Review

The Role of the Protein Corona in Fiber Structure-Activity Relationships

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Abstract: When nanomaterials enter biological fluids, they are immediately covered by biomolecules, particularly proteins, forming the so-called protein corona. The dynamic nature and complexity of the protein corona can impact upon the biological effects and distribution of nanomaterials with an organism. Therefore, the protein corona is an important factor in determining the biological impact of any nanomaterials. The protein adsorption pattern is determined by various factors, including the bio-fluids’ protein composition, the nanomaterials’ physicochemical properties, as well as the time and type of exposure. Predominantly, research has focused upon spherical nano-objects, however, due to their ever-increasing potential use within human based applications, and, therefore, heightening and inevitable exposure to the human body, little is known regarding how proteins interact with nanofibers. Therefore, the present review focuses on the current knowledge as to how the geometry of man-made (nano)fibers, carbon nanotubes (in comparison with asbestos fibers), affects their interaction with proteins within biological fluids. Summarizing state-of the art methodologies applied to dissect protein-binding signatures, it is further discussed whether the protein corona composition of fibrous and
non-fibrous materials differ, as well as what impact the protein corona has on (nano)fiber uptake, intracellular distribution and their subsequent toxicity.

**Keywords:** nanofibers; protein adsorption; carbon nanotubes; asbestos; toxicity; protein analysis

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1. **Introduction**

As soon as nanomaterials come into contact with biological fluids they are immediately covered by a large range of different biomolecules, such as proteins, peptides, and lipids. As this *bio-corona* is composed of proteins and peptides to a high extent, it is often referred as the *protein-corona*. The composition of the protein corona underlies certain dynamic fluctuations. Most importantly, it is widely accepted that the composition of the protein corona determines the biological identity of any (nano)material, and the resultant biological activity and distribution [1]. Various studies have been performed to identify the composition of the protein corona of a range of nanomaterials and to correlate the obtained protein adsorption pattern to the physicochemical properties of the materials, as reviewed by [2,3] and others. The aim of the investigation into the protein corona is to enable the correlation of the physicochemical parameters of a (nano)material type to the composition (quantitatively and qualitatively) of the protein corona, and to the resulting short- and long-term biological response. The goal is to obtain a biological fingerprint, or barcode of the individual (nano)materials. This, in turn, would enable to estimate or even predict the biological effects of the material, ranging from the level of toxicity to the efficiency as diagnostic tools or drug carriers. This is of importance for (nano)safety issues, as well as for pharmaceutical engineering.

Most studies on the composition of the protein corona have been performed on spherical (nano)particles. However, there are also several studies available that focused on the protein adsorption to fibrous materials. Due to their toxicological relevance toward humans, especially asbestos and carbon nanotubes (CNTs) have come under intense investigation.

Asbestos can be classified as a group of six silicate minerals: amosite, chrysotile, crocidolite, as well as actinolite, anthophyllite, and tremolite. Due to its high temperature and chemical resistance, in combination with its tensile strength, asbestos has been widely used within a large number of applications, e.g., as insulating material in building construction. Although, due to the severe health effects that have been shown to be caused by asbestos-related exposures (i.e., asbestosis, mesothelioma; it is defined as a human 1A carcinogen by the International Agency for Research on Cancer (IARC)/World Health Organization (WHO), the use of asbestos has undergone heightened regulations for its use, and furthermore banned from being used within numerous countries throughout the world.

Carbon nanotubes (CNTs), fibrous carbon allotropes, have been under intense investigation since their detailed description in 1991 [4]. Their diameter ranges from a few to several nanometers, to a length of up to several millimeters. The CNTs possess unique properties, such as tensile strength, high stiffness, temperature resistance, chemical resistance, and water resistance, amongst others. Due to these advantages these substances found various applications as reinforcement in polymers, in technical textiles, as sound absorbers, in cold-, heat- and fire protectors, and in medical applications.
Human Exposure to (Nano)fibers

Due to their physical properties some fibrous materials, such as asbestos fibers, have shown a severe impact towards human health, which also has been described in the literature as contributing to the fiber paradigm [5]. According to this, fibers that are thin enough or have an aerodynamic diameter small enough to enter the lung and which are too rigid and long to be taken up by phagocytes, have a high potential to damage the lung tissue, which can lead up to severe health effects based on persistence of the material within the lung.

For example, inhalation of asbestos fibers for prolonged periods has been clearly shown to be the specific cause of serious health effects, such as asbestosis and the development of malignant mesothelioma. Furthermore, erionite, a natural zeolite fiber, has been classified as a human carcinogen and is also reported to cause mesothelioma following long-term exposure [6,7].

In general, there are three main routes for fibers to enter the human body: by inhalation, by ingestion, or via penetration of the skin. The relevance of these portals of entry is dependent upon the kind of exposure scenario. Nonetheless, the human lung remains to be considered the primary portal of entry for any fibrous material. Entering the upper airways, fibers can be enveloped by mucus and cleared by mucociliary transportation, being coughed out or, alternatively they can be swallowed down into the stomach. It is assumed that most fibers with an aerodynamic diameter of a few micrometers are also able to reach the lower, non-ciliated airways (i.e., the alveolar region; responsible for gas exchange in the alveoli; Figure 1) The human lung consists of around 300 million alveoli, each with a diameter in the range of 200 µm. The air in the lumen of the alveoli has a close proximity (i.e., ~100 nm) from the bloodstream. The highly available surface for gaseous exchange at this air-blood tissue barrier is around 140 m² [8–10]. This surface is covered by the lung lining fluid or so-called surfactant, which is secreted by the lung epithelial cells. The lung lining fluid contributes to host defense, as well as regulates the surface tension at the air-blood barrier. Entering the alveoli, fibers and other foreign materials are coated with proteins (e.g., surfactant proteins), peptides, and lipids of the lung lining fluid. Although, the presence of over 40 different cell types, including important barrier cells types (epithelial and endothelial cells) [11], contribute to the normal homeostasis of this region following deposition of foreign materials, it is the interaction between important immune cells, specifically macrophages [12], and dendritic cells [13] that allows for the clearance of such materials. This clearance is, however, much slower (days to weeks) in comparison to the fast mucociliar clearance in the airways (minutes to hours). It is important to note, that although these lung defense mechanisms are highly active and efficient in removing foreign deposited materials, fibrous materials, due to their high aspect ratio, can have a different mode of action compared to non-fibrous particles.

Long, thin, and non-flexible (i.e., stiff) fibers are unable to be completely engulfed by lung (i.e., alveolar) macrophages and can incite the phenomenon known as “frustrated phagocytosis” [14,15]. If these materials are non-degradable, they will persist in the lungs (i.e., become bio-persistent) and can elicit a persistent inflammatory response. Prolonged exposure can therefore lead to an accumulation of these materials in the lung, which in turn can lead to severe health effects as the development of fibrosis and cancer [16]. For a complete review regarding the biological impact of fibers and nanofibers, please refer to [5,17].
As mentioned above, most forms of asbestos and erionite fibers are examples of fibrous materials that are known to cause the development of mesothelioma following prolonged exposure periods. Such effects are only known nowadays due to years of intense scientific research following unfortunate reports of worker ill-health in the asbestos industry since the 1950s. Therefore, due to the fibrous morphology of CNTs and their widespread use, safety concerns have been raised, leading to the discussion on the potential biological impact deriving from CNT human exposure. Over the past two decades, increased research has been performed on this issue [18]. Briefly, it has been reported that short, flexible and entangled CNT can be internalized by macrophages and cleared from the lungs. In contrast, stiff and straight CNTs might cause asbestos-like pathogenic effects [19]. Frustrated phagocytosis as well as simultaneous phagocytosis of single fibers by several macrophages could be observed [20]. By logistic regression models it could be demonstrated that differences in carcinogenicity of a fiber is a function of fiber characteristics, dimensions (thickness, length) and persistence in the lungs [21]. Fiber dimensions (thinner than 1 µm and longer than 20 µm) and the dissolution rate were the most important parameters in initiating malignant lung tumors and mesothelioma [22]. However, long, bio-soluble fibers such as rock-wool exhibit low pathogenic potential [23]. As there is no threshold for cancerous substances, there is no specific “sub-critical” dose. The risk therefore, to develop cancer can be estimated by the exposure duration and the amount (number) of inhalable fibers. Therefore, in theory, even low amounts and short exposure times can be sufficient enough to trigger cancer development [24,25].

Despite the increased research into the biological impact of fibers and nanofibers, the driven cellular interaction of these fibers is not solely related to their physical characteristics, but a combination with their interaction with the biological environment, i.e., their presence within biological fluid (e.g., biomolecules (proteins, enzymes, lipids) prior to any cellular entity. Thus, when considering the inhalation of any fibrous material, assuming that it deposits within the alveolar region of the human lung, this deposited fraction will primarily interact with the pulmonary surfactant layer, a mixture of surfactant proteins and lipids, and the underlying aqueous phase, prior to any subsequent
interaction with the cells of the epithelial airway barrier. At this point, complex competitive ad- and de-sorption processes will lead to the formation of certain protein adsorption patterns, which have been shown to be dependent on the physicochemical properties of the (nano)materials, as well as the structure and affinity of the proteins [26].

The aim of the present review is therefore, to highlight the role of the protein corona in the fiber structure-activity relationship. Especially both asbestos fibers and carbon nanotubes, due to their toxicological relevance towards humans, will be focused upon. This review will critically analyze the existing literature describing the interaction between fibers and proteins and their influence on cellular uptake, biological response (including adverse effects), as well as bioavailability. A short overview on the methods applied to investigate protein profiles is given within the next section to show how these profiles are obtained and where further progress might be needed to improve our understanding of the protein corona of different nanomaterials. This is a general section valid for the investigation of protein coronas of different nanomaterials, not only fiber materials.

2. Methods to Determine the Protein Coating of (Nano)materials

To gain insight into the mechanisms of protein binding at the solid-liquid interface, different approaches have been developed. In principle, they can be divided into single-protein studies, studies performed in complex medium, as well as mathematical modeling. To investigate the protein corona of a (nano)material in a complex medium, the main challenges of these analyses are the high complexity of biomolecules, as well as the highly dynamic nature of protein-nanomaterial interactions.

To investigate the protein-(nano)material interaction in complex medium, nanomaterials are incubated with biological fluids at protein concentrations that, in an ideal case, resemble the protein concentrations within the biological environment. After incubation, most approaches performed so far include a separation of excess non-bound proteins. For the separation of the protein-nanomaterial complexes from excess proteins, several techniques can be applied, including microfiltration, dialysis, magnetic separation, and centrifugation. With each method, after protein separation and several washing steps, the bound proteins are released from the nanomaterial surface by adding a denaturing buffer, separated from each other and identified by liquid chromatography coupled mass spectrometry (LC-MS) after trypsin treatment. This procedure does not take into account the dynamics of these protein fiber interactions and mostly represent a situation which is thermodynamically equilibrated and not representative of realistic physiological conditions.

The most commonly used method to separate the different proteins is one- or two-dimensional polyacrylamide gel electrophoresis (1D/2D-PAGE). In this method, proteins are identified according to their molecular weight or isoelectric point. To identify the proteins, the protein pattern on the protein gels can be compared to so-called master maps obtained from human plasma proteins or analyzed by MS [27]. Proteins are also separated by LC, size exclusion chromatography (SEC) or affinity chromatography. This can be performed on individual excised protein bands obtained by 1D/2D-PAGE, or directly coupled to liquid chromatography as so-called LC-MS or by Matrix-assisted laser desorption/ionization (MALDI) analysis combined with mass spectrometry with time of flight (TOF) analysis. The experimental mass spectra are analyzed and compared against data from protein sequence databases (e.g., UniProtKB/Swiss Prot, web.expasy.org). Additionally, these protein
sequences can be identified by N-terminal sequencing [28]. LC-MS is accepted as the most developed and commonly used method, therefore, the most relevant, although MALDI-TOF can be considered as the most sensitive method.

In general, the number of proteins reported to be associated with nanomaterials varies in the order of one magnitude. This variation is primarily based on the methodological approach of the analysis and therefore the relevant detection limits of the procedure and the equipment used [29,30]. Several studies have shown that protein binding to material surfaces is highly dependent on the ionic strength of the surrounding medium. Therefore, the type of buffers applied in experimental protocols can have a significant effect on the end result. It has been shown that the duration, volume and number of washing steps can affect the resulting protein profile, especially the reported values of albumin. Sempf et al. [27,29] suggested that high abundance proteins like albumin can occur as contaminations, but are not associated with the protein corona. In addition, Sempf and colleagues compared the available data on the protein adsorption patterns of a diversity of polymeric particles obtained by 2D-PAGE. Despite differences in body distribution based on material surface properties, similar proteins were reported to be in the protein corona. The authors therefore concluded that this is caused by a lack of sensitivity of the analytical method applied [27].

In summary, several methods are available to investigate the composition of the protein corona, each with its limitations. Further development of the analytic methods is necessary, especially those enabling time-dependent resolution, in order to gain a full understanding of the highly complex protein-(nano)material interactions.

3. Interactions of Proteins and Fibers

3.1. Asbestos-Protein Interactions

Most of the studies on the protein-material interactions of asbestos fibers available are single-protein studies elaborating the interactions of different proteins and asbestos fiber materials (Table 1). Only a few studies are available investigating protein adsorption onto asbestos fibers in complex protein mixtures. Even if single protein studies cannot give information about the adsorption behavior of a protein in a complex mixture, these studies are necessary to elaborate the general mechanisms of protein adsorption to fibers. Morgan et al. [31] investigated in the adsorption of human serum albumin on different types of asbestos fiber types (chrysotile, crocidolite, amosite, anthophyllite). It was reported that the difference in protein adsorption was due mainly to charge differences of the fibers’ surface. However, notable studies a few years later showed that hydrophobic interactions, nature and density of functional groups, as well as the absence or presence of ionic species significantly contributed to the protein adsorption upon asbestos fibers. In 1977, Light et al. [32] showed that by leaching of magnesium from the fibers, the surface charge of chrysotile and crocidolite fibers can be altered. Light and colleagues observed a significant correlation between the change in surface charge and the hemolytic activity of the fibers. These results were confirmed by Morgan et al. [33] who further demonstrated that Mg$^{2+}$-depletion of chrysotile fibers influences the albumin binding capacity of the fiber material, as well as the selective release of acid hydrolases from mouse peritoneal macrophages and the incidence of mesothelial tumors in rats. These findings show that the surface
characteristics of a fiber material can lead to a change in protein adsorption, which in turn can influence the biological effects.

**Table 1.** Key citations published in the last 40 years regarding the asbestos-protein interaction. Please note that this table is not a complete list, however highlights the important results concerning asbestos-biomolecule related research.

<table>
<thead>
<tr>
<th>Year of publication</th>
<th>Type of study</th>
<th>Type of adsorbed protein(s)</th>
<th>Major outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td>Protein adsorption</td>
<td>Human serum albumin</td>
<td>The surface charge of the asbestos fibers had a strong influence on the adsorption of proteins.</td>
<td>[31]</td>
</tr>
<tr>
<td>1977</td>
<td><em>In vitro, in vivo</em></td>
<td>Human serum albumin</td>
<td>The capacity of asbestos fibers to adsorb proteins is dependent from the magnesium content in the fibers.</td>
<td>[33]</td>
</tr>
<tr>
<td>1980</td>
<td>Protein adsorption</td>
<td>Bovine serum albumin, Ferritin</td>
<td>Magnesium depletion of the asbestos fibers leads to a decrease of albumin adsorption, while the specific adsorption of ferritin increased.</td>
<td>[34]</td>
</tr>
<tr>
<td>1986</td>
<td>Protein adsorption</td>
<td>Fetal serum proteins</td>
<td>Strong electrostatic interactions between the charges of the fibers and the proteins were responsible for the protein-fiber adsorption.</td>
<td>[35]</td>
</tr>
<tr>
<td>1987</td>
<td>Protein adsorption</td>
<td>Different types of proteins</td>
<td>The protein-fiber affinity was correlated with the specific area of the fiber and the protein charge density.</td>
<td>[36]</td>
</tr>
<tr>
<td>1990</td>
<td><em>In vitro</em></td>
<td>Serum proteins</td>
<td>The cytotoxic effects of asbestos fibers was serum-dose dependent.</td>
<td>[37]</td>
</tr>
<tr>
<td>1990</td>
<td><em>In vitro</em></td>
<td>Immunoglobulin G, Bovine serum albumin, Cytochrome c</td>
<td>Certain proteins were selectively adsorbed onto the asbestos fibers.</td>
<td>[38]</td>
</tr>
<tr>
<td>1995</td>
<td><em>In vitro</em></td>
<td>Vitronectin, Fibronectin</td>
<td>Vitronectin specifically enhanced the internalization of asbestos fibers via αvβ5 integrin receptors.</td>
<td>[39]</td>
</tr>
<tr>
<td>2000</td>
<td><em>In vitro</em></td>
<td>Vitronectin</td>
<td>The adsorption of vitronectin onto the asbestos fibers increased the fiber uptake and the cytotoxic effects of asbestos.</td>
<td>[40]</td>
</tr>
</tbody>
</table>

To determine whether there is a preferential adsorption of certain proteins in a complex mixture (such as blood or serum containing cell culture medium), protein adsorption on asbestos fibers in fetal calf serum was investigated by Valerio and co-workers. Compared to their abundance in serum, an enrichment of certain proteins on the fibers was found. Based on their results on different asbestos fiber types, Valerio et al. [35] concluded that the fiber dimensions is an important factor regarding the onset of cancer, but also, concomitantly, the specific protein adsorption characteristics to a fibrous material is a contributing factor to this biological effect. Boylan et al. [39] subsequently showed that vitronectin, a 75 kDa glycoprotein abundant in serum, can be easily adsorbed to crocidolite fibers. It was further noted that by pre-coating crocidolite fibers with vitronectin it significantly increases the internalization of these asbestos fibers by rabbit pleural mesothelial cells. Pre-coating of crocidolite with serum, which naturally contains vitronectin, had similar effects, whereas vitronectin-depleted serum did not lead to enhanced fiber internalization. Similar effects were also observed by Boylan and colleagues with vitronectin-coated chrysotile fibers underlying the importance of this finding. Despite
the role of proteins in determining their internalization, or not, it was still noted that the associated hazard with asbestos fibers remained, with intracellular oxidation, DNA strand breaks, cell-cycle arrest, and apoptosis being observed under these conditions [40,41]. In summary, these results demonstrate that the presence of proteins on the surface of asbestos fibers can significantly contribute to the subsequent biological effect measured.

3.2. CNT-Protein Interactions

3.2.1. Mechanism of Interaction

The size, chemical, and surface properties of CNTs are completely different compared to all forms of asbestos fibers. CNTs consist ideally of pure carbon, hexagonally arranged and formed as a tube with one or more layers [18]. The manner in which proteins interact with these nanofibers is complex and specific to their surface features. Table 2 gives an overview on the key literature on CNT-protein interaction.

It was shown that the dispersibility of CNT in aqueous environment can be improved either by covalent functionalization, but also by non-covalent binding of proteins and surfactants to the CNT surface. In contrast to covalent functionalization, non-covalent binding of proteins retains valuable technological properties of the CNTs (optical, electronic and mechanical). Therefore, CNT-protein interactions were intensively studied in the past by intentional attachment of several proteins, peptides, or other biomolecules to the CNT surface.

It has previously been demonstrated that certain serum proteins, (e.g., albumin, fibrinogen and apolipoproteins) show a higher affinity to the hydrophobic CNT surface than the rest of serum proteins [42–45]. It was proposed that serum proteins bind to CNTs by non-covalent π-π stacking hydrophobic interactions. Specifically, the interaction of aromatic residues of the proteins (e.g., phenylalanine, tryptophan, and tyrosine) with the hydrophobic surface of the CNTs was found to be important for the selective binding of proteins to CNTs [44,46–48]. Amphiphilic, α-helical peptides adsorb with their hydrophobic regions of the helix onto the aromatic surface of the CNTs and the more polar residues are located against the aqueous environment. The non-covalent binding of the hydrophobic amino acids to single walled carbon nanotubes (SWCNT) initiated a de-bundling and an increased dispersion of the tubes in water [49–52]. Just recently, Sacchetti et al. [53] showed that the amount of adsorbed proteins is correlated with the total number of hydrophobic, aromatic protein residues. Proteins that possess hydrophobic binding sites were found to attach to the slightly curved hydrophobic π-electron-rich graphitic surface of the CNTs [54]. Thus, the fact that hydrophobic regions of proteins adsorb readily to CNTs had been used to coat and solubilize CNTs [55–57]. Another group of scientists could demonstrate that the adhesion force between proteins and CNTs was a function of the pH. At low pH the protonated amine groups (–NH3+) of polylysine adsorbed strongly to the deprotonated carboxyl groups (–COO− + H+) of the oxidized (carboxylated) CNTs [58]. Salvador-Morales et al. [59] observed that binding of surfactant proteins to double walled carbon nanotubes (DWCNTs) was calcium-dependent. Control experiments in the absence of calcium ions showed no significant binding. Furthermore, comparison of different batches of pristine DWCNT showed that binding of surfactant proteins to DWCNT in the presence of calcium was linked to the
presence of surface functional groups as carboxyl groups. Therefore, binding might be mediated by calcium-bridging.

Witus et al. [60] synthesized special peptides that bind non-covalently with disulphide bonds to functionalized SWCNTs in order to make them water soluble without altering their electronic structure. It is reported that proteins with a high content of basic residues, such as histones or lysozyme, were suitable for the dispersion of CNTs. By this method, it was observed that the primary, secondary and tertiary protein structures play an essential role in forming a de-bundled CNT solution [61]. Proteins can bind with various amino acids to CNTs. By that, it is possible that the binding sequence is folding into a structure matching the geometry of the CNT to form a stable complex [18,62,63]. Conformational changes due to partial unfolding can lead to a reduction or complete blocking of the enzymatic activity [47]. There are several examples published reporting that enzymes showed a reduced activity or even a complete inhibition of the enzymatic activity after CNT-binding. Here some examples: Carboxyl-functionalyzed CNTs interacted with ribonuclease A and caused a reduction of the activity by a change of the protein conformation. The activity decreased further, when the enzyme adsorbed to larger CNTs [64]. Zhang et al. [65] also reported that different types of functionalized multi-walled CNTs (MWCNTs) were able to bind to, or near to the catalytic site of the digestive enzyme α-chymotrypsin and inhibited its proteolytic activity completely. In a similar study it could be shown that the loss of activity was a function of the change in secondary structure upon adsorption of the proteins onto the surface of the SWCNTs [66,67]. On the other hand there are also studies that report that different metalloproteins immobilized on carboxylated SWCNTs did not show a detectable retention of their activity [68].

In summary, the adsorbed proteins onto the CNTs are affecting the CNT properties, as well as their behavior towards biological systems. However, in contrast to the asbestos-protein interaction, and despite an ever increasing understanding of the CNT-protein interaction, it is not currently possible to correlate how the proteins attached to the CNT surface affect the subsequent biological response observed. In order to achieve this, the biological effects of the protein corona on CNTs have to be investigated on a case-by-case level. It is hypothesized however, that the effects are dependent upon the type of CNT (size, diameter, curvature, functionalization, etc.), from the type of adsorbed proteins (isoelectric point, aromatic amino acids, hydrophobic binding sites, primary-, secondary-, and tertiary structure) and from the environment (pH, presence of other nanoparticles, etc.). Although further research must be performed to confirm this and provide definitive understanding to fully comprehend the nanosafety profile of CNTs.

3.2.2. Influence of Solvents, Surfactants, Surface-Functionalization, and Pre-Coating on CNT-Protein Interactions

It is well established that pre-coating of nanomaterials with certain molecules can influence the further protein binding pattern, as well as nanomaterial uptake and distribution. This has also been demonstrated for CNTs [69]. In a study, where double-walled CNTs came in contact with natural human lung surfactant, it was shown that surfactant protein A (SPA) and the SPD selectively bound onto the surface of the CNTs [59]. Surfactant proteins contribute towards the immune defense system at the epithelial airway barrier and further enhance the phagocytosis of antigens by (alveolar)
macrophages. Further research that pre-coated bundled MWCNTs with porcine pulmonary surfactant (Curosurf®) found that this pro-protein corona affected the subsequent adsorption pattern of blood plasma proteins, as well as the cellular uptake by macrophages. It is important to note that whilst this protein coating had no effect on the cytotoxicity of the MWCNTs, it did mediate both a pro-inflammatory and oxidative stress effect in vitro. [70,71]. Further to this, studies by Holt et al. [72], investigated the internalization of bovine serum albumin (BSA) coated SWCNT. It was observed that cellular uptake of SWCNTs was proportional to the mass of SWCNT-BSA per cell. It could further be demonstrated that SWCNTs coated with BSA were internalized by different cell types, such as human mesenchymal stem cells or HeLa cells [73].

The protein-binding characteristics of nanomaterials can be significantly changed by surface functionalization or the addition of surface coatings [74]. To stabilize aqueous SWCNT dispersions, solvents or surfactant molecules are often applied [50]. Surfactant molecules consist of a hydrophilic head and a hydrophobic tail [75]. The adhesion of surfactants to the CNT is in principle similar to the adhesion of amphiphilic proteins as described above. Surfactant molecules adsorb with their hydrophobic tails onto the surfaces of the CNTs with the polar heads located against the aqueous environment. Dutta et al. [43] showed that pre-coating of SWCNT with the non-ionic amphiphilic copolymer surfactant Pluronic F127 reduced albumin adsorption. For SWCNT that had been modified with polyethylene glycol (PEG), the pattern of adsorbed proteins was affected in dependence of the conformation of the PEG [53,76]. The natural protein binding affinity to SWCNTs is reduced or even eliminated by covalent functionalization of the CNTs with PEG moieties [77], which in turn changes the cellular uptake [42]. In another study, it has been demonstrated that pre-coating of SWCNT’s with Triton X-100 prior to functionalization with PEG leads to a complete and uniform PEG-coating of the SWCNT sidewalls [78]. Practically no streptavidin or other proteins were able to adsorb onto SWCNT that had been treated with Triton X-100, as well as PEG. The importance here is if solvents or surfactants were used as a dispersion agent, these interactions ionic or non-ionic, rapidly or slowly exchanged by other compounds, such as proteins, has to be clarified in detailed in order to avoid misleading conclusions.

3.2.3. Alternative Theory to CNT Protein Interactions

In 2013, Cai et al. [79] released a study that was questioning the published theories concerning protein adsorption on CNTs surfaces. This was based on the fact that the authors did not observe a preference of hydrophobic protein moieties to bind onto MWCNTs. The hydrophobic aromatic amino acids (phenylalanine, tryptophan, and tyrosine) did not show higher affinity to CNTs than other amino acids. Interestingly another group made similar observations. Shannahan et al. [80] investigated the protein corona after incubating carboxylated CNTs (SWCNTs and MWCNTs), as well as pristine CNTs into fetal bovine serum. The quantities of adsorbed proteins on the carboxylated CNTs compared to the quantities of adsorbed proteins to pristine CNTs were similar. This indicates that hydrophobic interactions and π-π-stacking between the aromatic moieties of proteins and the CNTs did not play an important role in the CNT-protein interactions. In addition it could be shown that the carboxylated CNTs were able to bind a number of unique proteins such as Hsp60 or Hsp70 which did not bind to the unmodified pristine CNTs. This implies that hydrogen bonding and electrostatic
interactions as well as specific covalent bonding were involved. Further it had been proposed by Cai and co-workers [79] that the adsorbed protein binding patterns corresponded closely to the CNT-surface properties. MWCNTs with diameters of 20 nm to 40 nm or above were able to bind a significant amount of proteins. On the other hand, MWCNTs with diameters less than 10 nm and SWCNTs with diameters less than 2 nm showed no significant protein binding. Thus, the protein-CNT interaction depends on the size of the CNTs and on the three-dimensional arrangement of carbon atoms in the CNTs, and not on the chemical properties of carbon itself. Beside the diameter of the CNTs, a suitable surface curvature of the CNTs is required for a stronger protein binding [81]. Smoother curvature can induce larger protein conformational changes, while the protein adapts to the unfamiliar surface curvature. Peptides then re-orientate their structures to optimize their interactions with the SWCNTs through their aromatic residues [81].

From the available literature (Table 2) it can be summarized that protein-nanofiber interactions are highly dependent on various factors, which are (1) the inherent properties of the nanofiber (e.g., size, shape, curvature, diameter, surface-chemistry, zeta potential, density of functional surface groups, material composition, presence of impurities, surface functionalization/coating); (2) the properties of the proteins (e.g., size, isoelectric potential, primary, secondary and tertiary structure); (3) experimental/environmental conditions (e.g., type of dispersion medium, pH, presence and absence of ionic species); and last, but not least, the presence and amount of other proteins or amino acids that compete for available surface for binding on the nanomaterial. Therefore, one binding mechanism can be dominant for a certain nanomaterial type, while another binding mechanism may become more relevant with changes in the materials’ properties, proteins present (i.e., the biological environment). It is also important to note that the protein binding is a dynamic process and proteins can be exchanged constantly.

In summary, the reasons why the theories concerning the underlying mechanisms of protein-CNT interactions are quite diverse might be:

- Electrochemical and chemical nature of the CNT and proteins are essential for strong CNT-protein interaction
- Protein-CNT binding is based on non-covalent \( \pi-\pi \) stacking hydrophobic interactions
- The diameter, size and surface curvature of the CNT is essential for a significant protein-CNT binding
- Protein-binding is dependent on the three-dimensional arrangement of the carbon atoms of the CNTs

**Table 2.** Key literature (for the past two decades) concerning the carbon nanotubes (CNT)-protein interaction. Please note that this table is not a complete list, however highlights the key papers regarding CNT-biomolecule research.

<table>
<thead>
<tr>
<th>Year of publication</th>
<th>Type of CNT</th>
<th>Type of adsorbed protein(s)</th>
<th>Major outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>SWCNT</td>
<td>Ferritin, streptavidin</td>
<td>Proteins with primary and secondary amines adsorbed onto f-SWCNT via ( \pi-\pi ) stacking interactions.</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteins rich in surface amines (antibody for C60)</td>
<td>SWCNT with a curved hydrophobic ( \pi )-electron-rich surface bound on the hydrophobic binding sites of proteins.</td>
<td>[54]</td>
</tr>
<tr>
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<tr>
<td>2002</td>
<td>SWCNT</td>
<td>Metalloproteins, Enzymes</td>
<td>Enzymes immobilized on SWCNTs retain their catalytic activity.</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptavidin (various proteins)</td>
<td>Pre-coating of SWCNTs with triton X-100 prior PEG coating prevented the adsorption of small proteins onto SWCNT nearly completely.</td>
<td>[78]</td>
</tr>
<tr>
<td>2003</td>
<td>SWCNT</td>
<td>Amphiphilic (\alpha)-helical peptide</td>
<td>The apolar residues of amphiphilic proteins bound to the surface of SWCNT and the polar residues of the proteins were located against the solvent face.</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>MWCNT</td>
<td>Phage and other types of peptides</td>
<td>Peptides that were rich in histidine and tryptophan bound at special locations of the MWCNTs by hydrophobic interactions.</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\alpha)-Chymotrypsin, soybean peroxidase</td>
<td>The enzymes changed their secondary structures upon adsorption onto the SWCNTs, which caused a decrease or nearly complete loss of their activity.</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Ferritin</td>
<td>A covalent coating of SWCNT with PEG was alleviating or even completely eliminating the natural protein affinity of the SWCNTs.</td>
<td>[77]</td>
</tr>
<tr>
<td>2004</td>
<td>SWCNT</td>
<td>Amphiphilic (\alpha)-helical peptide</td>
<td>The binding of polar residues of amphiphilic proteins onto the surface of SWCNTs increased the dispersion of the SWCNTs in water.</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>CNT</td>
<td>Streptavidin</td>
<td>Protein adsorption onto CNTs occurred through interactions between the amine groups of the protein and the hydrophobic surface of the CNTs.</td>
<td>[83]</td>
</tr>
<tr>
<td>2005</td>
<td>SWCNT</td>
<td>Amphiphilic (\alpha)-helical peptide</td>
<td>Amphiphilic peptides bound non-covalently with their apolar residues onto the SWCNTs, which resulted in a better solubilisation of the SWCNTs.</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphiphilic peptide helix (nano-1)</td>
<td>The aromatic residues of the peptides interacted with the SWCNT surface, which was leading to a better dispersion of the SWCNTs.</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td>Model proteins</td>
<td></td>
<td>Protein coated SWCNTs were incorporated by the cells via energy dependent endocytosis through clathrin-coated pits.</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>Different types of proteins</td>
<td></td>
<td>Proteins adsorbed onto SWCNTs via (\pi-\pi) stacking as well as amine interactions, whereas the hydrophilic protein moieties were located towards the water face.</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td>Polyline, polytryptophan</td>
<td></td>
<td>A strong adhesive force was registrated between the protonated amine-groups of the protein (polylysine) and the carboxyl-groups of the oxidized CNTs.</td>
<td>[58]</td>
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<tr>
<td>2006</td>
<td>SWCNT</td>
<td>(\pi-\pi) stacking and hydrophobic interactions as well as protonated amine interactions between proteins and SWCNT were responsible for the dispersion of the SWCNTs.</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td></td>
<td>Protein binding onto SWCNT was highly selective.</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen, apolipoproteins (AI, AIV, CIII)</td>
<td></td>
<td>Hydrophobic as well as (\pi-\pi) interactions between proteins and SWCNTs were important for a selective protein binding onto SWCNTs.</td>
<td>[46]</td>
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<td></td>
<td>Peptides from phage libraries</td>
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<tr>
<td>2007</td>
<td>SWCNT</td>
<td>Foetal bovine plasma, human serum/plasma protein</td>
<td>The uptake of SWCNT occurred by pathways associated with the adsorbed proteins. The proteins modulated in addition the toxicity of the SWCNTs.</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Different types of proteins</td>
<td>The primary, secondary and tertiary structures of proteins and the pH of the dispersion medium were important to obtain a high yield of de-bundeled CNTs</td>
<td>[61]</td>
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<tr>
<td></td>
<td>DWCNT</td>
<td>Surfactant proteins A and D</td>
<td>Supernatant protein A and D adsorbed selectively onto DWCN Ts out of different pulmonary surfactant protein samples.</td>
<td>[59]</td>
</tr>
<tr>
<td>2008</td>
<td>SWCNT, MWCNT</td>
<td>Ribonuclease A</td>
<td>CNTs functionalized with carboxylic groups interacted with the enzyme and caused a reduction of its activity by changing its conformation.</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>MWCNT, f-MWCNT</td>
<td>Bovine serum albumin (BSA) and different types of proteins</td>
<td>Electrostatic and stereo-chemical properties of the MWCNTs and the proteins as well as the curvature of the MWCNTs were affecting the protein binding affinity onto the MWCNTs.</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human plasma and serum proteins</td>
<td>Functionalization of the MWCNTs affected the patterns of adsorbed proteins onto the MWCNT, which resulted in a better biocompatibility of the MWCNTs.</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>CNT</td>
<td>A-sub-domain of human serum albumin</td>
<td>The adsorption of proteins onto CNTs caused a conformation change of the secondary protein structure, which resulted in a decrease of the protein activity.</td>
<td>[67]</td>
</tr>
<tr>
<td>2009</td>
<td>MWCNT, f-MWCNT</td>
<td>α-Chymotrypsin</td>
<td>Enzymes bound onto MWCNTs through π-π stacking and hydrophobic interactions, which resulted in a competitive inhibition of the enzyme activity.</td>
<td>[65]</td>
</tr>
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<td></td>
<td>SWCNT</td>
<td>Model surfactant</td>
<td>Surfactants with a larger hydrophilic head group was leading to a significant better dispersion stability of SWCNTs.</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Model protein</td>
<td>Hydrophobic interactions between the hydrophobic core of the proteins and the SWCNTs formed stable complexes, which caused a blockage of the active sides of the proteins.</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>MWCNT, f-MWCNT</td>
<td>Pulmonary surfactant (Curosurf®)</td>
<td>The pre-coating of MWCNTs with a lung surfactant influenced the protein binding onto the MWCNTs and resulted in characteristic binding patterns.</td>
<td>[70]</td>
</tr>
<tr>
<td>2011</td>
<td>SWCNT, MWCNT</td>
<td>Serum proteins</td>
<td>The adsorption capacity of CNTs for proteins was dependent on the type, arrangement model, size and surface modification of the CNTs.</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Human serum proteins</td>
<td>Competitive binding of blood proteins onto the SWCNT surface can alter the cellular interaction pathways, resulting in a reduced cytotoxicity.</td>
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<td>2011</td>
<td>SWCNT</td>
<td>Bovine serum albumin (BSA)</td>
<td>Bovine serum albumin dispersed SWCNTs readily entered into the cells and did not acute deleterious cellular effects.</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>SWCNT, DWCNT</td>
<td>Serum proteins</td>
<td>The adsorption of enzymes of the immune system to the hydrophobic SWCNT surface didn’t caused an activation of the enzymes.</td>
<td>[85]</td>
</tr>
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<td></td>
<td>MWCNT</td>
<td>Blood proteins</td>
<td>A surface modification of the MWCNT affected their patterns of adsorbed proteins, which resulted in a modification of the biocompatibility of the MWCNTs.</td>
<td>[48]</td>
</tr>
<tr>
<td>2012</td>
<td>SWCNT</td>
<td>Bovine serum albumin (BSA)</td>
<td>Bovine serum albumin coated SWCNTs were taken up by the cells within seconds. However, the cells were able to expel the incorporated BSA-SWCNT complexes over hours and days.</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Different types of proteins</td>
<td>The stability of a SWCNT-protein complex had a substantial influence on the cellular uptake and the uptake of a certain protein was dependent from the cell type.</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>MWCNT</td>
<td>Pulmonary surfactant (Curosurf)</td>
<td>The pre-coating of MWCNTs with a lung surfactant affected the uptake of the MWCNTs without significantly altering the cytotoxicity of the MWCNTs.</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>MWCNT</td>
<td>Human cellular proteins (HeLa cells lysate)</td>
<td>Electrostatic, stereochemical properties, diameter and curvature of the MWCNTs were significantly affecting the adsorption of proteins onto the MWCNTs.</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>Plasma proteins</td>
<td>The surface PEG conformation of SWCNT-PEG complexes affected the pattern of adsorbed plasma proteins onto the SWCNTs and influenced the biodistribution of the SWCNT-PEG complexes.</td>
<td>[53]</td>
</tr>
<tr>
<td>2013</td>
<td>SWCNT, f-SWCNT, MWCNT, f-MWCNT</td>
<td>Foetal Bovine serum (FBS)</td>
<td>Functionalized CNTs were able to bind a number of unique proteins, which implied that electrostatic interactions and specific covalent bonding were involved.</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>CNT</td>
<td>Different types of proteins</td>
<td>π-π stacking and hydrophobic interactions were responsible for the adsorption of proteins onto CNTs. The protein adsorption led to a reduction of the cytotoxicity and to a loss of the enzymatic activity of the proteins.</td>
<td>[47]</td>
</tr>
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</table>

4. Discussion

According to the fiber paradigm, in addition to the geometry of fibers, their bio-persistence is the most important characteristic determining their biological impact towards the lungs. It has been shown that asbestos fibers, as well as long and thick MWCNT, can follow the fiber paradigm. As these fibers are made of different materials, the composition of the fibers is not considered as one of the most relevant factors, but in fact influences their persistence within the human body and the biological effects. The importance of a bio-corona that is formed around a nanomaterial entering a biological environment is undoubted and a view on the recent literature on fiber-protein interactions, as
summarized here, shows the importance of the protein corona on the fiber toxicity. Thus, the question is “how does the protein corona influence the fiber toxicity?”. Are there characteristics of the protein corona bound to (nano)fibers that differ from those obtained by other shapes? Is there anything that can be described as a fiber-specific effect (associated with the bound proteins)?

The majority of studies concerning the composition of the protein corona were performed on nanomaterials of similar shape but different size or surface characteristics. To answer the question, if there are certain fiber-specific characteristics within the protein-corona of fibers, we compared the “top-ten” or “hit list” of the proteins found on fibers and other nanomaterials of different shapes and materials: i.e., CNTs, silica nanoparticles [87,88], gold nanoparticles [89–91], polymeric particles [27,88,92], and iron oxide nanoparticles [93,94]. Despite the differences in morphology (shape and size), surface characteristics and material composition, several proteins (e.g., albumin, apolipoprotein AI and E, fibrinogen, fibronectin, vitronectin, complement factor C3, α2-macroglobulin) were found on nearly all protein coronas investigated. This leads to additional questions, regarding our knowledge on the protein corona, the methods applied to investigate the detailed composition of the protein corona as well as the comparability of the results obtained by different approaches.

4.1. Comparison of Protein Coronas

Comparison of the composition of protein coronas of different nanomaterials can be triggered by several factors. First, the experimental protocols applied can have a significant impact on the precision, reproducibility and comparability of the studies. Thus, the variability between protein coronas of identical materials but obtained by different protocols might be given.

Second, the protein corona formation is a very complex and dynamic process. Within less than few seconds after exposition of the nanomaterials to a biological medium, the protein corona is formed [88]. The composition of the protein corona can also vary over time. High abundant serum proteins can dominate adsorption on the nanomaterial surface at short incubation times, but with time they can be replaced by other proteins with lower abundance but higher affinity [2]. Testing strategies involving separation methods are not able to enclose the dynamic processes taking place at the protein-nanomaterial interface. Thus, the incubation time of the nanomaterial in the bio-fluid has an effect on the observed protein corona. Even if those studies are performed time-dependently, they only give us a semi-dynamic picture. Thus, the variation of the composition of the protein corona at different time points might be quite high, perhaps in the same range as the variation between different materials investigated or protocols being applied.

One factor that can further blur the picture obtained from the protein corona is the fact that the protein profile of nanomaterials is not necessarily the protein pattern found on each and every particle in the dispersion, but rather the sum of proteins bound to the sum of available surface area within the nanomaterial dispersion. This can be easily illustrated when the diversity of proteins found adsorbed to a particle dispersion is compared to the available surface area of a single particle, the particle size and the size of protein molecules detected in the protein corona. Tenzer et al. [88] detected almost up to 300 different proteins within the protein adsorption pattern of silica and polystyrene nanoparticle samples. They investigated the protein corona of silica nanoparticles of two different sizes, as well as
those of polystyrene nanoparticles with different surface functionalization, formed after exposure to human plasma [88].

As the adsorption pattern on material surfaces are dependent on the morphology and physicochemical properties of the nanomaterial, slight variations within a sample might also influence the resulting protein pattern. The measured physicochemical properties of nanomaterial dispersions are an average of the properties of all particles within the sample. In other words, the sum of the available particle surface cannot necessarily be regarded as a single and homogeneous surface area cut into equal pieces. The protein adsorption pattern of one particle might differ from the pattern adsorbed on another particle within the same sample. It is important to minimize the variability of fibers or particles within a sample to obtain results that can be linked to certain material properties. Therefore, the number of different proteins detected within the protein corona should be interpreted with care. A high number of different protein types within a protein profile does not necessarily reflect a higher precision of the methodology and might instead derive from a certain variability of physicochemical parameters in a sample.

In summary, the comparison of different studies is complicated by several factors and it was not possible to find any characteristic of the protein corona that could be clearly linked, specifically to fiber morphology alone.

4.2. Methodology and Challenges

The protein corona is often divided into a hard corona, which is composed of tightly bound proteins, and a soft corona, a layer of proteins which is subject to rapid exchange of its components. Monopoli et al. [2] suggest that the interface between the hard and soft corona is the key factor determining the biological effects of the nanomaterial. The residence time of biomolecules in the protein corona is seen as one of the most important determinants.

In other words, the interaction of proteins bound to the particle surface and those which bind to the adsorbed proteins is of high interest. This leads to the question if the recently applied methods to investigate the composition of the protein corona enable us to study such interactions. As described before, the methods recently applied are based on a separation of the tightly bound proteins from loosely bound protein fraction. As a consequence, these methods can only consider proteins tightly bound to the nanomaterial or nanomaterial-protein complexes. The obtained results do not allow us to determine if all proteins found within the corona are directly bound to the nanomaterials surface or whether their association with the nanomaterial is mediated by other proteins already bound to the surface. Information as to the conformational state of the bound proteins, as well as regarding the consequences of their binding to the nanomaterial for their biological function is, at best, limited. As mentioned above, the applied methods do not provide a resolution of the dynamic processes taking place at the solid-liquid interface. Without question, much progress has been performed in the investigation of the composition of the protein corona of nanomaterials, but extension of our recent tool-box is needed to gain further in-sight in the underlying mechanisms of the complex interaction of proteins at the solid-liquid interface and to correlate nanomaterial properties to the corona properties and resulting biological effects.
4.3. Influence of Shape on Nanomaterial-Protein Interaction

It is well accepted that several nanomaterial characteristics as size, material composition, and surface functionalization, determine the protein adsorption pattern to these materials in biological media. The influence of the shape of the nanomaterials, especially the difference between fibrous and spherical nanomaterials of similar elemental composition, is not well-understood. But in fact, there is evidence that the shape and morphology of the materials has a significant influence on the protein adsorption. In addition, it has been noticed that other factors, such as the atomic-scale surface topography, impurities, and structural defects, can have a significant impact on the protein adsorption to nanomaterials.

Recently, Gagner et al. [95,96] demonstrated that not only the size of a nanomaterial, but also its shape and crystal structure can have a significant effect on the nanomaterial protein adsorption pattern in biological fluids. In a first study, they systematically varied the morphology of gold nanomaterials and compared the binding of lysozyme and a-chymotrypsin to gold nanospheres and nanorods of comparable diameter. For both proteins, they observed differences in surface coverage dependent on the morphology of the nanomaterials. At normalized surface area, the amount of proteins bound to nanorods was higher than to nanospheres. Due to their cylindrical morphology, nanorods exhibited a relatively flat surface along the axis. This is thought to facilitate protein binding in contrast to the nanosphere surface, which is highly curved in all directions. In addition, enzyme activity was more preserved on gold nanospheres than on gold nanorods. These results are in line with reports that enzyme stability is higher on surfaces with high curvature than on those with more flat surface, demonstrated with silica [97], and gold nanoparticles [98], as well as by comparison of carbon nanotubes and graphite [99].

Comparison of the protein pattern found on titanium dioxide spheres, nanorods and nanotubes, by Deng et al. [100] showed that the shape of titanium dioxide nanomaterials can have an influence on the protein composition of the corona. Protein profiles obtained by 2D gel electrophoresis showed that spherical titanium dioxide nanoparticles (Ø ~ 21 nm) adsorbed qualitatively more proteins than titanium dioxide nanorods (Ø ~ 27 nm) or titanium dioxide nanotubes (Ø ~ 9 nm).

Taken together, there are several hints that the morphology of a material has an impact on the protein binding to its surface. For several materials, such as silica, titanium dioxide, gold, and carbon, nano-objects with different shapes are available. However, how the shape influences the protein adsorption pattern in a complex medium can only be deduced, due to the lack of systematic studies focused on this parameter. By a view on the available literature, clear fiber-specific characteristics of the protein-adsorption pattern within a complex medium could not be identified, but in fact, it could be shown that the protein corona has a significant impact on the biological effects of fiber materials (i.e., asbestos).

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Author Contributions

Peter Wick, Barbara Rothen-Rutishauser and Alke Petri-Fink elaborated the idea, structure and concept of the review. Melanie Kucki, Jean-Pierre Kaiser and Martin J. D. Clift performed the literature research & analysis, as well as wrote and prepared the manuscript. All authors read, commented on, and improved the manuscript. All authors are entirely responsible for the content of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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