Vertically Aligned Carbon Nanotubes as Platform for Biomimetically Inspired Mechanical Sensing, Bioactive Surfaces, and Electrical Cell Interfacing

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Vertically aligned carbon nanotubes (VACNTs) are one dimensional carbon objects anchored atop of a solid substrate. They are geometrically fixed in contrast to their counterparts, randomly oriented carbon nanotubes (CNTs). In this progress report, the breadth in which these one dimensional, mechanically flexible, though robust and electrical conducting carbon nanostructures can be employed as functional material is shown and our research is put in perspective to work in the last five to ten years. The connection between the different areas touched in this report is the biomimetic-materials approach, which rely on the hairy morphology of VACNTs. These properties in connection with their electrical conductivity offer possibilities towards new functional features and applications of VACNTs. To appreciate the possibilities of biomimetic research with VACNTs, first their material characteristics are given to make the reader familiar with specific features of their synthesis, the peculiarities in arranging and controlling the morphology of CNTs in a vertical alignment as well as a current understanding of these properties on a microscopic basis. In doing so, similarities as well as differences, which offer new possibilities for biomimetic studies of VACNTs with respect to multiwalled randomly oriented CNTs, will become clear.

1. Introduction

Vertically aligned carbon nanotubes (VACNTs) are 1D carbon objects which are anchored atop of a solid substrate. They are therefore geometrically fixed in contrast to their counterparts, randomly oriented carbon nanotubes (CNTs). VACNTs constitute the main material of this progress report. It is intended to show the breadth in which these 1D, mechanically flexible, though robust and electrical conducting geometrically constrained carbon nanostructures, could be employed as functional material. The connection between the different areas touched in this report is the biomimetic materials approach and the

2. Synthesis and Growth of VACNTs

Carbon nanotubes are accessible by a wide variety of different synthetic approaches. They all have in common that they are typically high-temperature synthesis processes. For the growth of freestanding VACNTs, a catalytically driven thermal CVD process is the most often used approach because it allows the widest tunability of the resulting CNT material properties. It can either be designed in a substrate fixed or a free flowing catalyst approach.[1] In the former, a catalyst composed of a single element or a specific binary or multinary catalyst composition containing a dedicated combination of elements is deposited on a solid growth substrate. The catalyst particles are either embedded or tethered on the growth substrate, sometimes onto a buffer or underlayer which is first deposited on top of the base substrate. In that way, the active catalyst typically remains located on the substrate or buffer layer during the CVD growth process which leads to a root or bottom growth mechanism where the catalyst is stationary with respect to the substrate surface but not immobile with respect to further catalyst growth, for example, by Ostwald ripening. However, it is not incorporated in the as-grown CNTs and as such does not disappear from the growth
zone as it is typically observed in processes where the catalyst is present in the gas phase (floating catalyst process\textsuperscript{14,15}). Nevertheless, catalyst particles are still able to move under high-temperature CVD process conditions employed in the root growth process. This is substantiated by the fact that catalyst particles enlarge themselves under typical growth conditions but still remain active for a CNT regrowth process.\textsuperscript{2} On the other hand, on certain substrates the deposited catalyst particles are prone to subsurface diffusion into additional lower substrate layers which influences CNT growth in height and density. Examples for such additional layers which support subsurface catalyst diffusion are TiN, MgO, or ZrO\textsubscript{2}.\textsuperscript{3}

Reacting carbon-rich precursor fragments generated at high temperatures from typically small molecular precursors like ethene, ethine, ethyl alcohol, methane, propane, or butane do react with the catalyst particles and form the growing CNTs on the catalyst surface.\textsuperscript{4–10} The growth rate and thus the CNT yield does depend on the chosen feedstock and is increased when larger and fully saturated carbon precursors are used.\textsuperscript{11} In addition, hydrogen gas serves as a growth promoter in CNT growth. It is able to reduce eventually formed catalyst oxides and thus helps to reconstruct the catalyst surface.\textsuperscript{12} A seminal discovery in VACNT growth was the observation that water could enhance the CNT growth significantly.\textsuperscript{13} This has led to a breakthrough in the variability of VACNT growth with respect to density and length. Later, it could even be shown that other oxygen-containing compounds are also able to enhance CNT growth and with that the alignment of VACNTs.\textsuperscript{14} The main influence of such oxygen growth promoter is their ability to clean the active catalyst surface from as-deposited carbonaceous residues introduced by incomplete decomposition or side reactions of the precursors and removing them as CO gas during the ongoing high-temperature CNT growth process. This cleansing allows for long-time active catalyst particles compared to an oxygen-free growth process. This is essential for a homogenous and dense CNT growth. Depending on the substrate and the morphology of the catalyst layer or the catalyst particle composition, size and density of the catalyst can be controlled. The catalyst may be deposited directly as such or may be formed from the as-deposited thin film catalyst layer by thermal processing and dewetting. In both of these cases, the catalyst particles change in form and size before and while the CNT growth proceeds.\textsuperscript{15–18} This has an influence on the growth kinetics of the CNT population in such VACNT array structures. Recent studies have revealed that after very short induction times of catalyst formation, which are well below 5 s, CNT growth is either initiated or the formed catalyst particles remain inactive over the entire process.\textsuperscript{12} The properties of the as-grown CNTs, namely, their length, height, density, distance, and diameter as well as the number of tube walls are prone to process variations based on these parameters over a wide range.\textsuperscript{19–23} As verified by a number of independent studies every growth parameter has an important impact on the individual properties of the generated CNTs and as such on the resulting material properties of a VACNT ensemble as a whole.\textsuperscript{24} It should also be mentioned that as in every CVD process in which multiple precursor species have to be managed in a concerted way, the chosen experimental setup also needs special attention, for example, with respect to size of the reactor and its thermal management both with respect to growth conditions as well as of the gas composition, dwell time,\textsuperscript{25} and for example, sample positioning in the reactor just to name a few.\textsuperscript{26} Thus, the CVD growth process of VACNTs is certainly best described as a complex situation in which all of these parameters have to be fine-tuned individually against each other to obtain a certain set of defined CNT material characteristics achievable in a most reproducible way (Figure 1).

Once this is achieved such a tweaked growth process of VACNTs with an optimized and reproducible set of parameters leads to constant material properties of such VACNT entities. Recently, systematic studies toward an even better understanding of the subtle interplay of experimental conditions have been undertaken and sweet spot conditions for the growth of VACNTs have been outlined.\textsuperscript{27} In these studies, a defined set of conditions could be determined which allow for a reproducible way of formation of VACNTs with regard to CNT diameter, alignment, length, and high surface area.\textsuperscript{27} To summarize, the overall process of VACNT synthesis relies on a concert of interdependent physicochemical properties and technologically feasible reaction conditions. Both determine the formation of the VACNT material under defined morphological control. Figure 2 shows the growth process of VACNTs in a nutshell schematically.

Contrary to the catalyst-driven growth process a thermal catalyst-free synthesis process of VACNTs is represented by the template-assisted growth of CNTs within ordered pores of a hexagonal array of porous alumina.\textsuperscript{28,29} This method works without a catalyst metal and is based on the deposition and agglomeration of carbonaceous species generated from a hydrocarbon precursor at high temperatures directly within the pores of the alumina template. Diameter, length, and distance of the CNTs are depending on the template geometry. After dissolution of the template the VACNT array is obtained as freestanding 3D arrangement.\textsuperscript{29} Compared to the water-assisted CNT growth
This approach generates multiwalled CNTs with a high defect content due to the direct molding process of the inner pores of the template by the deposited carbon species. In contrast to VACNTs from the water-assisted process, the so-obtained CNTs possess only short-range-order graphitic structures. In the following two main sections, selected examples on the usage of VACNT structures in different areas of biomimetically inspired research will be presented. The main motivation of this approach is to show the wide range of application-oriented biomimetically inspired research which can be covered with the VACNT material. The individual sections are however thematically not directly connected to each other but reside next to each other. They obtain their motivation from different scientific and technology-driven issues connected with the unique properties of VACNTs outlined in the preceding sections.

### 3. Mechanical Properties of VACNTs

#### 3.1. Pressure Sensing with VACNTs

CNTs display extraordinary mechanical and electrical properties. They exhibit a high mechanical stability as well as a high stiffness compared to their individual weight per single tube. Vertically 3D aligned arrays of CNTs are mainly stabilized by intertube van der Waals forces between individual CNTs. The elastic modulus of VACNTs longitudinal to the tube axis is lower when compared to individual CNTs and depends significantly on the CNT length, the aspect ratio, and the individual structure of the CNT ensemble comprising the 3D array of the VACNTs (Figure 3). As can be seen a persistent bending of the CNTs occurs in the bottom region close to...
the substrate upon applying a vertical load and remains after extended loading cycles (Figure 3). Due to the good electrical conductivity of CNTs their general usage in electromechanical devices in which function is based on compressibility (the longitudinal mode) as well as lateral moduli variation (the transversal mode) are current fields of intense research efforts.[33–40]

Interestingly, most of the past and current efforts have centered toward an understanding of the longitudinal compressibility of VACNT structures. In contrast, far much less efforts have been devoted to experimental and theoretical stress/strain studies in transversal, lateral deformation studies of VACNTs (see, e.g., Figure 4).[44–55] The bending situation which occurs upon such a mechanical distortion of a VACNT array is shown schematically in Figure 5. Typically, VACNTs show a straight tube alignment in the middle and an entanglement of individual tubes in the top region of such an array (Figure 5). This is due to the CVD growth process where the straight alignment of the CNTs develops over time during the beginning of the initial growth period and leads to an entanglement on top of the aligned VACNT array after final growth termination.

The movement of the CNTs by an external stimulus can distort the VACNT arrangement in lateral fashion and results in an increase of the intertube distances and a decrease of entanglement of the CNTs in the upper top region of the VACNT arrangement. When the bending is further increased, the de-entanglement increases, leading to a diminished intercontact area of the CNTs (Figure 5). The overall electrical resistance $R_{\text{eff}}$ which can be measured by contacting the VACNT array is composed of two contributions, one from the individual tube resistance $R_{\text{CNT}}$ and a second one $R_{\text{junction}}$ which is due to a resistance contribution from contacting and entangled CNTs. $R_{\text{junction}}$ contains contributions due to a physical contact resistance between the tubes and the tunneling resistance $R_{\text{T}}$.[57]

When the VACNT film is under distortion or strain, the change in the electrical resistance is a result of the change in $R_{\text{tube}}$ and $R_{\text{junction}}$. $R_{\text{tube}}$ changes due to band gap variations, $R_{\text{junction}}$ changes with the intertube distances. Thus both, contact and tunneling resistance should change. This effect also depends on the CNT length and the distance.

With respect to the effect of a lateral mechanical displacement induced by an external stimulus, VACNTs have been grown in a wall-shape arrangement placing two VACNT walls with 200 $\mu$m length and 4 $\mu$m width in a distance of 10 $\mu$m opposed to each other.[32] Applying a voltage leads to a lateral bending of the VACNT walls, resulting in a modulus change between 1 and 10 MPa with a capacity change of over 20%. This exemplifies the capability of VACNT to act as an electromechanical varactor device.[32,58] Measurement techniques to extract the in-plane horizontal mechanical modulus of VACNT arrays however may rely on sophisticated experimental set ups.[58]

Won et al. have developed a microfabricated resonator setup using a laser Doppler vibrometer to measure differences in the resonance frequency of a cantilever beam on which the VACNTs are directly grown.[32] Results show a strong modulus dependence on VACNT film thickness. A specific situation for VACNTs is the dependence of the internal nanotube density and the vertical CNT alignment in such arrays. Mechanical stress leads to a certain bundling of the CNTs and to an

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**Figure 3.** Left: Schematics of the compressive behavior of VACNT in longitudinal direction to the tubes axis. Outer right: Compressive behavior of VACNT. SEM of buckled CNTs under compression. Middle: SEM of pristine freestanding CNT film, lower middle: SEM of same CNT film compressed after 1000 cycles.[32,37] SEM images: Reproduced with permission.[36] Copyright 2005, AAAS.

**Figure 4.** Left: Schematics of a pillar-like VACNT arrangement showing a bundle of straight VACNTs with the typical morphological structure shown alongside the axial tube orientation. From left to right: Schematic drawing of an aligned VACNT bundle; SEM of a cylinder of VACNTs; SEM of the top view of the same VACNT cylinder; magnification of the top part of the same VACNT cylinder structure showing the parallel alignment of the CNTs together with the entanglement and top view of the CNTs with stronger entanglement.[41–43]
enhancement of entangled CNT regions on the top layer (crust layer) of such VACNTs. Altogether these effects modify the in-plane electromechanical properties of VACNTs. Their contributions are different for in-plane ($E_1$) and out-of-plane ($E_2$) moduli (Figure 6).[32] Systematic studies on how the individual contributions of CNT alignment, CNT entanglement, diameter, and substrate anchoring modify the piezoelectric properties of VACNTs are currently missing.

Generally, the mechanical stiffness of VACNTs crucially depends on the individual tube–tube contacts within the 3D array. These depend on the quality of the CNT alignment, the interface CNT/substrate, the CNT length, and the CNT diameter. The latter is directly influenced by the catalyst quality (size, morphology, shape).[15] A control over this set of properties is most important to manage the vertical as well as lateral displacement processes in VACNT arrays.

Initially, we had investigated the electromechanical properties of flexible microstructured VACNT arrays, prepared by the catalyst-free template-based CVD method, under mechanical stress conditions. The CNTs are arranged in a 3D array and are synthesized in parallel, hexagonally arranged, cylindrical pores of alumina using propylene as precursor. Their length is homogeneous and is given by the thickness of the alumina template and is about 50 µm. The CNT diameter and CNT density within the array is $6 \times 10^6$ mm$^{-2}$. They merge on both sides into a top and a bottom carbon layer of a few nanometer thickness each (Figure 7).[28,29]

This ultrathin carbon layer is essential for the mechanical stability of the VACNT arrangement. It allows fabrication of a thin and flexible skin-like pressure sensor for tactile sensing properties. In this, the VACNT array shows a highly sensitive piezoresistive behavior with a resistance decrease of up to $\pm 35\%$ and a spatial resolution of $<1$ mm (Figure 8). Such VACNT structures can be utilized for tactile sensing components or as vibration gauge for the measurement of indirect forces (resistance increase of 30% at an input signal of 0.15 V).[41]

The electromechanical gauge effect will occur when VACNT arrays are deformed in a transversal or lateral fashion perpendicular to the longitudinal individual CNT axis. In the latter case, the empty (air) interstitial space between the VACNT can be further modified by intrusion of different polymers as filler materials. For the pure VACNT structures, the gauge factor depends on tube diameter, CNT length, and tube distance. The size of the VACNT 3D microstructure might play an additional significant role in influencing the piezoelectric properties. For a VACNT/polymer composite material, the gauge factor also depends

![Figure 5. Schematics of the transversal mechanical behavior of a VACNT bundle during an in-plane bending perpendicular to the tube axis.][55,56]

The same situation exists when the tubes are actuated by an external stimulus, for example, a gas flow on a flat substrate. The drawing is enlarged and the entanglement is not to scale with respect to the overall CNT length. Contributions from $R_{\text{junction}}$ and $R_{\text{CNT}}$ to $R_{\text{eff}}$ will be influenced differently under lateral, in-plane bending.

![Figure 6. Situation exemplifying schematically different force contributions to the modulus in CNT nanostructures, from randomly oriented CNTs to VACNTs (lower left to upper right and lower right to upper left). $E_1$ denotes in-plane and $E_2$ out-of-plane moduli of VACNTs. Adapted with permission.[32] Copyright 2012, Elsevier.][32]

![Figure 7. Left: Flexible VACNT array after alumina template removal. Right: SEM side view of the self-supporting VACNT array; scale bar is 20 µm. Reproduced with permission.[41] Copyright 2012, IOP Publishing.][41]
on the type of the introduced polymer, acting as an interstitial filler. Strain sensors based on CNT/polymer hybrids are already known as important electromechanical devices for measuring quantities such as vibration, stress, or pressure. Recent studies indicate that the degree of curvature of CNTs within such random CNT percolation networks in polymers may be responsible for their different electromechanical behavior showing the greatest sensor sensitivity at the percolation threshold. We are currently not aware of studies on the stress/strain behavior of VACNT/polymer materials, in which the polymer is the minor component and is used to modify the mechanoelectrical properties of the VACNT systems. Systematic investigations on such model composite materials could give information on:

1. Influence of the loss of contact among CNTs due to the polymer interface (insulating vs conducting)
2. Change in tunneling resistance in neighboring CNTs due to a polymeric (insulating vs conducting) interphase
3. Change on piezoresistivity of CNTs due to a modification of their mechanical deformation behavior introduced by a polymer interphase.

Own work has so far focused on the intrusion of polystyrene (PS) and polymethyl-methacrylate (PMMA) of defined molecular weights into dense VACNTs. Dip filling of PMMA has shown that different polymer loadings can be intruded in the nanosized interstices between individual CNTs (Figure 9). Future research will focus on an understanding of the influence of isolating and conducting polymers or mixtures of those on VACNTs taking into account different parameters such as CNT diameter, distance, CNT height, etc. 2D percolation theory allows describing their intrinsic effects on a technologically valid basis. So far, a CNT/polymer composite, although comprised of randomly arranged CNTs, has been developed for strain sensing. The axial nanotube slippage in bare CNT structures degrades its strain response, this could be improved by means of a stronger interfacial CNT/polymer binding. Piezoresistive pressure sensor elements based on VACNTs which have been attached to a flexible electrically insulating parylene polymer film as backbone have been reported. This has allowed the formation of a piezoresistive microelectromechanical systems (MEMS)-based gauge sensor with positive and negative gauge pressures. However, in such a device the sensor characteristics rely on the individual properties of both the polymeric parylene support film and the VACNTs attached to it. A related MEMS setup was developed and its performance as resistance-dependent temperature and pressure sensor studied. Its bending deformation was studied on a free-hanging VACNT array which was again supported on a thin parylene film causing a resistance change due to the changing intertube distance in lateral direction of the aligned CNTs.

### 3.2. Flow Sensing with VACNTs

Flow sensing with artificial 1D objects mimicking hairy structures as they are found in nature has been an area of intense research over the last ten years. The most robust systems for this

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**Figure 8.** Left: Nanopressure sensor array contacted with a Frey filament which is mounted at a calibration robot demonstrator; middle: top view of the calibration system and schematic model of the wiring of the individual CNT pressure sensor elements, numbering 1: CNT contacts; 2: VACNTs; 3: top wiring; 4: bottom wiring; right: relative resistance change of the sensor element versus position of the Frey filament with respect to the VACNT sensor surface. Reproduced with permission. Copyright 2012, IOP Publishing.

**Figure 9.** SEM of VACNT/PMMA hybrid materials with different PMMA infiltration levels: a) bare VACNTs; c) PMMA concentration 100% increased compared to (b); d) PMMA concentration 50% higher compared (b); e) TEM cross section of a VACNT/polystyrene (PS) composite showing the alternate order —CNT/PS/CNT/PS/CNT— in a VACNT/polymer hybrid. e) Reproduced with permission. Copyright 2013, IOP Publishing. a–d) Reproduced with permission. Copyright 2010, Royal Society of Chemistry.
purpose rely on piezoresistive and capacitive principles using a variety of different materials, which have been employed for that. The hairy-like materials used so far for sensing purposes are typically made out of polymer, metal/polymer composites, carbon, or even pure metals or alloys. Different sensor designs available most often have in common that they work on an indirect basis meaning that the hairy structures of the device do not act as the piezoelectric primary material for the effect. Often the cilia structures transfer, through their lateral movement, the force to a cantilever at the bottom, and this deformation-induced change of resistivity of the cantilever can then be measured. Due to the necessary and sometimes laborious fabrication a larger number of different process steps are necessary until the final sensor device is obtained. In our recent work, we have devised an all carbon flow sensor device which is based on an arrangement of three VACNT architectures of \( \mu \)m dimensions which are in close proximity to each other. This approach allows a minimum of individual process steps and results in a sensor which relies on a direct strain/resistivity relationship solely based on a single material, namely, carbon. In these structures, the CNT bundles display a topo-tactical grading with different heights (150–1700 \( \mu \)m) and width (100–400 \( \mu \)m). This is achieved by control of the CVD growth time and catalyst composition. Underneath the catalyst layer a nickel contact layer is deposited which allows for the lateral electrical contacting of the individual CNT blocks (Figure 10). Due to the different growth heights of the CNTs which can be realized, the contact area of the VACNT blocks upon movement of the central sensor element can be varied which allows for a delicate fine-tuning of the sensor properties. In a multitude of individual VACNT block arrangements structured on a single wafer, each individual three-array CNT sensor unit is electrically automatically decoupled from the next. A VACNT array shows a high deflection capability with an individual bending angle close to the maximum of 90°. Although the Young’s modulus of such a VACNT bundle depends on its length and is in the range between 200 and 1600 kPa it is strongly controlled by the extreme buckling effect close to the substrate. This buckling could be further controlled and optimized employing a strain/strength-related growth process reported earlier by Hart and co-workers. In that process, different catalyst/substrate layer interactions are employed to manipulate the growth direction and VACNT morphology. These layers influence the growth process of VACNTs by creating different strain gradients during the continued CNT growth. These then cause the CNTs to bend and guide their growth into curvy VACNT arrays. This is caused by modulation of the growth rate by controlled catalyst/substrate interactions.

The VACNT sensor is able to realize fast deflections in the kHz regime. With a bundle height of 700 \( \mu \)m and a deflection of 2 kHz its mechanical stability over hours can be realized (Figure 11). With shorter VACNT bundle lengths of about 100 \( \mu \)m, 10^9 deflections have already been measured.

4. Bacterial Interaction, Cell Cultivation, and Electrical Contacting Using VACNTs

Due to the ease of surface modification and their length scales relevant for biological systems, CNTs and VACNTs have created
interest in bio-related applications.[88–92] Compared to randomly oriented CNTs, VACNTs have a higher available surface area, show a higher packing density and an overall controllable microstructure. These properties are important for the primary attachment of bacteria and archea. These microorganisms bind through their adhesins or through cell surface components to host surfaces. In case of pathogenic bacteria, such a binding may lead to infections by biofilm formation and are challenging in combating due to limited defense methods. Primary, initial biofilm formation is mainly governed by physicochemical properties of the host surface and the individual bacterial motility. Thus, the surface-controlled properties of VACNTs represent an interesting study object to examine the effects of hydrophobicity and hydrophilicity at different lengths on biofilm formation at human pathogens under continuous flow conditions in designed flow chambers. These conditions are close to those in an environmental setting as in tubes or medical catheters.[93] We have found that VACNTs do indeed inhibit biofilm formation of Pseudomonas aeruginosa, Klebsiella oxytoca, and Staphylococcus epidermis. However, we could show that this is not due to enhanced reactive oxidative species (ROS) formation by CNTs or even a CNT penetration into cells and therefore a reduced bacterial adhesion (shown by live/dead cell analysis in flow cytometry analysis of established biofilms).[93] Metal residues originating from the catalyst particles used in the VACNT synthesis as a source of ROS-generating species can also likely be excluded since these particles are buried underneath the CNT surface layer.[94] Consequently, we could rule out toxic effects of VACNTs. This is in contrast to earlier work.[92] We have proposed that the antiadhesion effect of the bacteria toward the CNT surface is due to the different lengths of the VACNTs. The increased flexibility of long VACNT hairy structures may not provide a static surface for the docking of the bacterial adhesins and therefore affects effective biofilm formation by contact mechanics. Similar observations of VACNT structures which show a reduced dead cell rate, compared to dispersed free-floating CNTs in contact with incubated bacteria point along the same direction. In addition, multi-walled CNTs dispersed in a cell culture medium led to a reduction in cell viability against sensory neurons.[95] These observations are backed by studies which show that the size of CNTs appears to play a vital role in affecting plantonic bacteria.[96] Flow channels, for example, in sensor devices which are able to sense liquid flows rich in bacteria are an area of further interest. With respect to surface-functionalized VACNTs bacteria biofilm formation is increased compared to pristine ones. This is in line with the assumption that ROS formation would be suppressed by a CNT surface functionalization due to an increased quenching and scavenging of free radicals and the further suppression of ROS activity by such surfaces compared to unfunctionalized ones.[97] It should be mentioned that these and other studies were done with bacterial monocultures. Indeed, natural systems are more complex and typically display a more diverse microbial community. In addition, knowledge about life cycles and environmental impact might be points of further concern.[98]

Randomly oriented carbon nanotubes, either single wall or multiwall, are materials which have been in the focus for cell cultivation and electrical contacting of cells for more than 15 years. The first report stems from 2000 and later other areas like stem cell differentiation, single neuron stimulation, spinal cord and artificial ganglia cell stimulation, as well as retina ganglion cell studies followed. Research into these fields spans a wide area ranging from a basic understanding of CNT/cell interaction toward studies of CNT microelectrodes for chronic intracerebral recording and microstimulation.[99] It is obvious that the well-established properties of VACNTs like large surface area, high electrical conductivity, good mechanical properties, tunable surface functionality, as well as tunable CNT hydrophobicity/hydrophilicity, chemical inertness, and electrochemical stability, and last but not least biocompatibility toward neuron cells are of utmost interest in that area of research.

As already mentioned in the preceding section, the metal catalyst necessary for the VACNT growth is buried underneath the CNT array and tightly anchored and thus almost completely shielded and immobile with respect to a substrate/cell interaction. Therefore, adverse effects coming from the proliferation of the catalyst particles can be excluded. This is important to mention since in earlier reported work on toxicity of CNTs such negative effects could be traced back to metal catalyst contaminations originating from raw samples.[94,98a–d]

Based on the advantageous physicochemical properties of VACNTs, these as well as randomly oriented CNTs have been subject to intense research in the area of basic neuroscience as well as in studies toward neuro-prosthetic applications.[100] The morphology of the interface between cells and 3D metal/nanoelectrode has been the subject of vital research activities.[101] The roughness of the CNT surface has impact on the neuron-CNT adhesion as was suspected in earlier work.[101b,103] When neurons interact with the CNT surface they spread along these structures and eventually make contact to each other.[103] Despite success already obtained so far, factors which govern further utilization span from improper cell adhesion to functional device stability. The low impedance of CNTs compared to conventional electrode materials as gold, platinum, titanium nitride, or iridium oxide which are typically used and represent Faradaic electrodes make CNTs highly interesting. With their higher charge storage capacity and high polarizability, the ability to inject enough current density to the cells at low overpotentials in order to avoid Faradaic reactions, for example, electrolysis which may cause cell damage qualify CNTs and VACNTs as interesting alternatives to more conventional metallic or metal oxide electrode materials. For basic neuroscience studies, high-performance multisite recording of various cell types with multielectrode arrays (MEAs) are the choice (diameter 20–200 μm). Other architectures for recording and stimulation have been developed and reviewed.[105] MEAs consist of an array of electrically conducting microelectrodes which can be used for recording and stimulation of cells. In the past decade, extracellular recording and stimulation with MEAs has developed into a standard technique for studying cell cultures and in the field of drug research.[106]

We could show that randomly grown CNTs on a conventional MEA substrate with planar gold electrodes resulted in a more than 200 times lower impedance than gold electrodes with the same diameter. The signal-to-noise ratio is also superior compared to conventional gold or TiN electrodes. Cardiac cells show a good adherence to these CNT islands. However, due to variations in the cell adhesion, the potential amplitudes...
showed some variation. In order to study influences and differences of the microstructure of CNT arrangements, we cultivated cortical rat neurons both on randomly oriented CNT islands as well as on VACNT structures with a length of about 400 µm. For the randomly oriented CNTs, neurons had accumulated after 21 d preferentially on the islands forming intimate cell clusters (Figure 12). In the case of an 6 × 5 array of 200 µm spaced VACNT pillars, after a 12 d incubation period 80% of the cells have moved from the interspace between the VACNTs onto individual VACNT pillar structures and settled on the side walls. They even seem to be able to climb onto the sidewalls of the CNT pillars however do not settle onto the tips (Figure 12, lower trace). Contrary to earlier reports, we have not observed any penetration of VACNTs into the active cell structure. As can be seen, engulfment of the cell agglomerate toward the VACNT pillar structure is obviously not perfect. However, for randomly oriented CNTs (Figure 12c) inclusion by the cells is more intimate than for VACNT structures with steep side walls. As observed earlier, neurons seem to entangle more efficiently with such rough CNT arrangements. In the case of the randomly oriented CNTs (Figure 12a), these were indeed curled and showed a high degree of entanglement compared to the VACNT side walls which are much smoother. It was speculated that the roughness of the CNTs in these randomly oriented structures are an important mechanical stimulus for cell processes and support their binding processes. Moreover, it could be shown that to some extent mechanical effects associated with the roughness of the CNT surface areas responsible for the development of the cell network exist.

Future studies need to address these issues for VACNT structures in more detail in order to gain insight how the engulfment process affects cell response and CNT/cell interaction/coupling. Attempts toward this end have also been undertaken with respect to the engulfment of cardiomyocyte HL-1 cells with mushroom-like and pillar-like gold electrode morphologies. It has been found that the size and shape of the electrode matters with respect to their interface to the electrogenic cells. This was explained by a different deformation of the cell membrane depending on the site of electrode engulfment.

It can be assumed that the VACNT arrays will not be deformed substantially in a way that the individual CNTs within a VACNT array cluster into aggregates upon cell adhesion on the VACNT array. Typical forces which cells would impart during the settling process are too low for that to occur. Considerable friction on the surface of VACNTs resulting in severe clustering of CNTs in the VACNT array is only observed when employing forces of 300 µN or larger. Since CNTs can be structured in various morphological shapes this allows for a directed, engineered growth of neuron cells (Figure 13). Neurons do indeed differentiate between various CNT structures as they are sensitive to spread differently along such growth patterns. Therefore, it can be envisaged that such 3D templates can be used to design cell patterns for neuron circuits in prosthetic applications.
Looking further toward the application of CNTs or VACNTs toward neural implants typically hard and rigid substrates like silicon on which the VACNTs are typically grown do certainly not show an in vivo compatibility due to their inherent rigidity. Their rigidity may cause damage to the surrounding tissue regions. Therefore, either biocompatible soft polymer matrices or a flexible soft polymer support or a ultrathin and therefore flexible and bendable silicon substrate on which the VACNTs are transferred or are directly grown, represent substrates of choice.\[111\] Probably, the intrusion of the void interspace of VACNTs with a suitable polymer, for example, polyethylene imine (PEI), or a substituted vinyl-pyridine, in a similar manner as has been shown for PMMA (see Figure 9) may lead to transferable VACNT/polymer composite for good cell adhesion.\[112\]

Certainly, further demands toward the VACNT/substrate interface arise. How can the CNT/substrate interface be tuned in order to allow for an utmost adherence of the VACNTs? How can the interfacial contacting be further modified (surface hydrophilicity vs hydrophobicity)? How can long-term stability under physiological conditions be achieved in order to avoid CNT leaching from VACNT electrodes? These and other unsolved questions currently pose some constraints on such flexible VACNT electrodes. Nevertheless, intense future research in that direction will certainly lead to solutions. Indeed, transfer of electrodes composed of randomly oriented CNTs initially grown on a hard substrate and then transferred onto a flexible parylene C film has been reported. Recording and stimulation of a crayfish nerve cord was also successfully demonstrated.\[113\] Crayfish nerve cell studies have been the subject of other investigations employing randomly oriented CNTs, this time by direct growth on a flexible polimide substrate.\[114\] Again by CNT transfer in which deliberately poorly attached randomly oriented CNTs were grown on a SiO$_2$ substrate followed by a transfer to a polydimethylsiloxane (PDMS) film resulted in an all-CNT electrode with 16 flexible single electrodes. These allow combining recording and stimulation of neuronal tissue. The obtained flexible electrodes were nearly purely capacitive.\[101\]

Challenging is the construction of an array composed of an even higher number of VACNT electrodes by a direct growth process. In each individual of the overall 60 VACNT electrodes, the CNTs are arranged and composed of up to $10^9$ double-walled CNTs of 5–8 nm overall diameter with a spacing of ≈15–20 nm and a length of about 2 μm (Figure 14). This electrode configuration showed dramatically decreased impedance caused by an enormous capacity increase compared to a similar arrangement of planar microelectrodes solely composed of polysilicon. DC capacities of 16 mF cm$^{-2}$ (normalized to the known surface

![Figure 13](https://www.advancedsciencenews.com/content/1700101/suppl/fig13.png)

**Figure 13.** Left: Neuron cell growth on a VACNT cross bar structure showing an oriented growth along the VACNT crossbar; Right: higher magnification showing the dense interconnection of the actin filament of a cell agglomerate on the CNT structure.

![Figure 14](https://www.advancedsciencenews.com/content/1700101/suppl/fig14.png)

**Figure 14.** SEM images of a contacted VACNT microelectrode array composed of 60 individual CNT electrodes. Visible circuit paths are covered with a 100 nm SiO$_2$ insulating layer. a) Complete microstructured electrode array with all 60 electrodes; b–d) Higher magnifications of (a). d) A contacted single VACNT electrode. Reproduced with permission.\[115\] Copyright 2015, AIP Publishing.
area) were observed which is more than two orders of magnitude higher than for planar polysilicon electrodes.\(^{[115]}\) The capacity increase can be assigned to a dramatically increased surface area compared to planar gold electrodes on standard MEA substrates. Based on the above dimensions the ratio of such a CNT electrode area in comparison to a planar one is\(^{[115]}\)

\[
A_{\text{ratio}} = 45.24 h \, \text{µm}^{-1} + 1
\]  

where \(h\) is the electrode (CNT) height. This shows that 3D VACNT microelectrodes offer a tremendous gain in electrochemically active surface area over planar ones. Such ultradense VACNT microelectrodes were fabricated in a direct growth process using photolithography employed in MEMS technology.\(^{[116]}\) The transfer of pregrown VACNTs from a growth substrate to eventually a prestructured soft electrode structure with only minor electrode degradation is possible, but the transfer itself is a delicate process.\(^{[117]}\) Nevertheless, especially neuronal cells are very sensitive to mechanical stiffness and rigidity especially on electrical contacts.\(^{[118–120]}\) Due to this fact, certainly further efforts are necessary to devise processes which allow for a better transfer of VACNTs to soft surfaces.

Alignment and structural integrity and uniformity of the VACNT electrode architecture is certainly superior compared to other electrode materials using nanosized material, however one major challenge toward implantable electrodes relates to the VACNT electrode housing to protect the VACNTs during handling and further organ implantation. It has been shown that nonembedded VACNT electrodes experience a strong compression upon implantation which cause them to lose a certain degree of their form factor. Insertion of a VACNT electrode into a rat brain leads to a compression by about 50% of its original height of 2.5 µm.\(^{[121]}\) This is in contrast to parylene-embedded a rat brain leads to a compression by about 50% of its original height.\(^{[121]}\) Furthermore, the direct growth approach is limited to substrates which allow for a thermal growth of VACNTs directly on the electrode material.

5. Conclusion

VACNTs represent a well-established carbon nanomaterial. Their synthesis is highly reproducible and the adjustment of a variety of structural parameters of these 1D carbon nanomaterials can be controlled over a broad parameter range. Their flexible mechanical properties depend on the length of the CNTs as well as their distance and diameter. These parameters can be controlled by choosing from a number of different catalyst and interlayer combinations whose subtle interplay during synthesis controls the properties of the resulting VACNTs as CNT length, diameter, height, and distance to each other. With respect to their mechanical properties, the combination of catalyst composition and growth substrate plays an important role in adjusting these different physical parameters. The response of VACNTs to external forces or stimuli like flow of liquids, gases, or the detection of sound thus relies on the combination and exact setting of these parameters. Here, the method of strain-engineered growth represents a valuable technique toward tuning and understanding the properties of VACNTs and generating complex 3D architectures by a bottom-up growth technique.\(^{[87]}\)

For areas such as microbiological or cell growth studies, the surface of VACNTs can be modified from (super)hydrophobic to (super)hydrophilic under mild conditions using gas-phase techniques like an rf plasma. Such gas-phase methods allow to keep the overall VACNT architecture as 3D object intact, whereas in contrast to these, solution-phase techniques for VACNT functionalization change their overall architecture and integrity due to the elastocapillary effect which may exert strong capillary forces on the individual CNTs.\(^{[122]}\) In this regard, it is also a current challenge to maintain the mechanical integrity of VACNTs as 3D structured material with a high aspect ratio under any functional operation where direct contact to the VACNT structures is a strong issue. This is especially important when VACNTs are employed as active electrodes in implantable neural microsystems. Here, the application of VACNT/polymer composites might offer a good opportunity to strengthen the mechanical properties of VACNTs in such applications further. With respect to microbiological interactions VACNTs hold great promise too. Their easy to control aspect ratio as well as the possibility to fix them as 3D ordered arrays with micrometer dimensions in a fluid environment together with their potential for biofilm prevention caused by human pathogens qualify them as interesting materials in such type of applications.

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Conflict of Interest

The author declares no conflict of interest.

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