. This strategy is dependent on the nucleotide sequence, enabling the separation of SWCNTs with different tube diameters and electronic properties through anion-exchange chromatography,<sup>[55,56]</sup> or aqueous two-phase systems.<sup>[57]</sup> Furthermore, alignment of DNA-wrapped SWCNTs can be achieved by hybrid deposition onto hydrophobic SiO<sub>2</sub> surfaces and

the application of an external electric field to direct the process. This approach is used for the cost-effective fabrication of SWCNT patterns on solid substrates.<sup>[58]</sup>

DNA conjugation allows the design of sequences with appending fragments or DNA "handles" available to hybridise with complementary sequences, thus directing the aggregation or recognition. For instance, SWCNTs were grafted with DNA sequences containing both single and double-stranded domains, which acted as SWCNT recognition and spacer parts, respectively (**Figure 3**b).<sup>[59]</sup> Upon surface deposition onto mica, the otherwise individual SWCNTs were able to diffuse and form many dimers and trimers of parallel SWCNTs, with distances depending on the length of the dsDNA domain. Larger multi-nanotube assemblies were observed upon increasing the surface mobility, which was achieved by NaCl addition

(Figure 3b).



**Figure 3**. a) dsDNA–CNT wrapping model. Adapted with permission.<sup>[56]</sup> Copyright 2009, Springer Nature. b) DNA–SWCNT hybrids with overhanging strands (top) that are able to

hybridise, leading to a parallel alignment (bottom). Adapted with permission.<sup>[59]</sup> Copyright 2012, American Chemical Society c) Hexagonal 2D pattern obtained upon hybridisation of SWCNT with three-way-junction DNA. Adapted with permission.<sup>[60]</sup> Copyright 2018, American Chemical Society d) Perpendicular SWCNT orientation assisted by DNA origami. Adapted with permission.<sup>[61]</sup> Copyright 2010, Springer Nature e) Parallel SWCNT orientation assisted by DNA origami. Adapted with permission.<sup>[62]</sup> Copyright 2013, American Chemical Society and f) controllable SWCNT orientation assisted by DNA origami. Adapted with permission.<sup>[63]</sup> Copyright 2020, John Wiley and Sons.

In addition to the alignment of SWCNT hybrids, DNA functionalisation enables control of their relative orientation. In a first approach, ssDNA was grafted onto oxidised SWCNTs through amidation, and was subsequently hybridised with gold nanoparticles (AuNPs) functionalised with the complementary strand.<sup>[64]</sup> Interconnected structures were observed, where the AuNPs served as node for the growth of branched aggregates, resulting in a space between the SWCNT and AuNP of 70 Å, which is similar to the length of dsDNA. This strategy was used to successfully prepare SWCNT/MWCNT hybrid materials. The same principle was applied in the absence of AuNPs, by covalent modification of oxidised SWCNT with two complementary ssDNA molecules, producing two sets of hybrids.<sup>[65]</sup> The functionalisation occurs either at the open end or a wall defect of the SWCNT, directing the self-assembly of the complementary sets of ssDNA–SWCNT hybrids into branched structures. Noticeably, the thickness of the junction between two SWCNTs is equivalent in thickness and length to those of hybridised dsDNA.

Furthermore, full control of the SWCNT concentration was achieved by ultrasonication in the presence of DNA three-way junctions containing oligo-guanine overhangs.<sup>[60]</sup> After

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interaction with the overhangs, the SWCNTs arranged themselves in the junction with an angle of ~120° between them. These dsDNA structures were subsequently labelled by intercalating a fluorescent marker (YOYO-1) within the strands. This strategy avoids typical fluorescence quenching near the SWCNT. However, if the hybridisation occurs with the ssDNA sequences, and is subsequently subjected to annealing, the system results in a two-dimensional hexagonal network instead of the individual three-armed hybrids (**Figure 3**c).

#### 3.1.3. DNA origami–SWCNT

DNA origami is a self-assembled and water-soluble nanosized structure, which consists of ssDNA (usually a long scaffold strand) folded into the desired shape with the aid of multiple short staple strands.<sup>[66]</sup> The unique programmability and addressability of the DNA origami<sup>[67]</sup> have been exploited for a wide range of applications, including controlled drug delivery, targeting, and cargo release.<sup>[68–72]</sup> Higher-ordered structures<sup>[73]</sup> are achieved by hybridisation with small molecules,<sup>[74]</sup> inorganic nanoparticles<sup>[75]</sup>, or DNA-functionalised SWCNTs. The latter were reported to self-assemble and align as cross-junctions on DNA origami tiles of 75  $\times$ 95 nm<sup>2</sup> (Figure 3d).<sup>[61]</sup> The rectangle contains two crossing lines of ssDNA "hooks" at a distance of about 6 nm from each other. Each line of the hooks is designed to bind with the specific sequences of the DNA labels and to align the ssDNA-SWCNT. To prevent aggregation of the DNA origami, the rightmost column of staples is replaced by DNA strands, which leads to growth of the DNA ribbon made of DNA tiles. The length of the ribbon is typically 500 nm, helping the image interpretation and increasing the deposition rate of DNA-SWCNT constructs. The two different ssDNA–SWCNTs (*i.e. red* and *blue* in Figure 3d) aligned with the preferred hook array. Statistical analysis indicated that 86% of the templates had at least one red ssDNA-SWCNT attached, with 50% of them horizontally aligned  $(\pm 15^{\circ})$ , while 80% of the templates had at least one *blue* ssDNA–SWCNT attached, with 56% of them vertically aligned  $(\pm 15^{\circ})$ .

Successful parallel alignment of SWCNTs was achieved by direct conjugation of ssDNA– SWCNT and a linear assembly of cross-shaped DNA origami (**Figure 3**e).<sup>[62]</sup> The highly directional assembly (over 500 nm in length) was only observed in the presence of ssDNA– SWCNTs and sticky-end DNA staples decorating the origami, after thermal annealing. Based on limited observations, only ~2% of the hybrids had aligned SWCNTs attached. A similar strategy was later used with triangular origami and discrete-length ssDNA–SWCNT.<sup>[76]</sup>

AuNP-mediated conjugation was employed to deposit ssDNA–SWCNT onto rectangular DNA origami tiles (**Figure 3**f).<sup>[63]</sup> To this end, ssDNA–AuNPs were synthesised and precisely aligned ( $\pm 10^{\circ}$ ) onto DNA origami tiles, giving a total height of 13 nm, which was in good agreement with the expected dimensions. The highly packed ssDNA grafted on the AuNP provided a 5-fold binding affinity compared to the direct ssDNA–SWCNT/DNA origami conjugation. Further validation of this methodology to precisely control the SWCNT-SWCNT angle was achieved by the arrangement of cross-linked SWCNTs at 75°. This system, with one SWCNT modified with fluorescein amidite electron donor dye, and the other with guanine as an electron acceptor, showed a 1.28-fold decrease in the fluorescence, due to efficient electron transfer (30%) upon SWCNT cross-linking.

The research reviewed here has shown that SWCNT-based biohybrid materials are used to separate complex mixtures of SWCNTs, achieve bi-dimensional networks, pH-responsive branched structures, and straightened SWCNTs on DNA origami. Complementary DNA sequences are used for connecting the DNA–SWCNT components into more complex structures.

#### 3.2. Peptide-based biohybrids

The water solubility of CNTs can be increased by hydrophobic and  $\pi$ - $\pi$  stacking interactions between a peptide backbone and the CNT.<sup>[77, 78]</sup> A seminal paper published in 2011 described

the peptide decoration of SWCNTs in a virus-like coating approach. This geometrically defined assembly of peptides directs the mineralisation of AuNPs into helical arrays along the SWCNT axis, employing cysteine to direct the metal nucleation (**Figure 4**a and b).<sup>[79]</sup> Controlled assembly of  $\alpha$ -helical peptide–SWCNT composites was achieved by hydrophobic-hydrophobic and electrostatic interactions, resulting in highly ordered fibre-like structures (**Figure 4**c and d).<sup>[77]</sup> The fibre size increased in the presence of 120 mM NaCl due to the enhanced crystallisation forming larger structures. In contrast, DMF interferes with peptide-peptide interactions, reducing the size of the aggregates to flat ribbons (20–75 nm) of aligned SWCNTs (**Figure 4**c).



**Figure 4.** a) Schematic representation and b) TEM images of AuNP nucleation directed by self-assembled peptides (scale bars: 10 nm). Adapted with permission.<sup>[79]</sup> Copyright 2011, The

American Association for the Advancement of Science c) Molecular model of the interaction between an amphiphilic  $\alpha$ -helix peptide and a SWCNT; microscopy images of the aggregate in the d) absence and e) presence of DMF. Adapted with permission.<sup>[77]</sup> Copyright 2003, American Chemical Society f) SEM image of phosphate-induced aggregation of phenylalanine-containing peptide–SWCNT structures. Adapted with permission.<sup>[78]</sup> Copyright 2008, John Wiley and Sons g) AFM micrograph of aligned self-assembled cyclic peptide (SCP)–SWCNT complexes (scale bar: 2 µm); (*inset*) a schematic representation of the interaction. Adapted with permission.<sup>[80]</sup> Copyright 2014, American Chemical Society h) Modified morphology of Aβ peptides in the presence of SWCNT; (*inset*) a schematic representation of the interaction. Adapted with permission.<sup>[81]</sup> Copyright 2014, Royal Society of Chemistry.

Phenylalanine-containing peptides effectively solubilise SWCNTs through  $\pi$ - $\pi$  interactions between the aromatic moieties.<sup>[78]</sup> The biohybrids self-assemble into aligned fibrous structures in the presence of a phosphate buffer, as observed by SEM (**Figure 4**f). In contrast, dispersion in water yielded anisotropic dispersions, indicating that the self-assembly was induced by the electrostatic interaction between the negatively charged phosphate ions and positively charged lysine residues in the peptides. An unusual approach to direct the assembly of biohybrids is combining SWCNTs and peptides with a natural tendency to aggregate. First, cyclic peptides (SCPs) self-assemble into tube-like supramolecular polymers held together *via* hydrogen bonding of individual cyclic peptides.<sup>[82,83]</sup> SCPs with an appended pyrene moiety were reported to interact through  $\pi$ - $\pi$  stacking with SWCNTs, yielding a dual tubular structure.<sup>[80]</sup> The aromatic pyrene moiety provides higher binding affinity towards semiconducting rather than metallic SWCNTs.<sup>[84]</sup> Highly directional arrays of SCP–SWCNT biohybrids were

dual tubular biohybrids provide a promising approach to produce conducting nanowires without short circuits. Other self-assembling peptides include the A $\beta$  peptides that tend to aggregate into amyloid plaques, which are responsible for conditions such as Alzheimer's disease.<sup>[85]</sup> However, conjugation of A $\beta$  peptides with SWCNTs results in non-amyloid aggregates, thus offering a promising therapeutic approach to treat such diseases.<sup>[81]</sup> In detail, SWCNT triggers a conformational change from the natural random coil of the peptide to a more stable parallel  $\beta$  sheet, as confirmed by circular dichroism and AFM (**Figure 4**h). This stabilisation is due to the interaction of hydrophobic residues of the A $\beta$  peptide and the SWNT surface. Consequently, this inhibited the formation of cross- $\beta$ -sheet conformation that is the pathologic basis for amyloid plaques. The composites resulted in reduced cytotoxicity in neuroblastoma SH-SY5Y cells, thereby providing new avenues for biocompatible SWCNTs.

#### 3.3. Protein-based biohybrids

Proteins fulfil structural, recognition, transport, and catalytic functions, and their self-assembly results in a wide range of materials.<sup>[14,86–89]</sup> Protein–SWCNT biohybrids were widely reported elsewhere<sup>[22,90–92]</sup> and reviewed for their use in nanobiotechnology, material science, and bioelectronics.<sup>[93–95]</sup> Therefore, this section is limited to the preparation of highly ordered structures that have not yet been reviewed in detail.

Collagen is an elongated triple helix that forms a fibril, and plays a crucial role in the extracellular matrix of various tissues,<sup>[96]</sup> with both structural and functional importance in cell adhesion and migration. However, collagen fibres are mechanically weak materials that lack electrical conductivity, which limits their applications. Therefore, the assembly of SWCNTs by self-aligning collagen fibres was pursued.<sup>[97]</sup> First, oxidised SWCNTs interact with the hydrophobic domains of the collagen proteins located inside the protein fibre.<sup>[98]</sup> Further stabilisation of the collagen–SWCNT fibre was accomplished by hydrogen bonding or electrostatic interaction between the carboxyl groups of the oxidised SWCNTs and the 15

positively charged amino acids of the collagen proteins (**Figure 5**a, top). The composite prepared in the presence of 0.4 wt% of SWCNTs was characterised by SEM (**Figure 5**a, bottom), which shows the entangling of SWCNTs within the naturally occurring collagen fibre. Although the composites showed no visible disruption of the collagen structure, the presence of SWCNT was confirmed by Raman spectroscopy. Collagen–SWCNT composites underwent cell-mediated gel-compaction, which is characteristic of smooth-muscle-cell-seeded Type I collagen gels. The cell viability of rat aortic smooth muscle cells was above 85% over a period of a week for every SWCNT concentration tested (0.2–2.0 wt%).



**Figure 5.** a) Schematic of SWCNT inclusion within collagen fibres (top) and SEM micrographs (bottom) of the composite (the arrows indicate embedded SWCNTs). Adapted with permission.<sup>[97]</sup> Copyright 2005, John Wiley and Sons b) Schematic of the external forces that direct the silk–SWCNT alignment (top) and SEM images of the SWCNT reinforced fibres (bottom). Adapted with permission.<sup>[99]</sup> Copyright 2006, American Chemical Society c) SEM 16

micrographs of cross-linked lysozyme crystals (CLLCs, top) and photographs of the crystals with increasing SWCNT payload (bottom, from left to right). Adapted with permission.<sup>[100]</sup> Copyright 2018, John Wiley and Sons d) Schematic (left) and micrographs (right) of a hydrophobin-mediated cellulose–CNT nanofibril composite. Adapted with permission.<sup>[101]</sup> Copyright 2018, Elsevier.

Silk consists of large regions of hydrophobic amino acids, and has attracted interest in the field of biomaterials due to its remarkable mechanical properties and biocompatibility.<sup>[102,103]</sup> Composites have been produced by electrospinning a mixture of natural silk from a silk moth and SWCNTs in the presence of formic acid, yielding structures with SWCNTs aligned along the fibre axis (**Figure 5**b).<sup>[99]</sup> The stiffness of the resulting nanosized silk–CNT fibres containing 1 wt% SWCNT was 1.1–4.6 times higher than that of the native silk fibre.

Methoxypolyethylene-glycol-grafted SWCNTs promote the growth of 3D crystals, and provide an advantageous approach for preparing thaumatin and lysozyme crystals for X-ray crystallography.<sup>[104]</sup> The post-crystallisation cross-linking was shown to increase the mechanical and chemical stability of the protein crystals, making them more accessible for manipulation and improving their applicability. In particular, cross-linked lysozyme crystals (CLLCs) were prepared by encapsulating several metal and semiconducting NPs, with potential applications in catalysis, optical devices, and electron conduction.<sup>[105,106]</sup> A method was developed to incorporate a peptide–SWCNT hydrogel within CLLCs to take advantage of the strength and electronic conductivity of the SWCNTs at the macroscale.<sup>[100]</sup> First, SWCNTs were dispersed into a peptide hydrogel (Fmoc-diphenylalanine) by a pH switch protocol.<sup>[107]</sup> Second, lysozyme protein was allowed to diffuse into the hydrogels, followed by a buffered NaCl solution to induce lysozyme crystallisation. Macroscopic crystals were observed, which

showed a darker colour with increasing SWCNT concentration (**Figure 5**c). The Young's modulus of the CLLC–SWCNT composites (measured by AFM) gradually increased at higher SWCNT concentration, suggesting that the SWCNTs interact with the protein and not only the peptide. Additionally, lysozyme maintained its enzymatic activity, resulting in enhanced performance at 90 °C. Finally, the SWCNTs provided the CLLC composite with an exponential increase in electron conductivity.

Hydrophobin is a globular amphiphilic protein containing a hydrophobic section that can interact with SWCNTs.<sup>[108]</sup> A glycosylated hydrophobin was used to solubilise SWCNTs and disperse them in a nanofibrillated cellulose (CNF) matrix, providing electronic conductivity.<sup>[101]</sup> А 5-fold increase in conductivity was observed in nanocellulose/hydrophobin-SWCNT hybrids compared to nanocellulose-SWCNT composites. The nanocellulose/hydrophobin-SWCNT showed higher ductility and lower stiffness than the native nanocellulose materials. This modification produces a plasticising effect via noncovalent functionalisation; this effect is enhanced at elevated humidity due to the increased lubrication provided by the protein layer at the CNF-SWCNT interface.

#### 3.4. Carbohydrate-based biohybrids

Carbohydrates can be classified according to the complexity of their structure into monosaccharides, oligosaccharides (*e.g.* cyclodextrins (CDs), and polysaccharides (*e.g.*  $\beta$ -glycan, cellulosic derivatives, dextran, starch, and chitosan). The first two groups are molecularly well defined, making their lack of microscale structure unsuitable to direct the SWCNT arrays. In contrast, CD-containing polymers and polysaccharides can direct SWCNT self-assembly, even though their molecular weight and structure differ greatly.

#### 3.4.1. Oligosaccharides – cyclodextrins

One of the most interesting types of oligosaccharides used in materials science is CDs, which contain a toroidal oligomer with glucose units linked by  $\alpha$ -1,4 glycosidic bonds.<sup>[109–111]</sup> Typical 18

CDs contain six, seven, or eight glucose units ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -CD, respectively). The toroid has a hydrophobic inner cavity and a hydrophilic outer surface, making it an extraordinary host for supramolecular chemistry. The combination of SWCNTs and  $\beta$ -CD was recently investigated with respect to applications ranging from wastewater treatment to electrochemical detection.<sup>[112–114]</sup>

The selective adsorption of organic pollutants was demonstrated by covalently linking a linear O-carboxymethyl- $\beta$ -CD polymer (poly- $\beta$ -CD) with amino-functionalised SWCNTs *via* an amide bond.<sup>[113]</sup> The composite shows a fibrous structure with aligned SWCNT patterns, as determined by AFM and TEM (**Figure 6**a). This combination resulted in a 6-fold increase in specific surface area compared to aminated–SWCNTs, along with enhanced regeneration sorption and desorption for organic pollutants such as estradiol, alizarin red, toluene, benzene, phenol, nitrobenzene, aniline, rhodamine, and nitrophenol. Additionally, aminated–SWCNTs selectively adsorb metal ions including Hg<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup>. The modified SWCNTs show a considerable range of selectivity for organic pollutants and metal ions. The resulting supramolecular carbon nanofibers were efficiently recovered, regenerated, and reused at least seven times without any significant loss of removal efficiency, which demonstrates their potential for use as adsorbents for water treatments.



**Figure 6.** a) Schematic of the host-guest chemistry and structure of the poly-β-CD–SWCNT composite. Adapted with permission.<sup>[113]</sup> Copyright 2019, Elsevier b) Schematic and corresponding HR-TEM micrograph of boronic acid–SWCNT composite showing the SWCNT alignment. Adapted with permission.<sup>[115]</sup> Copyright 2008, Royal Society of Chemistry c) Schematic and corresponding HR-TEM micrograph electrostatically schizophyllan (SPG)–SWCNT cross-linked composite showing the SWCNT alignment. Adapted with permission.<sup>[116]</sup> Copyright 2008, John Wiley and Sons d) Photograph of an iridescent CNC–SWCNT composite (top) resulting from the deposition of the biohybrid nematic phase (bottom). Adapted with permission.<sup>[117]</sup> Copyright 2017, American Chemical Society.

#### 3.4.2. Polysaccharides

Linear polysaccharides such as curdlan (Cur) and schizophyllan (SPG) were investigated to solubilise hydrophobic moieties such as SWCNTs.<sup>[118]</sup> These glycans consist of a repetitive linear chain of  $\beta$ -1,3-glucose (Cur), where every third glucose links to one  $\beta$ -1,6-glucose sidechain (SPG). Such  $\beta$ -glucans have a triple helix structure in biological media, with a strongly hydrophobic cavity inside, similar to those of CDs. Denaturalisation of Cur and SPG into its constituent strands is achieved by solubilisation in DMSO, and re-naturalisation in aqueous media in the presence of SWCNTs produces the aforementioned hybrids.<sup>[119,120]</sup> Characterisation of the composites by microscopy techniques such as TEM, AFM, and SEM showed helical wrapping of both cut and as-grown SWCNTs.<sup>[119]</sup> SPG–SWCNT conjugates were assembled in tightly packed arrays through covalent cross-linking of the SPG  $\beta$ -1,6-glucose sidechain with boric or boronic acid derivatives at a pH of 9.3.<sup>[117]</sup> The covalent approach was validated using several boric/boronic-acid-containing moieties, such as boric acid, phenyl-1,4-diboronic acid, and 5,10,15,20-tetrakis(4-boronylphenyl)porphyrin. In all cases, the composites have sheet-like structures with periodical stripes.<sup>[115]</sup> High-resolution TEM (HR-20

TEM) images showed that the distance between SWCNTs was proportional to the length of the boric/boronic acid linker (**Figure 6**b). Additionally, the porphyrin derivative showed optical activity in circular dichroism spectroscopy, suggesting that the dye was incorporated into the helical structure of the hybrid.

The presence of Cur was used as a platform to endow chemical variability to the composite without disturbing the SWCNT properties. For instance, the reaction of Cur with propargyl bromide provides alkyne moieties suitable for "click chemistry", and was used to decorate the hybrid either with trimethylammonium or sulfonate groups, producing cationic (Cur-N<sup>+</sup>) or anionic (Cur-SO<sub>3</sub><sup>-</sup>) derivatives, respectively.<sup>[116,121]</sup> In fact, the combination of oppositely charged hybrids resulted in densely packed hierarchical self-assembled composites, interacting through electrostatic interaction.<sup>[116]</sup> Well-ordered Cur–SWCNT sheets were observed that consisted of parallel aligned hybrids with a periodicity of *ca*. 2 nm, consistent with the height of the hybrid observed by AFM. Additionally, periodic patterns with a spacing of *ca*. 10 nm were observed, due to the moiré pattern created by the overlap of a few sheets (**Figure 6**c). In a similar approach, negatively charged tetraphenylporphyrin sulfate (H<sub>2</sub>TPPS<sup>2-</sup>) was used to bind Cur-N<sup>+</sup>–SWCNT, which, under acidic conditions, triggers self-assembly due to the H<sub>2</sub>TPPS<sup>2-</sup> columnar aggregation.<sup>[121]</sup> The helicity of Cur-N<sup>+</sup> is transferred to the dye, as observed in the circular dichroism spectra. Increasing the pH to 6.9 resulted in the disassembly of the composite.

Cellulosic materials such as cellulose nanocrystals (CNCs) have attracted considerable attention in the last decades for a wide range of applications.<sup>[122]</sup> Rod-like CNCs obtained from hydrolysis of cellulose have a high aspect ratio, excellent mechanical properties, colloidal stability, and an organised structure. The latter was used to direct the assembly of unidimensional molecules such as SWCNTs into chiral nematic liquid crystals (**Figure 6**d).<sup>[117]</sup>

First, SWCNTs were oxidised in acidic media to provide hydrophilic functional groups compatible with the saccharide structure of the CNCs, and then subsequently dispersed in 3 wt% CNCs under mild sonication. The combination yielded isotropic dispersions with long-term stability and no birefringence when observed using cross-polarised light microscopy. However, upon solvent evaporation, spherulite-like textures appeared, indicating the formation of the CNC-directed lyotropic phase (**Figure 6**d). As a consequence of the aligned nanostructure observed in the films, which was thoroughly characterised by polarised optical and electron microscopy, anisotropic conductivity was observed by electrical resistance measurements, indicating the alignment of the SWCNTs along the CNC matrix. In a similar study, fibre alignment was achieved by controlled dip coating,<sup>[123]</sup> as well as ambient dry and shear blade deposition techniques, where additional aqueous stability was attributed to hydrophobic interactions between the CNC (200) surface and the aromatic SWCNT.<sup>[124]</sup>

#### 3.5. Lipids and other surfactants

Lipids are amphiphilic molecules including fats and oils that consist of a hydrophilic head and a hydrophobic hydrocarbon tail. In the presence of water, lipids form micelles and other selfassembled structures, where the hydrophilic head faces outwards and the hydrophobic tails are hidden inside to avoid contact with the water.<sup>[125]</sup> Electrostatic interactions drive the recognition between cationic liposome complexes and anionic SWCNTs, which produce two types of centred rectangular columnar phases: an intercalated lamellar structure with a centred rectangular columnar superlattice of SWCNTs, and an inverted centred rectangular columnar packing with SWCNTs at the column centres.<sup>[126]</sup> Anionic SWCNTs were individually isolated in water by radical polymerisation of cetyltrimethylammonium 4-vinylbenzoate in the presence of sodium styrenesulfonate. In contrast, cationic liposome complexes with finely tuned spontaneous curvature and surface charge density were prepared by combination of zero spontaneous negative lipids curvature and spontaneous curvature 22

(dioleoyltrimethylammoniumpropane (DOTAP) and dioleoylphosphatidylethanolamine (DOPE), respectively), which were consecutively extruded through a 200-nm filter. Combination of the anionic SWCNTs and the aforementioned cationic liposomes formed aggregates near the isoelectric point, as confirmed by dynamic light scattering and zeta potential measurements. Small-angle X-ray scattering (SAXS) diffraction patterns measured at different DOTAP/DOPE mass ratios of 0.33, 0.5, and 0.67, showed a multilamellar structure with repeat distances of ~10 nm. For the ratio of 0.75, a topological phase transition was observed and consolidated at the mass ratio of 0.80 (**Figure 7**a). At these ratios, the spontaneous curvature of the lipid monolayer becomes more negative, resulting in an inverted centred rectangular columnar packing. This morphology can only be explained by the demixing of the lipid mixture, creating anisotropic pockets surrounding the SWCNT.

In a similar manner, polymeric nanotubes (pSWCNTs) were prepared by dispersion in aqueous cetyltrimethylammonium 4-vinylbenzoate and subsequent free-radical polymerisation. The individually dispersed pSWCNTs were combined with cylindrical micelles of penta(ethylene glycol) monododecyl ether ( $C_{12}E_5$ ) at different ratios in deuterated water.<sup>[127]</sup> The crystallinity of the mixture was evaluated by temperature-dependent SAXS. A 45:55 mixture of  $C_{12}E_5/D_2O$  produced a structure with hexagonal packing at 10 °C (6.07 nm lattice parameter), evolving to a lamellar phase (6.27 nm lamellar spacing and 3.23 nm water gap) at higher temperatures. Upon addition of the pSWCNT, phase transitions occurred at higher temperatures as a result of an increase in the stability of the array. At a pSWCNT/ $C_{12}E_5/D_2O$  ratio of 15:45:55, new peaks were observed in the hexagonal phase, suggesting a hierarchical hexagonal structure with two coupled hexagonal arrays (**Figure 7**b). This hypothesis was further confirmed by contrast-matched small-angle neutron scattering and SAXS measurements under an oscillatory shear field.



**Figure 7.** a) Crystalline phases of liposome–SWCNT composites, depending on the DOTAP/DOPE lipid ratio. Adapted with permission.<sup>[126]</sup> Copyright 2012, Royal Society of Chemistry b) SAXS diffraction pattern of intercalated hexagonal binary arrays of SWCNT and cylindrical micelles. Adapted with permission.<sup>[127]</sup> Copyright 2014, John Wiley and Sons.

#### 4. Applications

Highly ordered SWCNT biohybrids are an ideal platform for technical and biomedical applications, due to their unique biocompatibility, and electrical and mechanical properties. The following section reviews some well-studied biocomposites for various applications.

#### 4.1. Tissue engineering

Tissue engineering is a medical technology that aims to improve or replace biological tissues by combining cells, signalling, and scaffolds.<sup>[128,129]</sup> The latter is necessary to support and direct the cellular proliferation, and therefore biocompatible and highly directional composites are of paramount importance.<sup>[130–132]</sup> The incorporation of SWCNTs into such scaffolds provides enhanced mechanical properties (*e.g.* stiffness and flexibility) and functionalities such as electronic conductivity, which are highly desirable in cardiac and neural tissue.<sup>[133]</sup> Furthermore, a key feature of the bundle-like structures obtained by the self-assembly of the CNT biohybrids, is that they can provide a tailorable platform to direct cell growth.

Pelvic organ prolapse and other medical conditions associated with collagen disorder can possibly be treated using silk-SWCNT fibres.<sup>[98]</sup> This common disease prevents the collagen fibre network from forming coordinated interactions, which weakens the connective tissues. Well-aligned SWCNT-based composites from spider dragline silk protein were produced via electrospinning. Such biohybrids helped the patients to retain cell function and achieve tissue repair (Figure 8a).<sup>[98]</sup> The addition of only 0.05 wt% SWCNT with silk protein yielded a 48fold increase in conductivity (Figure 8b) compared to the previously described collagen-SWCNT materials (Section 3.3). In addition, silk-SWCNT fibre is stronger and more elastic than silk-SWCNT fibre. The superior features of the silk-SWCNT fibres are most likely due to hierarchical β-sheet structures, which improve the silk–SWCNT interactions and SWCNT alignment. The silk-SWCNT fibres act as a conducting scaffold for fibroblast growth, stimulated by the electric field along the fibre direction (Figure 8b). This was confirmed by a 20-fold increase in collagen production by fibroblasts. The cell viability of silk-SWCNT fibres remained above 90% after 24 h and prolonged times produced better cell polarisation/elongation compared to collagen, due to the superior alignment and mechanical properties (Figure 8c).



**Figure 8**. a) Silk–SWCNT fibre morphology. b) Fibroblast cell growth aligned along the composite fibre direction. Adapted with permission.<sup>[98]</sup> Copyright 2018, Elsevier c) BetaVhex self-assembly in the absence and presence of SWCNTs (top) and HR-TEM images with overlapped helical wrapped model (bottom) d) Neural *in vivo* signal intensity upon seizure in the presence of betaVhex (grey) and betaVhex–SWCNT composite (red). Inset: schematic cortical circuit. Adapted with permission.<sup>[134]</sup> Copyright 2020, American Chemical Society e) Results of an *in vitro* fibroblast gel contraction test, showing the stiffness of collagen–SWCNTs composites (inset) and its suitability for bone regeneration. Adapted under the terms of the Attribution 4.0 license.<sup>[137]</sup> Copyright 2009, Springer Nature f) SEM image of an aligned polycaprolactone-silk fibroin–SWCNT fibre (top) and 3D confocal microscope model of interconnected neurones. Adapted with permission.<sup>[136]</sup> Copyright 2019, Elsevier.

A challenge in neural interfacing technology is to mimic the viscoelastic behaviour of brain tissue with long-term stability.<sup>[137,138]</sup> To address this, a biocompatible hydrogel was prepared

by supramolecular conjugation of a non-biodegradable  $\beta$ -peptide (betaVhex) and pristine semiconducting SWCNTs.<sup>[134]</sup> TEM images showed that the betaVhex nanofibers, with viscoelasticity similar to brain tissue, helically wrapped the SWCNTs into helical bundles (**Figure 8c**). The betaVhex–SWCNTs hydrogel increased the contact area with the cerebral tissue compared to conventional metal electrodes, thus improving the tissue-hydrogel coupling. This resulted in changes in the amplitude and frequency of the signal recorded *via* an extracellular field (**Figure 8**d). The improved biomimicking conditions maintained the cerebral microvasculature and neural recording was sustained over six weeks. The applied hydrogel showed little degradation over three weeks and no microglial cells (*i.e.* symptom of systemic response to injury) were observed over a 12-week period, confirming its advantage over conventional metal electrodes.

One goal in cardiac tissue engineering is to take advantage of the mechanical properties of the extracellular matrix of the native myocardium to endure the contractions while responding to electric impulses.<sup>[139]</sup> This is of great importance in the regenerative therapy of damage caused by myocardial infarction. Gelatin–SWCNT hydrogels showed promising results due to the inherent toughness, electrical conductivity, and aligned cell growth.<sup>[140]</sup> The incorporation of SWCNTs into the microporous structure of gelatin resulted in a tubular structure and uniform dispersion in the hydrogel. The viability of murine neonatal ventricular cardiac cells remained stable at around 80% after a 3D culture for SWCNT contents up to 2 mg mL<sup>-1</sup>. Additionally, incorporation of SWCNTs into the gelatin–SWCNT hydrogel provided structural integration of the tissue scaffold, evidenced by the migration of both transplanted cells and SWCNTs into the infarcted myocardium, along with infiltration of host cells and macrophages into the hydrogel.<sup>[140]</sup>

infarction. Similar results with a collagen–CNT matrix showed the capability to align cells on the scaffold, which also has major implications for cell communication, cell viability, and hence their ability to regenerate tissue.<sup>[141]</sup> In a similar fashion, electrospun silk fibroin-MWCNTs fibers direct the alignment of cardiomyocytes. The aligned cardiac tissues showed enhanced functionalities, including the expression of cardiac specific proteins, and the formation of sarcomeres and gap junctions.<sup>[142]</sup>

Collagen-CNT composites similar to those described in Section 3.3 showed potential as scaffolds for bone-tissue regeneration.<sup>[135]</sup> The composites are bioresorbable (*i.e.* naturally dissolving materials), biodegradable, and mechanically stiff, while simultaneously showing tuneable stability and swelling in aqueous solution by varying the SWCNT content. This was confirmed by conducting gel contraction studies, revealing that the biocomposites with mouse fibroblast cells presented structural stability in the presence of SWCNTs, thereby indicating that the scaffold would maintain its original size and 3D structure in vivo (Figure 8e). Additionally, it was observed that under physiological conditions, the collagen-SWCNT scaffold activates the *in vitro* mineralisation of hydroxyapatite crystals, which accounts for 65-75 wt% of human bone and is of paramount importance in bone regeneration and engineering.<sup>[143]</sup> Induction of SWCNTs into rat-derived collagen improved the stress resistance by a factor of three. Overall, such properties can be exploited by integrating a bone craft implant into the native bone, which activates the growth of new bone tissue. Furthermore, composites consisting of hydroxyapatite-MWCNTs can be aligned via agarose electrophoresis, and subsequently covered by collagen. The composites influenced the differentiation of rat bone mesenchymal stem cells into osteoblasts in vitro and in vivo, showing a potential acceleration of bone repair.<sup>[144]</sup>

Three-component composites were also developed, containing polycaprolactone and silkfibroin–SWCNT, which are suitable materials for neural tissue regeneration (**Figure 8**f).<sup>[136]</sup> The composite, organised into fibres by shear flow, mimic the hierarchically aligned 3D structure of native nerve tissue, which is crucial for neuronal alignment and elongation. In a similar approach, agarose–SWCNT hydrogels were shown to mimic the mechanical properties of brain tissues, but were stiff and hard upon drying.<sup>[145]</sup> In addition, the scaffolds were biocompatible, biologically modifiable, and non-toxic. These scaffold properties, along with their gel formation and electrical conductance, offer promising applications in nerve guidance conduits and directed nerve repair.

#### 4.2. Sensors and wearable electronics

In addition to tissue engineering, SWCNT-based biohybrid materials are applied in the fields of sensors and wearable electronics.<sup>[146]</sup> Reducing the size of the electronic circuits allows faster and more sophisticated electronic applications. For this purpose, SWCNT-based biohybrid materials are ideal due to their molecular scale and excellent electrical properties, together with their inherent biocompatibility. SWCNT-based biohybrids enable sensor applications for healthcare monitoring, including measurements of the body temperature and electrical activity of the heart.<sup>[146]</sup>

Stimuli-responsive biohybrids, such as the pH-responsive DNA-SWCNTs,<sup>[147]</sup> can be applied as versatile sensor-actuator smart materials to fabricate artificial muscles (Figure 9a-b). In this work, dsDNA-SWCNT biohybrids were covalently cross-linked through a pH-responsive ssDNA strand, which decreased the SWCNT-SWCNT distance at pH 5, thus controlling the porosity of the material. Consequently, the electrochemical properties determined by cyclic voltammetry were shown to be pH dependant. Fibres fabricated by wet spinning showed electronic conductivity and excellent actuation stability, with only ~0.15% expansion/contraction. The composite also showed pH-dependent performance as a H<sub>2</sub>O<sub>2</sub> 29

sensor, resulting in a simultaneous detection of pH and targeted peroxide (**Figure 9**c). Biosensors based on gelatin-MWCNT fibers coated with BSA have been shown to achieve lower detection limits of  $H_2O_2$  than previously reported biohybrid sensors.<sup>[148]</sup>



**Figure 9**. a) Schematic representation of a dsDNA–CNT pH actuator, b) TEM micrograph and c) pH-dependent H<sub>2</sub>O<sub>2</sub> sensing response. Adapted with permission.<sup>[147]</sup> Copyright 2018, American Chemical Society d) Schematic diagram, e) SEM image of the microstructure, and f) electromagnetic shielding effect of a fabric composed by a synthetic polymer and a CD– SWCNT conjugate. Adapted with permission.<sup>[149]</sup> Copyright 2020, Elsevier.

Most wearable electronics require fibres that are conductive, light, and sufficiently elastic to bend and allow the fibre to be woven. Such properties were achieved by a 3D-printed nanofibrillated cellulose (NFC) composite with aligned NFCs and SWCNTs along the direction of the microfibre.<sup>[150]</sup> The 3D-printing method enables the pattern of the fibres to be controlled. This dual alignment of both SWCNTs and NFCs resulted in increased mechanical strength/toughness (247 MPa) and electrical conductivity (216.7 S cm<sup>-1</sup>, respectively). The orientation at the microscale of the dual microfibre was confirmed using SAXS. Such 3D-

printed conducting fibres have potential applications in supercapacitors, embedded circuits, embedded electronic sensors, and 3D-printed batteries.

Overexposure to electromagnetic radiation, specifically ionising radiation, is harmful to the human body,<sup>[151–153]</sup> and also causes malfunction in microdevices.<sup>[154]</sup> It was recently demonstrated that using carbon nanomaterials as fillers for manufacturing hybrid fabrics improves the electromagnetic interference (EMI) shielding activity.<sup>[155–158]</sup> Synthetic 2-hydroxyethyl methacrylate fabric with appended adamantane was doped *via* host-guest interaction with  $\beta$ -CD–SWCNT derivatives (**Figure 9**d).<sup>[149]</sup> The resulting fabric was produced using magnetic-field-assisted electrospinning (**Figure 9**e), which cross-stacked aligned fabric layers and formed an aligned unilateral orientation of SWCNT stacks with excellent EMI shielding behaviour at a very-low SWCNT loading (0.17 wt%, **Figure 9**f). The non-covalent interactions between the SWCNT and the fabric increased the fabric adherence, dispersion of SWCNTs, and provided a self-healing ability under a 100% relative humidity environment.

Diffusion of lipids within aqueous bundles of SWCNTs results in a liposome-like fibrous material with the ability to host other biomolecules such as enzymes, which can be used as solid-state reusable sensors.<sup>[159]</sup> In particular, glucose-1-oxidase has been immobilised within a permeable lecithin–SWCNT composite, and the structure was thoroughly characterised by TEM, HR-TEM, and Raman and fluorescence spectroscopy. In this study, soft lipid immobilisation was achieved in the absence of harsh sonication by simple diffusion of the lipid into the SWCNT bundle, followed by sonication in the presence of the desired biomolecule. The reported sensor detected glucose concentrations between 0.1 and 2.5 mg mL<sup>-1</sup>, and the activity of the enzymatic composite after the second and third reuse was 3- and 2-fold higher than that of the corresponding supernatant (**Figure 9**d). This strategy was further validated by

decoration with alkaline phosphatase and citrate synthase, opening a new direction in soft enzymatic immobilisation.

#### 5. Conclusions

Although CNTs were discovered more than three decades ago, their mechanical properties and electric conductivity are still unmatched, making the development of CNT biocomposites a highly promising field. However, their low hydrophilicity and poor biocompatibility limits their application in the biomedical field. The biohybrids reviewed here highlight the enhanced colloidal stability and biocompatibility of this approach, enabling their use in biomedical applications. Additionally, the rich chemistry available at the solvent interface opens a path for SWCNT self-assembly into higher-order structures, which enables property transfer from the nanoscale to the micro- and macroscale.

However, the CNT-based biohybrids have several development areas that have been consistently hindering the true potential of the field. The intrinsic heterogeneity of the SWCNT scaffold represent the main bottleneck for some of the most promising applications: even if there are single-chirality enriched fractions commercially available, their purity and scalability is far from optimal. Only selected bioconjugation strategies have contributed to mitigate this issue (e.g. section 3.1.2), and it remains a major challenge to be answered by the preparation methods for general SWCNT-based materials. Besides, specific challenges that belong to the biohybrid field amplify the intrinsic SWCNT heterogeneity, instead of focusing on the systematic study and protocol development.

First, choice of the employed chemistry greatly affects the SWCNT properties thus its suitability for the targeted application. While oxidised SWCNTs are water soluble and suitable for mechanical reinforcement or scaffold, the degree of oxidation is challenging to assess and difficult to reproduce between batches. Additionally, covalent modification alters the electron 32

conductivity, rendering non-covalent modification the desirable strategy for biohybrid electronics. Second, the selected dispersion (milling, tip or bath ultrasonication; use and nature of surfactant) and separation (sedimentation, centrifugation or filtration) technique will benefit from a thorough description, due to its effect on the bundle size and SWCNT average length. Third, the biohybrids have reasonably well-defined diameters, but present polydisperse lengths due to the strong dependence on all the aforementioned factors. This greatly impacts the properties of certain structure morphologies, such as networks.

This review is focused on the chemical interplay between the biomolecules and the SWCNT, highlighting the modularity of the bioconjugation approach. Hydrophobic SWCNTs are dispersed and organised by amphiphilic biomolecules, interacting mainly *via* van der Waals and  $\pi$ - $\pi$  interactions. In contrast, chemical SWCNT modifications induce hydrophilic groups, which can achieve covalent bonding and hydrogen bond recognition. Regarding hierarchical self-assembly, most of the reviewed composites consist of linear biopolymers such as DNA, proteins, and polysaccharides that tend to form elongated fibrous materials. Thus, the desired properties can be achieved by embedding low fractions of SWCNT within the biomaterial, without disrupting its structure. However, there are examples of biomolecules with limited self-organisation properties (certain peptides, CD, and lipids) that acquire or modify their long-range structural order in the presence of SWCNTs. In these cases, the SWCNT plays a key structural role, rather than doping the pre-existing structure.

In this review, we focus on the principles of the SWCNT–biohybrid design, aiming to promote the diversity of the research field and impact of the applications. Unravelling the forces governing the SWCNT-biohybrid formation and self-assembly, together with a thorough compilation of the existing synthesis strategies, creates a roadmap towards more efficient and scalable materials. Eventually, applications that require specific biomolecules will benefit from

the alignment with the synthetic strategies detailed in this review, to enable applications ranging from sensors to responsive materials. This knowledge is expected to promote research at the interdisciplinary boundary of biotechnology and materials science, and provide a foundation for further development in the field.

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#### **Biographies**

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Self-assembled and biocompatible carbon nanotube (CNT) composites are promising materials for a range of biomedical technologies, such as tissue engineering, sensors, and wearable electronics. This review analyses the biomolecule-CNT interactions governing the conjugation and assembly, providing a framework for the development of future materials.

Keyword: Bioconjugation

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Biomolecule-directed carbon nanotube self-assembly

