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Toward peptide-based bioelectronics: reductionist design of conductive pili mimetics

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Abstract

Proteins and peptides possess inherent properties which can benefit medical devices that interact with electro-responsive tissues. However, proteinaceous materials are typically electrically insulating and hence are not suitable to be utilized as conductive elements in electromedical and other bio-interfacing devices. The discovery of intrinsic electrical conductivity in bacterial protein nanofibers, termed e-pili, could give rise to mimetic reductionist design and thus provide an opportunity to improve the function of existing electromedical devices. In this Special Report we review key aspects concerning the properties of e-pili and present the ongoing effort toward the design of mimetic conductive nanostructures. We highlight the advantages of using self-assembling peptides as building blocks for this purpose and discuss the prospect of the envisioned mimetic nanostructures.

Keywords

bioelectronics; biomimetics; biotechnology; materials science; nanomaterials; self-assembly

Nonmetallic materials with conductive properties and nanoscale features emerge as next-generation materials for biomedical and bioelectronic medicine applications that involve intimate contact with electro-responsive cells, including tissue engineering, tissue regeneration and *in situ* sensing. In terms of device fabrication, such materials can contribute significantly to miniaturization, controlled degradation and tissue interfacing due to their morphology, chemical composition and high surface area, respectively [1]. Conductive polymers and carbon nanotubes are the main candidate materials studied in this regard owing to their favorable electronic properties. Yet, they each possess specific shortcomings which have so far precluded their broad implementation in biomedical devices. For example,

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there are difficulties in preparing isolated conductive polymer nanostructures by nanolithography or in functionalizing conductive polymer and carbon nanotube materials for specific applications [1,2]. Ideally, a conductive material suitable for the above applications would comprise nanoscale structures which resemble the native extracellular environment, be easily functionalizable for specific applications, possess inherent biocompatibility and softness and include modifiable biorecognition elements to promote cell adhesion. These properties are highly characteristic of biomaterials based on proteins, but such materials are considered electrically insulating. Recent developments in protein bioelectronics and peptide self-assembly set the stage for the design of conductive proteinaceous materials based only on the 20 coded amino acids. Such conductive biomaterials could potentially improve the function of existing devices used in bioelectronic medicine.

Throughout evolution, the vast landscape of amino acid configurations had given rise to diverse structure–function relationships in proteins, peptides and assemblies thereof. Structure–function relationships can result in unique physicochemical properties, atypical of proteins, such as strong visible-range photoluminescence [3], firm adhesion to different surfaces [4] and high physicochemical stability [5]. A relatively recent addition to this repertoire is long-range electrical conductivity as observed in several instances of type IV (T4) pili, a class of polymeric protein nanofibers. Conductive T4 pili, recently termed e-pili by Lovley, are reported to possess conductivities ranging from tens of $\mu\text{S}/\text{cm}$ to hundreds of S/cm per nanofiber [6–9] and hence challenge the conception that proteins are electrically insulating. Although the structure–function relationship in e-pili is currently unknown, the very discovery of native conductive protein nanofibers inspires mimetic design toward electronic applications, as we have recently begun to pursue using self-assembling peptides.

Since the conductive e-pili are a subset of T4 pili, the latter should be introduced first. In bacteria, T4 pili are flexible extracellular protein nanofibers that emerge from the cell surface. The biological role of T4 pili is to mediate interactions of the bacterium with its extracellular environment, such as in twitching motility, adherence to surfaces, DNA uptake and biofilm formation processes [10]. The typical structure and assembly of T4 pili are illustrated in Figure 1A–C. Structurally, the T4 pilus (singular of pili) is usually several μm in length and has a diameter of 5–8 nm. This nanofiber is a helical supramolecular polymeric assembly of the protein pilin [11], in other words, assembly of pilin monomers that are organized as a helix and held together by noncovalent interactions. The pilin monomer generally consists of a long α -helix ($\alpha.1$) and a globular head domain. The N-terminal region of the long α -helix, or polymerization region, is hydrophobic and evolutionary conserved (i.e., possesses an amino acid sequence with high similarity across bacterial species). In contrast, the C-terminal region of $\alpha.1$ is polar and more evolutionary variable. This region is usually wrapped by an antiparallel β -sheet to form the globular head domain. The T4 pilus is assembled in the bacterial inner membrane by enzymatic machinery that facilitates the association between pilin monomers *in vivo* [11]. In the assembled T4 pilus, pilins are associated noncovalently mainly through their N-terminal regions, which partake in hydrophobic and electrostatic interactions and thus form the hydrophobic core of the mature pilus. Copies of the head domain form the exposed surface of the mature T4 pilus and come into contact with the extracellular environment to carry out one of the aforementioned biological functions.

e-Pili were originally discovered in *Geobacter sulfurreducens* (GS) [13], the only organism to date in which they were thoroughly studied. In GS and related subsurface species, e-pili fulfill the divergent biological function of long-range extracellular transport of respiratory electrons. These electrons are transported along e-pili and subsequently reduce nearby insoluble acceptors, such as oxidized metals in minerals that are found in the habitats of GS and other species. The terminal transfer of electrons from e-pili to the insoluble acceptors is a redox process that appears to be mediated by pili-associated cytochromes [14], a type of iron-containing proteins which participate in various biological electron transfer processes. However, the pili-associated cytochromes are not required for electron transport along e-pili themselves, as strongly supported by recent studies of highly purified e-pili, free from associated proteins or metals [6,8,15]. e-Pili additionally function in direct interspecies electron transfer, a metabolic coupling process between different microorganism types that requires direct cell-to-cell electrical connection [16]. The existence of e-pili in bacterial species not belonging to the *Geobacter* genus has been suggested in a recent study, implying that electrical conductivity along protein nanostructures may be a broad phenomenon in microorganisms [17]. While the intrinsic conductivity of GS e-pili has been established, their underlying structure–function relationship remains unclear. Compared with characteristic T4 pili, GS e-pili are only approximately 3 nm in diameter [18] and their pilin monomer is considerably shorter [12], such that it lacks most of the globular head domain and is therefore almost entirely helical (Figure 1D). Despite the existence of an atomic structure for the pilin monomer, the organization of pilins within an intact e-pilus is currently unknown: a high-resolution structural model for e-pili is not available and there is very little structural information on these nanofibers altogether as they are not amenable to various structural methods. Moreover, charge transfer properties reported by different research groups appear to contrast and have invoked two contradictory structure–function models, suggesting metallic-like electron delocalization [19,20] or consecutive electron hopping [8,21] as the charge transfer process underlying conductivity. Importantly, according to both models, amino acids with aromatic side chains are crucial for e-pili conductivity, as was indeed observed experimentally [22].

Although e-pili are not fully understood, their conductivity and proteinaceous nature make them attractive for different applications. Yet, utilizing intact e-pili specifically for biomedical applications may prove difficult for several reasons. First, obtaining highly pure intact e-pili in large quantities could be challenging and purity issues may impair the functionality of even very simple devices [23]. Second, there is a concern that bacterial-derived impurities, which may be generated in the process of intact e-pili purification, might trigger an immune or other unwanted response in higher organisms, hampering the utilization of e-pili for biomedical purposes. Moreover, even if purified native e-pili would prove as safe for biomedical applications, regulatory impediments stemming from their biological origin are likely to arise and thus complicate their practical use. Finally, native e-pili might not form proper physical contact with target tissues or other device components. This is because very specific amino acid sequences are typically required to achieve substantial adhesion to various cells [24], synthetic organic or inorganic materials [25]. Due to these potential issues, it would be most desirable to develop mass-producible, highly pure and easily functionalizable synthetic conductive nanofibers which mimic the structure–

function relationship of e-pili. Synthetic short peptides that correspond to the GS pilin sequence are the obvious candidate building blocks for this purpose. Peptides are generally advantageous due to their facile and scalable synthesis and commercial availability. Peptides can be produced at very high purity in a cost-effective manner and their amino acid sequence can be readily modified for specific purposes. The self-assembly of peptide building blocks into well-ordered structures at the nanoscale is a spontaneous supramolecular process. Peptide self-assembly can hence be tuned by environmental conditions, yet this process is capable of delivering physicochemically stable nanostructures [5] that in some cases can also resist biological degradation [26]. Self-assembling peptides have been successfully used in mimicking various polymeric protein assemblies, including helix-based assemblies. Instrumental to such efforts is the reductionist study of the parent protein monomer sequence by corresponding synthetic peptides. As we and others have shown, this approach can lead to the identification of short self-assembling peptides that form native-like configurations in a test tube [27–29]. Such work gave rise to accessible model systems for studying native protein assemblies as well as to novel biomaterials that mimic the native functionality.

Recently, we began to explore the route toward GS e-pili synthetic mimetics by following a reductionist approach and predicating on a serendipitous discovery made more than a decade ago. In a pioneering work, Audette and co-workers have shown that a recombinant pilin protein from the bacterium *Pseudomonas aeruginosa*, which lacks its conserved N-terminal polymerization region, self-assembles into functionally mimetic filaments in the presence of hydrophobes [30]. This work demonstrated that pilins, which do not self-assemble *in vitro* generally [31], can be induced to do so by substantial sequence reduction and environmental tuning and thus form functional mimetic assemblies. This malleability, combined with the clear advantages of short synthetic peptides, motivated us to explore the ability of peptides derived from the GS pilin sequence to self-assemble *in vitro*. In our work [32], we have assessed the self-assembly propensity of pilin-derived peptides. We reduced the GS pilin protein to a 20-mer peptide, which conjoins sequences from the two distinct regions of the protein, namely the conserved N-terminal polymerization region and the more variable C-terminal region that is generally associated with functionality. The latter sequence included three out of the five aromatic amino acids of the GS pilin C-terminal region. This minimized representation of the intact pilin successfully self-assembled into nanofibers. The 20-mer was further reduced into shorter N- and C-terminal peptides to better understand the contribution of each sequence to the self-assembly process. We observed that peptides which correspond to the N-terminal polymerization region present strong propensity to self-assemble into nanofibers, adopting a non-native β -type conformation. The self-assembly of these peptides was in line with their high hydrophobicity, yet the folding into β -type structures was unexpected. In contrast, peptides which correspond to the C-terminal functionality-related region did not self-assemble under identical or alternative environmental conditions. However, these peptides did adopt a native-like helical conformation, in line with the designed substitution of native alanines into α -aminoisobutyric acid (Aib) residues, which nucleate and stabilize helical conformations. Figure 2 summarizes the above described study.

Our reductionist approach has shown for the first time that short and synthetic peptide building blocks, which correspond to a pilin protein sequence, can self-assemble into

nanofibers. The nanofibers indeed exhibit several of the morphological and structural features characteristic of native GS e-pili. While the reported peptide nanofibers are not conductive, likely due to differences in their amino acid composition and internal organization as compared with native e-pili, their very assembly may be considered as a first step toward the development of synthetic peptide assemblies that fully mimic e-pili, structurally and functionally. The importance of C-terminal aromatic amino acids for e-pili conductivity merits a systematic investigation of related sequences, which can now be pursued by modifying our peptide design. Increased helicity of the self-assembling peptide building block is another important factor in mimicking e-pili since GS pilin is almost entirely helical. Interestingly, the combination of building block aromaticity and helicity indeed appear to facilitate long-range charge transfer as demonstrated in another system, where nanofibers assembled from a *de novo* peptide building block of coded amino acids formed low-conductance films [33]. We believe that investigating self-assembling pilin-derived peptides would lead to the identification of highly conductive synthetic nanofibers, which are simpler to obtain, study and modify than native e-pili. The reductionist approach could therefore bring about novel bioelectronic materials and also have the benefit of providing constructionist insights into the unique structure–function relationship of native e-pili.

Although further development is required to obtain pili-mimetic peptide-based electronic materials, the potential application of these materials in bioelectronic medical devices can already be envisioned. Conductive peptide nanofibers could potentially be used as nanoscale electrodes to stimulate and record electrical activity of single cardiac or neuronal cells in engineered cultures or as part of implantable devices. By modifying the peptide building block sequence, these nanoelectrodes may be conveniently functionalized to adhere to other device components made of various materials [25]. Other sequence modifications may tune the cell adhesion efficiency of the nanoelectrodes [24] or may confer guided adhesion to specific cell types by mimicking T4 pili receptor binding [34]. Moreover, implantable devices with supramolecular peptide-based electronic components could be designed to fully degrade inside the body following short-term treatment, or be programmed to respond to environmental stimuli such as specific molecules and ions [35]. Looking forward, conductive pili mimetics formed by self-assembling peptides are expected to combine the obvious advantages of protein-based materials for medical applications with unprecedented electronic properties. As such, they are envisioned as next-generation materials for bioelectronic medicine.

Conclusion

The electrical conductivity observed in native e-pili paves the way toward the design of proteinaceous nanostructured materials, which could potentially improve the function of tissue-contacting devices as used in bioelectronic medicine. Such materials are envisioned to combine electrical conductivity with the characteristic properties of proteins and peptides, such as biocompatibility and softness. Mimetic nanofibers formed by synthetic self-assembling peptide building blocks are likely to possess important advantages over native e-pili from the applied perspective. The ongoing effort to design mimetic conductive peptide nanofibers involves a reductionist approach, which aims to design simple self-assembling

peptide building blocks that correspond in sequence to the GS e-pilus monomer. While this new niche in the fields of biomimetics and bioelectronics has only recently emerged, it has the potential to revolutionize the development of electromedical devices.

Future perspective

Basic research of bioinspired amino acid sequences, especially with aromatic content and helical conformations, would lead to the identification of self-assembling peptides that form conductive nanostructured materials. These basic research efforts will contribute to the elucidation of the mechanism underlying native e-pili conductivity and uncover general design principles for producing conductive proteinaceous materials. This process is also expected to yield candidate materials for translational studies, aiming at the development of application-relevant devices and technologies. Increasing attention from the commercial sector would likely lead to improvement in material properties and costs.

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Executive summary

- Proteinaceous materials are typically electrically insulating, but bacterial protein nanofibers termed e-pili present electrical conductivity of up to hundreds of S/cm.
- e-Pili are a special case of T4 pili, a class of extracellular nanofibers abundant in bacteria with nanometric diameters and micrometric lengths.
- e-Pili diverge from characteristic T4 pili in function and apparently in structure, as indicated mainly by studies in *Geobacter sulfurreducens* (GS) bacteria.
- The exact structure of e-pili and the mechanism underlying their conductivity is unclear, but aromatic amino acids appear to play an important role in their function.
- The conductivity and proteinaceous nature of e-pili marks them as a potentially suitable material for electromedical applications and devices, but utilizing native intact e-pili may prove difficult from technical and regulatory aspects.
- Mimetic nanostructures from synthetic self-assembling peptide building blocks could provide a safer, more scalable, and more easily modifiable alternative for intact native e-pili.
- Simple self-assembling peptide building blocks, derived from the GS e-pilus monomer sequence by reductionist approach, represent a first step toward fully mimicking e-pili.
- Mimetic conductive nanofibers are envisioned as biocompatible and functionalizable nanoelectrodes or electrical components in implantable devices.

Figure 1. Pilus structure and assembly.

(A & B) High-resolution 3D structure of a typical T4 pilus (A) and its pilin monomer (B) from the model species *Neisseria gonorrhoeae*. The long helix, $\alpha 1$, comprises a highly conserved and hydrophobic N-terminal polymerization region (amino acids 1–22, red) and a more variable and polar C-terminal region (amino acids 23–53, blue). The latter region, together with the remaining $\alpha\beta$ loop and consecutive antiparallel β -sheet (amino acids 54–158, green), form the globular head domain. The hydrophobic polymerization regions form the pilus core while the head domains form its exposed surface, as seen in (A). (C) Illustration of pilus assembly *in vivo*. Pilin monomers (gray) are associated with the bacterial inner membrane (green). Membrane-associated enzymatic machinery (teal) assembles the monomers. (D) Structure of *Geobacter sulfurreducens* (GS) pilin (gray) superimposed on *N. gonorrhoeae* pilin (black). GS pilin, the conductive GS e-pilus monomer, is only 61 amino acids long, with most of the typical globular head domain lacking. GS pilin is therefore less than half the sequence length of typical pilins. Structure of assembled e-pilus is currently unknown. The pilin proteins were superimposed using PyMOL software package. In (B) and (D) the N- and C-terminal ends are indicated by *N* and *C*, respectively. Structures in (A & B) and (D) correspond to Protein Data Bank accession numbers 2HIL [11], 2HI2 [11] and 2M7G [12], respectively.

Figure 2. Reductionist approach for mimicking pili using synthetic self-assembling peptides.

(A) Designed sequence reduction of GS pilin (left, amino acid color coding as in Figure 1B) into a 20-mer peptide (right), which conjoins amino acid sequences from the two distinct regions of *Geobacter sulfurreducens* (GS) pilin. The included sequences correspond to the segments in the dashed frames. Structural investigation of the 20-mer peptide, in part by using shorter derived peptides, revealed a non-native β -type conformation in its assembly driving N-terminal region, and a native-like helical conformation in its nonassembling C-terminal region. The 20-mer structure is illustrated based on its reductionist study. (B) Illustration of the 20-mer peptide self-assembly into nanofibers *in vitro*. This highly pure synthetic peptide self-assembles in a test tube into nanofibers by virtue of its assembly driving N-terminal region. Modifying the peptide design may ultimately lead to the formation of synthetic assemblies that fully mimic e-pili in terms of structure and function. GS: *Geobacter sulfurreducens*.