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From antigen uptake to immune modulation: the multifaceted potential of peptide nanofibers as vaccine nanocarriers

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Spanning from the mitigation of pathogenic diseases *via* protective immunity to the provision of therapeutic solutions for other debilitating ailments, immune-based interventions have showcased promising and significant outcomes. Peptide nanofibers constructed from self-assembled biocompatible peptide chains have garnered considerable attention. Evaluation of the peptide nanofibers' capabilities has revealed their aptitude to enhance antigen uptake by antigen-presenting cells (APCs), such as dendritic cells (DCs), and their potential to manifest immune adjuvant-like effects. These results suggest that peptide nanofibers could amplify the efficacy of vaccines administered through diversified approaches and potentially obviate the necessity of co-administering antigens and immune adjuvants to APCs. This review highlights the potential utility of peptide nanofibers as an approach to augment immune responses, with the potential for the effective and safe enhancement of vaccine potency. Furthermore, the opportunistic application of emerging peptide nanofibers to enhance the therapeutic outcomes of recently uncovered immune modulators is also deliberated upon.

1. Introduction

Cancer is recognised as a predominant cause of mortality and poses a significant challenge to enhancement of life expectancy globally.[1] In 2020, approximately 19.3 million novel cancer diagnoses and nearly 10.0 million cancer-related fatalities were reported worldwide.[2] Neoplasms linked with socio-economic transitions exert considerable strain on the healthcare systems and economic frameworks, particularly in lower to middle income nations.[3] Conventional therapeutic approaches, such as radiotherapy and chemotherapy, possess inherent constraints.[4–7] For instance, chemotherapy inflicts DNA damage upon cancer cells, impeding their ability to proliferate. However, it also has the potential to harm normal cellular DNA and disrupt cell division, leading to significant health hazards.[8] This underscores the necessity of investigating innovative therapeutic strategies to optimise patient prognosis and counteract the expansive global impact of cancer.

The pivotal functions of immune cells stem from their inherent ability to swiftly identify and eliminate a diverse array of threats encompassing pathogenic and neoplastic agent-affected cells.[9] In this context, APCs, specifically DCs, emerge as central orchestrators in instigating the commencement of immune reactions.[10]

Serving as professional APCs, DCs bridge the innate and adaptive immune responses.[10] Pattern recognition receptors (PRRs) are a set of receptors that are expressed by APCs such as DCs and play a crucial role in the antigen recognition process.[11] PRRs' interactions with pathogen-associated molecular patterns carried by invading pathogens initiate the process of DC stimulation and maturation.[12] Similarly, the immune response can be triggered by PRR signalling mediated *via* the recognition of damage-associated molecular patterns released by damaged cells or stressed cells.[13] In addition, PRR signalling promotes the expression of DCs' antigen uptake receptors that enable antigen internalisation for subsequent processing and presentation. Hence, the presence of PRR-stimulating molecules in vaccine formulations is crucial for antigen recognition and immune response induction.

Once a tumour cell is pinpointed, APCs engulf and process it, presenting antigens to other immune cells such as $CD8^+$ T lymphocytes (Fig. 1).[14] Subsequently, $CD8^+$ T cells transform into specialised cytotoxic T lymphocytes (CTLs) that are capable

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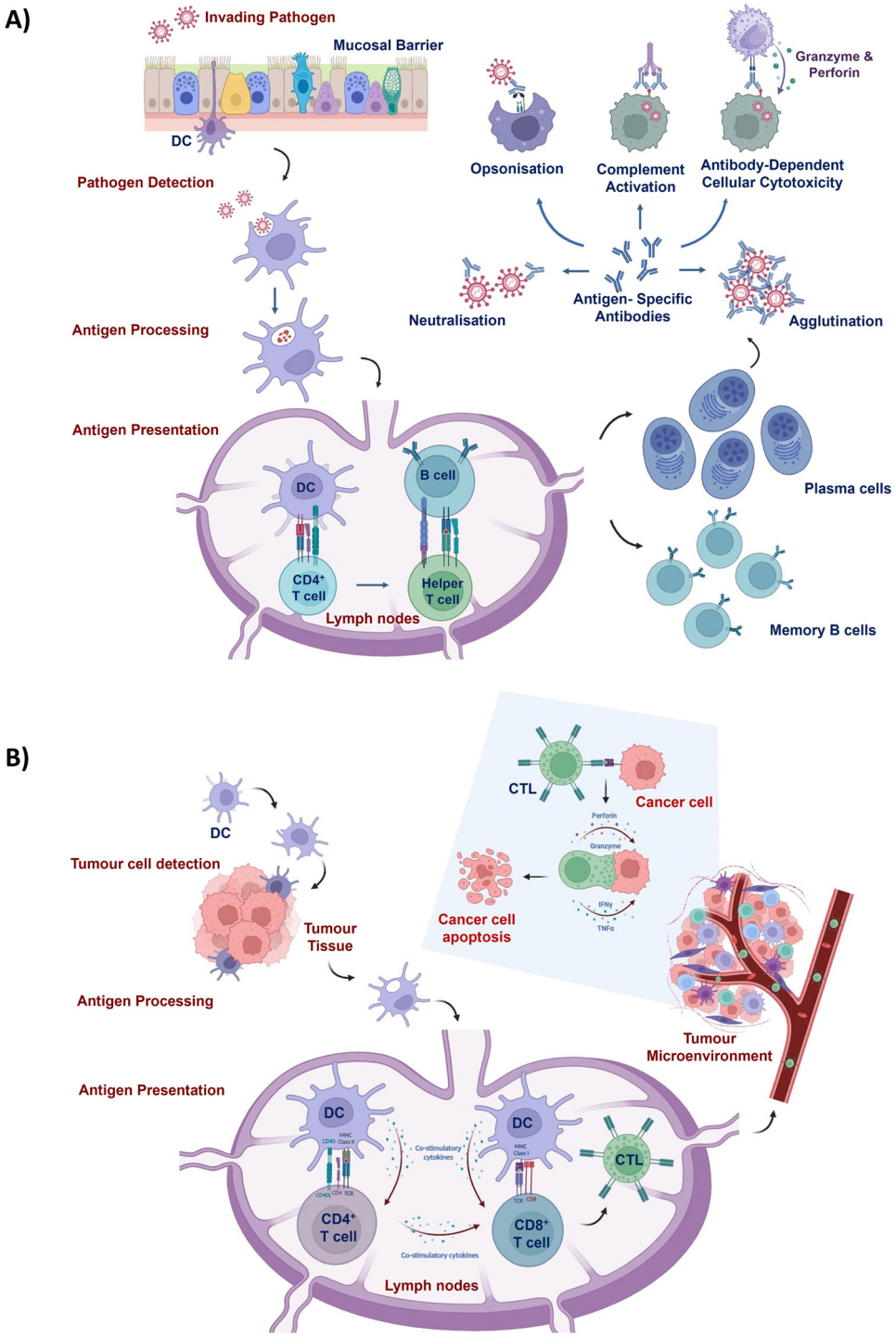

Fig. 1 DC-mediated immune responses. (A) DC-mediated pathogen detection and B cell activation. Following invading pathogen detection and consequent antigen presentation by APCs such as DCs, $CD4^+$ T cells undergo differentiation into $CD4^+$ T-helper cells that provide essential stimulatory signals to B cells. Activated B cells differentiate into plasma cells and memory cells that immediately execute humoral adaptive immune response and provide long-term protection, respectively. Plasma cells produce antigen-specific antibodies that enhance immunogenicity and detection by immune cells. These antibodies neutralise pathogens, enhance phagocytosis by macrophages, and activate the complement system that disrupts pathogen integrity. (B) DC-mediated antitumour immune response induction. DCs actively survey the local environment and engulf the detected tumour cells to present their antigens through the major histocompatibility complex class (MHC) I and II to $CD8^+$ and $CD4^+$ T cells, respectively. Upon activation, $CD4^+$ T cells further stimulate $CD8^+$ T cells that differentiate into tumour-specific cytotoxic T lymphocytes (CTL). Once tumour cells are detected, the stimulated CTLs induce tumour cell killing as part of the antitumour immune response.

of selectively eliminating cancer cells. Stimulated CTLs possess the ability to undertake comprehensive surveillance of the entire body to identify cells expressing specific tumour antigens, exclusively targeting cancer cells for annihilation while preserving the integrity of normal cells. The profound safety margin exhibited by this approach, coupled with its

efficacy in eliminating both localised and metastasised tumours, has spurred a growing interest by leveraging the body's immune process against cancerous cells. Nonetheless, tumour progression coincides with the emergence of immune suppressive mechanisms that pose substantial impediments to immune cell activity.[15] As a result, imperative strategies aimed at bolstering the antitumour immune response have become indispensable to surmount the compromised immune milieu and effectuate the eradication of established neoplastic growths.

Immunologically mediated mechanisms hold the potential to confer therapeutic benefits in dealing with proliferating tumour tissue, as well as to establish a defensive barrier against impending infectious agents[16,17] (Fig. 1). To amplify the immune response of prophylactic vaccine formulations, incorporation of natural or synthetic immune adjuvants is imperative.[18] The effective elicitation of safeguarding immunity against pathogens, such as the emergent severe acute respiratory syndrome coronavirus (SARS-CoV), necessitates the administration of prophylactic vaccines in a profoundly immunogenic state.[19] Moreover, the feasibility of administering additional vaccine booster doses is circumscribed by concerns over heightened reactogenicity and inflammatory reactions associated with the administered vaccines.[20] Accordingly, there is a call for alternative strategies to augment prophylactic immune responses against infectious diseases.

Multiple research endeavours have explored the application of peptide nanofibers as carriers for nano-vaccines, encompassing a spectrum of investigations involving model antigens, antigens sourced from infectious agents, antigens derived from tumours, and/or immune adjuvants.[21–26] Concomitantly, in-depth scrutiny has been dedicated to the examination of physicochemical attributes inherent to these nanofibers, including surface charge and mechanisms underpinning immune enhancement. Previous studies have ascertained the pronounced capacity of nanofibers to notably amplify the uptake of antigens by APCs.[27,28] Moreover, these studies have unveiled the potential of nanofibers to assume the role of immune adjuvants upon interaction with APCs. This could offer the prospect of potentially diminishing the necessity for concurrent administration of supplementary immune adjuvants alongside the antigen, while upholding the desired degree of immune response potency.

In the present review, we undertake a critical evaluation of the capabilities exhibited by peptide nanofibers in their role as nanocarriers for vaccines. Furthermore, we carried out a comprehensive assessment of peptide nanofibers' latent potential to catalyse transformative advancements in the domain of nanovaccinology. This exploration is approached through a multifaceted lens, encompassing innovative inclusion of immune modulators and the exploration of alternate administration routes.

2. Peptide nanofibers

Nanoscale materials have been used in crafting both preventive and remedial vaccine formulations.[29,30] Depending on the constituent materials and the methods of synthesis, nanomaterials can manifest as diverse nanostructures, such as nanospheres, nanorods, nanotubes, and nanosheets.[31–41] Among these, peptide nanofibers have emerged as prominent nanoarchitectures that arise from the spontaneous arrangement of peptide chains into secondary structures such as α-helical structures, peptide micelles or β-sheets, forming distinct patterns.[42,43] The design and evaluation of self-assembled peptide nanofibers have been the subject of prior reviews.[44,45] Peptide nanofibers constitute inherently self-assembled nanostructures that leverage the innate capacity of their molecular constituents to engage in spontaneous cohesion through intermolecular interactions (Fig. 2).

2.1. Structures

2.1.1. Coiled-coils. Peptide chains forming a secondary α-helical structure rely on the formation of intramolecular hydrogen bonds within the peptide backbone to maintain the α-helical configuration.[46,47] Driven by an interplay of factors, including amino acid sequence and concentration as well as the applied temperature and pH, α-helical peptides can self-assemble into nanofibers.[46] The structural integrity of the self-assembled peptide chains is preserved *via* the establishment of intermolecular hydrogen bonds between the adjacent chains. These self-assembled helical structures can be described as chains aligned in parallel and stacked atop one another, ultimately forming the nanofiber structure. Altering the pH and temperature of the self-assembly conditions or the amino acid sequence can induce variations in the intensity of intermolecular hydrogen bonding that can impact the structure and stability of the resulting nanofiber structures.[48–50] Several additional factors exert significant influence over the self-assembly process. Varying the length of the α-helical peptide chains can adjust the equilibrium between intermolecular and intramolecular interactions, thereby fundamentally impacting the self-assembly dynamics.[51] Moreover, the presence of high ionic strength in the assembly conditions can limit the orderly arrangement and peptide nanofiber formation *via* the induction of electrostatic interactions among the peptide chains.[52] The solvent employed also plays a pivotal role by modifying the peptide chains' hydrophobic interaction, hence affecting the shape of the resulting structures.[53] These factors can significantly impact the complex interplay of molecular forces that govern the self-assembly processes and the nanoscale structure formation.

2.1.2. Cylindrical micelles. Peptide amphiphiles consist of a hydrophilic peptide sequence linked to a hydrophobic chain capable of spontaneous assembly into supramolecular self-assorted nanostructures such as spherical micelles.[54] In aqueous conditions, the genesis of peptide micelles arises from the hydrophobic effect, which facilitates the aggregation of the hydrophobic tails at the core site, leaving the hydrophilic peptide sequences exposed to the surrounding aqueous solvent.[55] Subsequently, the transition of these spherical micelles into nanofibers is steered by intermolecular hydrogen bonding among the amino acids. The formed micelles undergo self-organisation and serve as nuclei that allow the growth and extension of nanofibers measuring nanometers in diameter

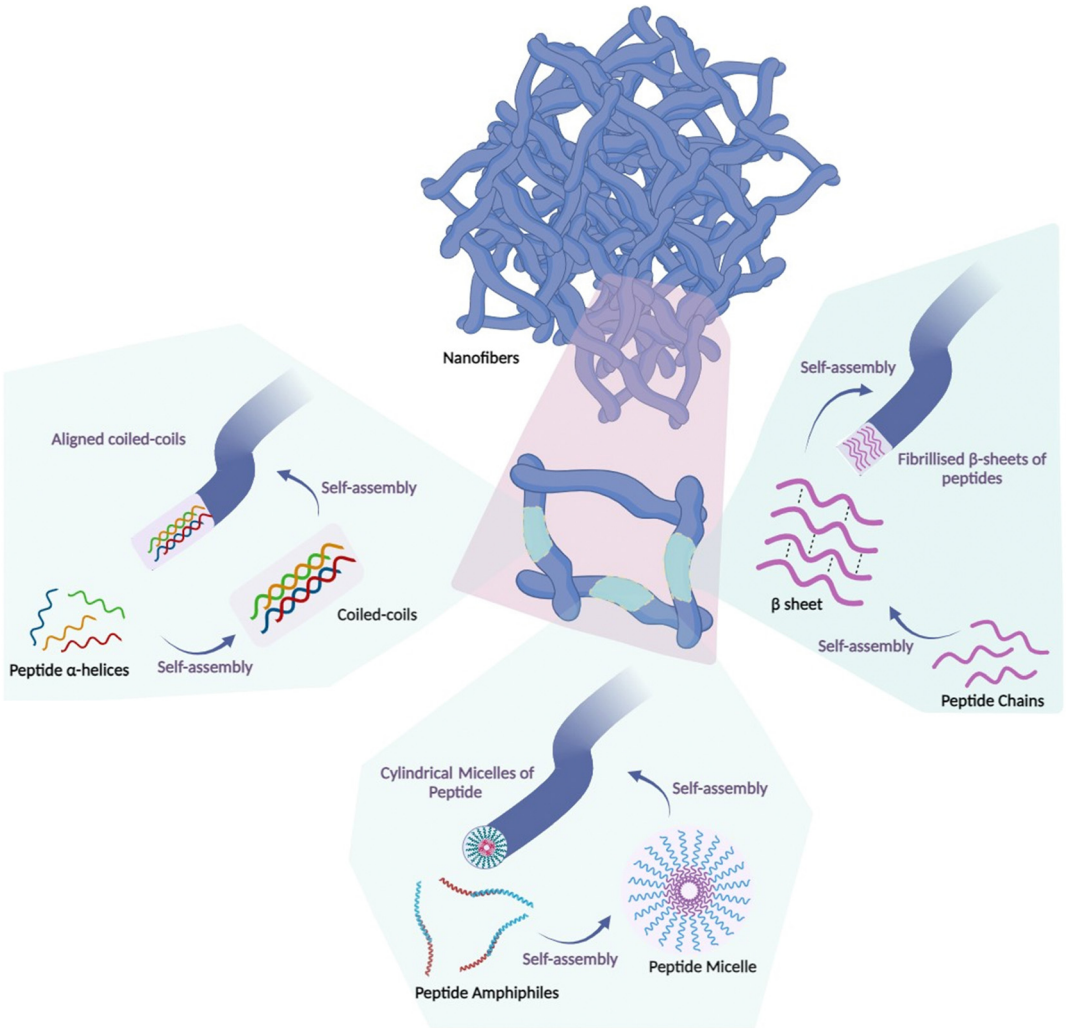

Fig. 2 Peptide nanofiber structures. Peptide nanofibers are self-assembled nanoscale materials formed from spontaneous arrangement of α-helices, peptide micelles or β-sheets into a well-defined fibril pattern, driven by intermolecular interactions such as hydrogen bonding. Peptide chains adopting a secondary α-helical structure depend on the creation of intramolecular hydrogen bonds connecting the carbonyl oxygen and amide hydrogen functional groups along the peptide backbone. Coiled-coils of peptides established by intermolecular hydrogen bonds between neighboring α-helices possess the ability to self-assemble into nanofibers *via* parallel orientation and sequential stacking. The key step in β-sheet formation is the interaction between peptide chains that align side by side, with the amide and carbonyl groups of adjacent strands forming intermolecular hydrogen bonds. This structural conformation results in highly ordered, stable nanofibers with various potential applications. Peptide amphiphiles are molecular constructs consisting of a hydrophilic peptide sequence tethered to a hydrophobic chain. This structural arrangement provides them with the capacity to self-orient into spherical micelles when placed in aqueous solutions. This self-assembled structure is maintained by intermolecular hydrogen bonding interactions among neighboring amino acids. The resulting micelles serve as nucleation sites for the additional self-assembly of micelles, culminating in the development of a fibrous pattern characterised by dimensions on the order of nanometers in diameter and micrometers in length.

and several micrometers in length.[56,57] The size and length of the resulting nanofibers can be influenced by the intensity of the intermolecular hydrogen bonding among the adjacent spherical micelles.[56,57] In addition, the peptide amphiphiles' self-assembly process can be controlled by other variables such as the contained amino acid sequence, hydrophobic domain size, hydrophilicity of incorporated spacers, applied temperature, and solvent polarity.[58,59] These factors can dramatically affect the formation as well as the properties of the micelle-based peptide nanofibers.

2.1.3. Fibrillised β-sheets. Peptide β-sheets are morphologically characterised by the presence of peptide chains arranged either parallelly or antiparallelly that are structurally stabilised by hydrogen bonding between the amino acid residues.[60–62] With the aid of intermolecular hydrogen bonds, spontaneous alignment and aggregation of these β-sheet structures form the foundation for the construction of fibrillised peptide chains.[60,61,63] Additionally, hydrophobic interactions exert their influence by facilitating the cohesion of nonpolar segments within peptides, thereby contributing to the overall process of self-assembly.[64] The equilibrium between hydrogen bonding and hydrophobic interactions is instrumental in maintaining the structures of the fibrillised β-sheets and nanofibers. The assembly of self-assembling peptides into these

fibrillised β-sheets and nanofibers can be highly impacted by several critical factors such as the contained amino acid sequence, solvent polarity, pH, temperature and added salt concentration.[62,65] The amino acid sequence can alter the peptide chains' tendency to form β-sheets. The applied temperature can further modulate the assembly kinetics that shape the self-assorted structures. Furthermore, the used salt concentration can also alter the peptide solubility and the subsequent ability to self-aggregate. Additionally, the pH of the self-assembly conditions can exert a substantial impact by altering the amino acids' degree of ionisation and, consequently, the molecular interactions. These variables have the potential to modulate the mode and strength of the interchain interactions, thereby orchestrating the distinctive attributes of the resultant peptide nanofibers. To this end, solvent conditions act as a critical determinant, either enhancing or reducing peptide chain interactions that constitute the driving force of the self-assembly process.

2.2. Influence of peptide nanofibers' properties on immune modulation

Understanding the influence of peptide nanofibers' properties on immune modulation is crucial for optimising vaccine design and immune-based therapies. In this section, we provide a comprehensive exploration of the multifaceted relationship between the peptide nanofibers' morphological and physicochemical properties and their impact on immune modulation.

2.2.1. Morphological properties. The effects of peptide chains' morphological features on the ensuing immune response were previously assessed. Fries *et al.* studied the impact of the structural characteristics of nanofiber-forming peptides on the magnitude of the evoked immune response, using the fibre-forming peptide Coil29.[66] The results showed that mice subjected to immunisation with short Coil29, featuring terminal hydrophilic residue capping and $OVA_{257–264}$ linkage, demonstrated a heightened $CD8^+$ T cell response in comparison to the long uncapped variant. Furthermore, the longer nanofiber exhibited a more efficacious internalisation by APCs *in vitro*.

Previous studies had also shed light on the influence of the self-assembly process on the peptide carrier's immune modulation capabilities. Rudra *et al.* showed that mice subjected to subcutaneous immunisation using free $OVA_{323–339}$ or $OVA_{323–339}$ linked to nanofibers comprised of the β-sheet-forming SGSG-Q11 peptide combined with CFA manifested a comparable and robust OVA-specific IgG response that endured for a span of 52 weeks.[67] Furthermore, parallel *in vivo* investigations revealed commensurate $CD4^+$ T cell proliferation. Notably, $OVA_{323–339}$-specific IgG detection witnessed a reduction in T cell receptor-knockout mice, implying an imperative role of $CD4^+$ T cells in its initiation. On the other hand, mice immunised with the $OVA_{323–339}$-loaded non-fibril-forming P3-Q11 peptide showed a reduced IgG response in contrast to those receiving the $OVA_{323–339}$-incorporated fibril-forming Q11 peptide, underscoring the dependence of immune response elicitation on the formation of peptide β-sheet secondary structure. Remarkably, mice subjected to immunisation with $OVA_{323–339}$ covalently tethered to KFE8 (an alternate nanofiber) exhibited an elevated magnitude of specific IgG responses compared to those receiving separate injections of unlinked $OVA_{323–339}$ and KFE8.

In a more advanced study, Wu *et al.* assessed the inherent immunogenicity of two distinct self-assembled peptide nanocarriers using the loaded OVA as a model antigen.[68] In the conducted comparative studies, α-helix-based nanofibers (Coil29) induced higher levels of OVA-specific IgG in murine subjects than β-sheet-based (Q11) nanofibers. The study highlighted that Coil29 nanofibers were more readily internalised by DCs from immunised mice, than Q11 nanofibers. Moreover, Coil29 nanofibers were more effective in the costimulatory molecules CD80 and CD86 expression upregulation on DCs as well as antibody response induction. It was suggested that the T cell epitope inherently contained in the Coil29 nanofibers' structure contributed to their observed increased immunogenicity. These findings propose a promising strategy for enhancing the immune responses by incorporating specific T cell assistance within the self-assembling units of fibrillar peptide materials.

These studies set the stage for in-depth mechanistic investigations. Exploring how altered nanofiber characteristics impact cellular internalisation, endosomal escape, and antigen presentation could provide crucial insights into the immune response mechanisms, enabling more efficient vaccine designs. A thorough analysis of these studies underscores the prospective merits conferred to vaccine formulations through the integration of peptide nanofibers as adept cargo carriers.

2.2.2. Physicochemical properties. Understanding the effect of peptide nanofibers' physicochemical properties on immune response elicitation has sparked considerable attention. Wen *et al.* explored the immune responses triggered by $OVA_{323–339}$ complexed with β sheet-forming Q11 peptide-based nanofibers that were characterised by diverse charges.[69] The induction of immune response *via* subcutaneous immunisation of mice using positively charged nanofibers-$OVA_{323–339}$ resulted in elevated levels of OVA-specific IgG, surpassing those observed with negatively charged nanofibers-$OVA_{323–339}$. Correspondingly, the peak levels of IFN-γ and IL-4 were recorded within *ex vivo* stimulated lymphocytes from mice treated with positively charged nanofibers-$OVA_{323–339}$. Additionally, the uptake of positively charged nanofibers-$OVA_{323–339}$ by APCs was superior, as evidenced by both *in vitro* and *in vivo* assessments.

In a separate study, the immunogenic potential of nanofibers constructed using the self-assembled positively charged Nap-GDFDFDYDK (YK) peptide or the negatively charged Nap-GDFDFDYDE (YE) peptide was investigated by Yang *et al.*[70] Mice subcutaneously injected with nanofibers exhibited an increase in OVA-specific IgG levels. Notably the YK-conjugated OVA induced a more pronounced IgG response than YE-OVA. *In vitro* assays using FITC-OVA revealed that nanofibers enhanced the internalisation of OVA by DCs, where the uptake of positively charged YK-OVA by DCs surpassed that of negatively charged YE-OVA.

The impact of nanofiber's hydrophobicity on the immune response induced using the β-sheet-forming peptide sequence

FVIFLD was also studied.[71] The investigation revealed that the *in vitro* immune response, triggered by a covalently attached $OVA_{257–264}$ antigen, was more potent when associated with a short rather than long oligo-ethylene glycol chain. The authors suggested in another study that long hydrophilic chains incorporated into the peptide nanofiber structure could limit the hydrophobic interaction with the APCs' cell membrane.[72] These studies investigated the effects of peptide nanofiber components on cellular interactions, aiming to bridge the gaps between materials science and immune modulation.

3. Peptide nanofibers as a vaccine carrier

3.1. Antigen and/or immune adjuvant loading approaches

The inherent characteristics of amino acids in the peptide chain can facilitate covalent bonding such as disulfide bridges or non-covalent interactions *via* hydrophobic interactions or hydrogen bonding through various means.[73–76] For example, non-covalent interactions can be prompted by adjusting the peptide-surrounding factors such as pH or temperature. The structural attributes and physicochemical characteristics inherent to peptide nanofibers are subject to multiple influences, including the amino acid sequence delineating the peptide chains and the specific conditions governing their self-assembly. The distinctive configuration and chemical attributes exhibited by peptide nanofibers facilitate the entrenchment of antigens and/or immune adjuvants in a manner that offers versatility (Fig. 3). This is manifested in the potential for antigens and/or immune adjuvants to be integrated through non-covalent interactions with the molecular domains of the peptide nanofibers. Moreover, the functional moieties inherent in the amino acid constituents that underpin the nanofiber architecture offer the prospect of covalent incorporation of antigenic fragments and/or immune adjuvants. An integral advantage of peptide nanofibers resides in their amenability to accommodate and dispense numerous copies of the antigen and/or immune adjuvant, which could ultimately elevate intracellular concentrations and augment the effectiveness of the resultant immune response.[22,77]

Notably, the nanoscale dimensions characterising peptide nanofibers hold the potential to expedite lymphatic drainage, thereby facilitating accessibility to APCs.[78] Furthermore, the compositional makeup of peptide nanofibers, which undergo degradation into their constituent amino acids, underscores their inherent biocompatibility.[79]

3.2. Validation of vaccine deliverability using model antigens

The potential of peptide-based nanofibers as a vaccine carrier, with a focus on model antigens such as the epitope derived from ovalbumin (OVA), has been previously explored (Table 1). Using nanofibers constructed using a fibrillised β-sheet forming peptide composed of the SGSG (spacer)-linked QQKFQFQFEQQ peptide (Q11, fibril forming or assembly domain), Rudra *et al.* conducted a study in which $CD4^+$ T-cell epitope $OVA_{323–339}$ was employed as a model antigen.[80] Through an *in vivo* evaluation, it was elucidated that mice administered with nanofiber-$OVA_{323–339}$ *via* subcutaneous injection demonstrated OVA-specific immunoglobulin G (IgG) levels akin to those exhibited by mice immunised with a blend of $OVA_{323–339}$ and complete Freund's adjuvant (CFA). Furthermore, mice immunised with nanofiber-$OVA_{323–339}$ displayed heightened OVA-specific immunoglobulin M (IgM) levels in comparison to their counterparts vaccinated with the $OVA_{323–339}$ and CFA amalgamation.

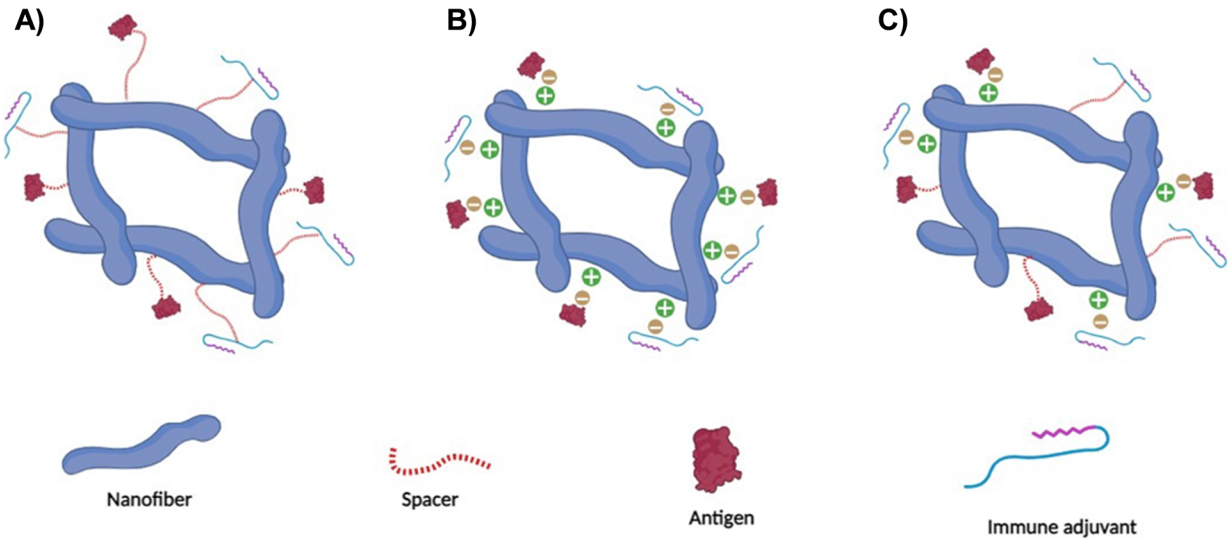

Fig. 3 Antigen and/or immune adjuvant incorporation onto peptide nanofibers. (A) Covalent conjugation. This approach relies on the covalent interaction between the chemical functional groups contained in the peptide chains along with the antigen and/or immune adjuvant. (B) Non-covalent conjugation. This strategy utilises the electrostatic or hydrophobic interaction to facilitate loading of the antigen and/or immune adjuvant onto nanofiber chains. (C) Hybrid binding. The versatile peptide nanofiber surface could be harnessed in the concurrent incorporation of the antigen and/or immune adjuvant *via* both covalent and non-covalent conjugation methods.

Table 1 Model antigens delivered *via* peptide nanofibers

Nanofiber	Antigen	Immune adjuvant	Ref.
VVAGKK	OVA[a]	—	21
Lauryl-VVAGKK-biotin	OVA[a]	CpG[a]	22
SGSG – Z1	$OVA_{257–264}$[b]	—	81
Q11	GFP or cutinase enzyme[b]	—	76
SGSG – Q11	$OVA_{323–339}$[b]	—	80
GGAAY – Q11	$OVA_{257–264}$[b]	—	82
p-Nitrophenyl phosphonate-Q11	GFP[b]	—	83

Abbreviations: OVA, ovalbumin; $OVA_{257–264}$, class I-restricted peptide epitope of ovalbumin; $OVA_{323–339}$, class II-restricted peptide epitope of ovalbumin; GFP, green fluorescent protein; CpG, cytidine-phosphate-guanosine-containing oligodeoxynucleotides; SGSG, spacer; Z1, RVQVRVQVRVQV; Q11, QQKFQFQFEQQ. [a] Non-covalently conjugated. [b] Covalently conjugated.

Chen *et al.* presented findings wherein the impact of $OVA_{323–339}$ linked to nanofibers formed using fibrillised β-sheet-forming SGSG-Q11 on J774.1 macrophage viability was examined through *in vitro* incubation at concentrations spanning from 0.007 to 0.7 mg ml^{-1} over a duration of 4 hours.[77] Notably, the administration of nanofiber-$OVA_{323–339}$ to mice did not elicit localised inflammatory responses, which were conversely observed with alum-adjuvanted $OVA_{323–339}$. Subsequent to intraperitoneal administration to mice, nanofibers were shown to heighten the uptake of $OVA_{323–339}$ by DCs and macrophages, as discerned through flow cytometric analysis conducted 20 hours post-administration. Comparative analysis with $OVA_{323–339}$ alone unveiled that subcutaneous injection of nanofiber-$OVA_{323–339}$ into mice substantially bolstered the differentiation of $CD4^+$ T cells, evinced by elevated expressions of PD-1 and CXCR5. Additionally, nanofiber-$OVA_{323–339}$ exhibited a marked escalation in the production of interferon-gamma (IFN-γ) and interleukin-4 (IL-4) upon *ex vivo* stimulation of lymphocytes. It was also observed that immunisation involving alum-adjuvanted nanofiber-$OVA_{323–339}$ demonstrated enhanced efficacy in inducing T cell differentiation and cytokine production. Furthermore, immunisation with nanofiber-$OVA_{323–339}$ resulted in an augmented percentage of differentiated B cells specific to $OVA_{323–339}$ ($GL\text{-}7^+$ and FAS^+ cells), along with elevated $OVA_{323–339}$-specific IgG titers, in comparison to vaccination utilising alum-adjuvanted $OVA_{323–339}$.[77] The non-inflammatory nature of peptide nanofibers could render them a promising candidate not only for prophylactic or therapeutic vaccines but also for immune therapies targeting autoimmune disorders, where excessive inflammation can worsen the symptoms.

In another study, Zhang *et al.* presented the $CD8^+$ T-cell epitope ($OVA_{257–264}$) to DCs using peptide nanofibers formulated using the β-sheet forming RVQVRVQVRVQV peptide (Z1)-conjugated SGSG spacer.[81] The nanofiber-$OVA_{257–264}$ demonstrated a discernible augmentation in the presentation of $OVA_{257–264}$ by DCs to $CD8^+$ T cells *in vitro*. Murine subcutaneous immunisation with nanofiber-$OVA_{257–264}$, as well as $OVA_{257–264}$ alone, yielded commensurate *in vivo* T-cell proliferation outcomes.

Furthermore, Chesson *et al.* studied the covalent conjugation of $OVA_{257–264}$ with Q11-based nanofibers.[82] The implementation of nanofiber-$OVA_{257–264}$ instigated heightened proliferation of OVA-specific $CD8^+$ T cells in mice, as evidenced by flow cytometric analysis of lymphocytes within lymph nodes subsequent to footpad immunisation. This effect was notably superior to the administration of $OVA_{257–264}$ alone or its co-administration with incomplete Freund's adjuvant. This discernment was substantiated by the observation of mice attaining protection against the infectious OVA-expressing influenza PR8 virus subsequent to vaccination with nanofiber-$OVA_{257–264}$, in contradistinction to the outcomes achieved by mice subjected to exclusive $OVA_{257–264}$ vaccination or its combination with incomplete Freund's solution.[82]

Hudalla *et al.* achieved successful conjugation of green fluorescent protein (GFP) to β sheet forming *p*-nitrophenyl phosphonate-linked Q11, leading to the formation of nanofibers that elicited an augmented antibody response targeting GFP within murine subjects.[83] Furthermore, the same research team demonstrated the capability of these nanofibers to enhance the production of antibodies specific to GFP or cutinase subsequent to subcutaneous administration in mice.[76] The administration of nanofibers physically mixed with GFP yielded a significantly diminished specific antibody response compared to treatment involving nanofiber-bound GFP. Through the utilisation of an alternative amphiphilic peptide sequence, specifically VVAGKK, nanofibers were successfully conjugated with OVA using a biotin–streptavidin interaction mechanism, resulting in the induction of a $CD8^+$ T cell response that was specific to OVA within murine subjects.[21]

The non-covalent conjugation of OVA and CpG (an immune adjuvant) to lauryl and biotin-modified amphiphilic VVAGKK peptides by Tohumeken *et al.* unveiled the capacity of CpG-nanofiber-OVA constructs to augment the uptake of CpG by DCs.[22] Subsequent outcomes included the elicitation of cytokine production, an upregulated expression of CD40 and CD86 co-stimulatory molecules, and an enhancement in antigen presentation by murine splenocytes during *in vitro* assessments. Furthermore, administration of the nanofiber construct led to a heightened production of OVA-specific IgG after subcutaneous immunisation with CpG-nanofiber-OVA in mice. Following *ex vivo* restimulation of splenocytes with OVA after immunisation with CpG-nanofiber-OVA constructs, a notable augmentation in cellular proliferation and an increased production of IFN-γ became evident. Moreover, the CpG-nanofiber-OVA entities exhibited the ability to enhance IFN-γ production within $CD8^+$ T cells upon *ex vivo* re-stimulation with OVA. The non-covalent attachment of antigens to the nanofiber system could offer modularity and versatility. This flexibility can be advantageous in creating vaccines for various diseases, including emerging infectious diseases, as it enables the rapid development and adaptation of vaccines to changing pathogenic threats.

The research studies presented in this section highlight the potential and versatility of peptide-based nanofibers as potent vectors for antigen delivery and immune modulation. The investigations discussed consistently demonstrated the ability

of these nanofiber constructs to effectively enhance the immune responses triggered by various antigenic epitopes. The utilisation of different peptide sequences, such as Q11 and VVAGKK, in conjunction with model antigens including OVA and GFP, revealed the capacity of nanofibers to stimulate robust and targeted antibody and T-cell responses. Moreover, the integration of immune adjuvants such as CpG further amplified the immunogenicity of the nanofiber constructs, facilitating enhanced antigen presentation, cytokine production, and cellular proliferation.

3.3. Applications

3.3.1. Infectious disease vaccines. The delivery capabilities inherent in peptide nanofibers with respect to antigens derived from infectious agents were previously investigated within more complex and demanding vaccination strategies (Table 2). For example, FLIVIGSIIGPGGDGPGGD (H9e) amphiphile-based nanofibers synthesised from a combination of an elastic segment of spider silk and a trans-membrane segment of the human muscle L-type calcium channel were examined for their capacity to facilitate the delivery of the killed H1N1 swine influenza virus.[84] The results showed that mice subjected to immunisation with the nanofiber-H1N1 virus or those administered an oil-based adjuvant-mixed H1N1 virus displayed comparable levels of H1N1-specific IgG1 antibodies.

Harnessing the H9e peptide amphiphiles, Li *et al.* studied the use of peptide nanofibers as an adjuvant to enhance the efficacy of the modified live porcine reproductive and respiratory syndrome virus (PRRSV).[85] The implementation of the nanofiber formulation exhibited a capacity to prolong the duration of PRRSV circulation, a phenomenon substantiated through the detection of viral load in pig serum *via* polymerase chain reaction subsequent to intramuscular vaccination. Notably, the nanofiber-PRRSV vaccine demonstrated the ability to elicit a state of protective immunity against both the VR-2332 and MN184A strains of PRRSV. This was evidenced by a discernible reduction in the circulating viral load. In stark contrast, vaccination involving PRRSV alone conferred protection solely against VR-2332 infection. Remarkably, administration of the nanofiber-PRRSV vaccine yielded notably heightened proportions of memory helper T cells ($CD4^+$ $CD8^+$) alongside correspondingly reduced proportions of regulatory T cells ($CD4^+CD25^+FoxP3^+$), as compared to vaccination employing PRRSV alone.[85]

In a study by Rudra *et al.*, the immunogenicity of the malaria peptide epitope $(NANP)_3$ when conjugated to the self-assembling fibrillised-β sheet-forming peptide Q11 was assessed in a murine model.[86] Their findings indicated that when mice were immunised with equivalent quantities of Q11 devoid of the $(NANP)_3$ epitope, no antibody response was observed, even after a booster dose, underscoring the essential role of the epitope in antibody generation. Further, the research highlighted that the initiation of an antigen-specific antibody response in mice immunised subcutaneously necessitated the involvement of T cells and the activation of the myeloid differentiation primary response gene 88 (MyD88), without the need for TLR2 or TLR5 interactions. Importantly, when mice were exposed to malaria sporozoites and subsequently administered a booster of the vaccine containing $(NANP)_3$-Q11 nanofibers, there was a pronounced presence of antigen-specific antibodies, an observation not seen in unboosted mice.[86] While the study focuses on the $(NANP)_3$ epitope from *Plasmodium falciparum circumsporozoite* that could possess significantly different immunogenicity in humans, the ability to co-assemble different epitope-bearing peptides opens avenues for developing multi-epitope vaccines. This approach could enhance the immune system's ability to target multiple strains or variants of a pathogen, ultimately leading to increased effectiveness against diseases with high antigenic variability.

In a different study, Pompano *et al.* established a covalent linkage between PADRE (a $CD4^+$ T cell epitope) and E214 (a B

Table 2 Vaccines against infectious diseases delivered *via* peptide nanofibers

Nanofiber-forming peptide	Antigen [conjugation]	Immune adjuvant	Ref.
KFE8	Ag85B[a]	—	23
Q11	HIV envelope-derived-antigen gp120[a]	Squalene,[b] R848[b] and CpG[b]	24
SGSG-Q11	$(NANP)_3$[a]	—	86
SGSG-Q11	PADRE ($CD4^+$ T cell epitope)[a] and E214[a]	—	87
SGSG-Q11	TNF4-23 (B cell epitope)[a] and PADRE ($CD4^+$ T cell epitope)[a] or vaccinia I1L7-21 ($CD4^+$ T cell epitope)[a]	—	89
GGAAY-KFE8	Ag85B[a]	—	88
H9e	Killed H1N1 swine influenza virus[b]	—	84
H9e	PRRSV[b]	—	85
Nap-GFFY-OMe	DNA encoding the HIV-1 envelope protein gp 145[b]	—	90
GGAAY-KFE8	HSV gB peptide[a]	CpG[b] or gardiquimod[b]	92
$(RADA)_4$ oligopeptide	rHBsAg[b]	CpG[b]	73
SGSG – EAK16-II	SL9[a]	R848[b]	91

Abbreviations: H9e, FLIVIGSIIGPGGDGPGGD; KFE8, FKFEFKFE; EAK16-II, AEAEAKAKAEAEAKAK; GGAAY, spacer; Ag85B, *mycobacterium tuberculosis*-derived $CD4^+$ T cell epitope; E214, *Staphylococcus aureus* B cell epitope; HSV gB peptide, herpes simplex virus-derived $CD8^+$ T cell epitope; rHBsAg, recombinant hepatitis B virus surface antigen; SL9, HIV-1 CTL epitope; $(NANP)_3$, malaria peptide epitope; R848, resiquimod. [a] Covalently conjugated. [b] Non-covalently conjugated.

cell epitope derived from *Staphylococcus aureus*) with the fibrillised-β sheet-forming SGSG-Q11.[87] Mice that underwent subcutaneous vaccination with the co-assembled PADRE-Q11 nanofiber showed a significant elevation in the $CD4^+$ $PADRE^+$ $CD44^+$ T cell count within the lymph nodes, in contrast to those vaccinated with PADRE alone. Remarkably, mice administered the nanofiber-conjugated PADRE and E214 demonstrated an E214-specific IgG titer comparable to that seen in mice vaccinated with a combination of E214 linked to a diphtheria toxin carrier and alum. The results also illustrated the potential importance of epitope content modulation in influencing the magnitude and nature of the immune response.[87] Utilising modular self-assembly to incorporate B- and T-cell epitopes into vaccines offers a promising avenue for the construction of efficient vaccines. By precisely tailoring immune responses, this approach could lead to the development of vaccines capable of eliciting appropriate and protective immune responses against a wide range of diseases.

Rudra *et al.* synthesised a conjugate that incorporated the GGAAY spacer and the β sheet-forming FKFEFKFE (KFE8) peptide, which was covalently linked with the $CD4^+$ T cell epitope derived from *Mycobacterium tuberculosis* (Ag85B). The study sought to elucidate the fundamental mechanisms governing the initiation of the immune response.[88] Notably, inhibiting autophagy within APCs resulted in a significant reduction in the enhanced $CD4^+$ or $CD8^+$ T cell responses induced by nanofibers *in vitro*. Furthermore, inhibition of proteasomal activity compromised the capacity of macrophages treated with nanofibers and antigens to effectively elicit stimulation of $CD8^+$ T cells. A recent study illustrated the ability of Ag85B delivered using KFE8-based β sheet-forming peptide nanofibers to significantly augment the $CD4^+$ T cell response in *Bacillus Calmette-Guérin* (BCG)-primed mice following intratracheal administration, suggesting the suitability of the peptide nanofibers for efficient pulmonary vaccination.[23] Current models highlight the need for multivalent tuberculosis vaccines capable of eliciting immune protection from both $CD4^+$ and $CD8^+$ T cells, along with functional B cells and other immune cell populations. Incorporating these components into a vaccine formulation poses a challenge. The KFE8 nanofiber construct provides a modular vaccine platform capable of accommodating additional immunogenic *Mycobacterium tuberculosis*-derived antigens and adjuvants. This modularity offers an opportunity to customise the vaccine composition for optimal immune responses against *Mycobacterium tuberculosis*. Mora-Solano and colleagues have demonstrated that the administration of β sheet-based nanofiber-conjugated TNF4-23 (a B cell epitope) alongside either PADRE (a $CD4^+$ T cell epitope) or vaccinia I1L7-21 (a $CD4^+$ T cell epitope) to mice led to the induction of antigen-specific IgG production and cytokine release from *ex vivo* stimulated lymphocytes.[89] Furthermore, mice that received nanofiber-based vaccinations and subsequently challenged with LPS exhibited reduced signs of inflammation, as evidenced by mitigated hypothermic responses and prolonged survival durations. The ability to elicit protective antibody responses without triggering strong T-cell responses that could lead to autoimmune reactions represents a significant advantage. These approaches pave the way for developing immune therapies that minimise adverse effects and ensure patient safety.

Peptide nanofibers were also previously studied as delivery vehicles for HIV-1 vaccines. Tian *et al.* conducted an investigation in which mice were vaccinated using intramuscular, intradermal, or subcutaneous injection with nanofibers conjugated to DNA encoding the helical HIV-1 envelope protein gp145.[90] This approach resulted in a more robust IgG response, alongside heightened production of IFN-γ and IL-4 by restimulated lymphocytes, in comparison to administering HIV-DNA alone. *In vitro* incubation of nanofiber-conjugated HIV-DNA with T cells, B cells, or macrophages for a duration of 72 hours did not exert an adverse influence on cellular viability. Importantly, the histological analysis of muscles, epidermis, and dermis following nanofiber administration showed no evident pathological features.

In order to improve vaccine efficacy, Ding *et al.* integrated the HIV-1 CTL epitope SL9 with the TLR7 and TLR8 agonist R848 (Resiquimod) using a β sheet-forming peptide nanofiber-based delivery approach.[91] The incubation of nanofibers carrying SL9 and R848 with peripheral blood monocyte-derived DCs showed the highest propensity for triggering the production of immune-stimulatory cytokines by $CD8^+$ T cells, sourced from individuals with HIV infection. Additionally, nanofibers demonstrated the capacity to facilitate the expansion of antigen-specific $CD8^+$ T cells subsequent to subcutaneous vaccination in murine subjects. Understanding the durability of these responses and the long-term effectiveness of the vaccine could provide insights into the longevity of the elicited CTL responses. Using fibrillised-β sheet-forming Q11-based peptide nanofibers conjugated to the HIV envelope-derived-antigen gp120, Chen *et al.* illustrated the capacity of a single dose of the nanofiber vaccine to enhance the capacity of the induced antibodies to bind to the heterologous HIV envelope antigen in rabbits.[24]

Rudra *et al.* studied the use of the GGAAY spacer-linked and fibrillised-β sheet-forming KFE8 peptide to develop a nanofiber-based vaccine formulation targeting herpes simplex virus (HSV).[92] The results showed that subcutaneous administration of the nanofiber-conjugated HSV-derived $CD8^+$ T cell epitope (HSV gB peptide) combined with CpG or Gardiquimod (TLR7 agonist) to mice induced an antigen-specific CTL response in an *in vivo* setting. Subsequent *ex vivo* re-stimulation of lymphocytes extracted from the immunised mice revealed significant production of IFN-γ. Using a β sheet-forming $(RADA)_4$ oligopeptide that self-assembles into nanofibers, Grenfell and colleagues demonstrated a novel approach where nanofibers facilitated the delivery of the recombinant hepatitis B virus surface antigen (rHBsAg) in conjugation with CpG.[73] This method yielded elevated levels of rHBsAg-specific IgG and IgM when compared to an alum-based combination of rHBsAg and CpG, following subcutaneous administration in murine subjects.

Collectively, the findings presented in this diverse array of studies demonstrated the multifaceted potential of peptide nanofibers as versatile vehicles for antigen delivery and immune modulation. Through their capacity to effectively

facilitate antigen transport and presentation, these nanofibers showed their utility across a spectrum of infectious disease models. From influenza and porcine viral infections to malaria, tuberculosis, HIV and herpes simplex virus, the results consistently highlighted the ability of peptide nanofibers to enhance immune responses, evoke antigen-specific antibody production, and promote cell-mediated immunity. Furthermore, the strategic incorporation of various adjuvants and epitopes into nanofiber formulations has unveiled novel avenues for fine-tuning immune outcomes. These studies presented the peptide nanofibers as a promising platform for next-generation vaccine development, offering a tailored approach to combatting a diverse range of infectious agents. Nevertheless, despite the encouraging outcomes observed in murine studies, transitioning to human clinical trials represents a complex challenge. Several variables, such as pre-existing immunity, age and health status, may significantly influence the vaccine's efficacy in human subjects. Addressing these factors requires careful consideration to ensure the successful translation of research findings into practical and effective immunisation strategies.

3.3.2. Cancer vaccines. The ability of peptide nanofibers to enhance antitumour immune responses has gained substantial attention (Table 3). Subcutaneous administration of coiled coil-forming QARILEADAEILR-AYARILEAHAEILRAQ (Coil29) peptide-based nanofibers incorporating PEPvIII (B cell epitope), Trp2 (melanoma-derived T cell epitope) and Td (tetanus-derived $CD4^+$ T cell epitope) dramatically retarded the growth of subcutaneous B16vII tumours in C57BL/6NHsd mice.[25] Song *et al.* developed a trivalent cancer vaccine using fibrillised-β sheet-forming FEFEFKFK peptide-based nanofibers incorporating various antigenic epitopes, namely glycoprotein 100209-217, tyrosinase369–377 and melanoma-derived MART-126-35.[26] The trivalent nanofiber vaccine augmented the $CD8^+$ T cell response and significantly delayed the growth of subcutaneously inoculated B16 cells in C57BL/6J mice. The ability to design vaccines tailored to specific epitopes represents a gateway to personalised cancer immunotherapies. Customising treatments based on individual tumour profiles could significantly improve the effectiveness of immunotherapeutic interventions.

Using the nanofibers constructed from amphiphilic peptide VVAGKS, Gunay *et al.* showed that nanofiber-mediated delivery of melanoma-derived glycosphingolipids and mannose exhibited efficient uptake by DCs that stimulated maturation signal initiation *in vitro*.[27] While DC activation is crucial, the complexity of immune responses *in vivo* poses challenges. Understanding the broader immune reactions elicited by these carriers, including potential regulatory mechanisms, is essential for predicting their overall therapeutic impact accurately. Mice subcutaneously immunised with MUC1 glycopeptides non-covalently mixed or covalently linked with nanofibers constructed using the β sheet-forming naphthylacetic acid-GFFYK peptide displayed heightened production of antigen-specific IgG, IgM, and immunostimulatory cytokines in comparison to counterparts immunised solely with MUC1 glycopeptides.[93] However, the covalently linked nanofiber-MUC1 glycopeptide formulation demonstrated superior potency compared to the noncovalent mixture. The antisera derived from mice immunised with covalent nanofiber-MUC1 glycopeptides exhibited the most pronounced level of complement-dependent cytotoxicity upon incubation with MCF-7 cells.

Amphiphilic peptide-based nanofibers were also used as carriers for the plasmid vector encoding a melanoma-specific tumour antigen and the immune adjuvant HMGN1-gp100.[94] Under physiological conditions, the nanofiber formulations exhibited sustained DNA retention of approximately 90% over a period of 14 days. Following subcutaneous administration, the co-delivery of DNA with the peptide MAX8 (VKVKVKV-$KV^DPPTKVEVKVKV$-NH_2) or HLT2 (VLMx0054;KVKKV^{D}PP-TKVEVKVMx004C;V-NH_2)-based nanofibers resulted in enhanced *ex vivo* lymphocyte proliferation, an effect not observed with DNA injection. However, administration of DNA-conjugated MAX1-(VKVKVKVKV^{D}PPTKVKVKVKV-NH_2)-based nanofibers did not show this response. Importantly, lymphocytes and splenocytes harvested from mice subjected to HLT2-DNA injections demonstrated no detectable cytotoxicity towards co-cultured B16F1 melanoma cells during *in vitro* assessments. In another study, fibrillised β sheet-forming Q11 peptide-contained nanofibers conjugated with glycosylated MUC1-derived B-cell epitope (MUC1 VNTR) induced a pronounced specific IgG response in murine subjects.[95] The antibodies generated through this process demonstrated the capacity to selectively target MUC1-expressing MCF-7 breast cancer cells, leading to the induction of complement-dependent cytotoxicity.

Table 3 Anticancer vaccines delivered *via* peptide nanofibers

Nanofiber-forming peptide	Antigen	Immune adjuvant	Ref.
FEFEFKFK	Glycoprotein 100209-217,[a] tyrosinase369–377[a] and melanoma-derived MART-126-35[a]	—	26
Coil29	Melanoma-derived T cell epitope,[a] B cell epitope[a] and tetanus-derived $CD4^+$ T cell epitope[a]	—	25
VVAGKS	Melanoma-derived glycosphingolipid[b]	Mannose[a]	27
Naphthylacetic acid-GFFYK	MUC1 glycopeptide[a]	—	93
MAX1, MAX8 or HLT2	Plasmid vector encoding for melanoma-specific tumour antigen gp100[b]	HMGN1[b]	94
SGSG-Q11	Glycosylated MUC1-derived B cell epitope (MUC1 VNTR)[a]	—	95
Q11	Human papillomavirus oncoprotein E7(44–62)[a]	—	96
Naphthylacetic acid-D- or L-GFFY-methyl amide	OVA[b]	—	28

Q11, QQKFQFQFEQQ; Coil29, QARILEADAEILRAYARILEAHAEILRAQ; MAX1, VKVKVKVKVPPTKVKVKVKV; MAX8, VKVKVKVKVPPTKVEVKVKV; HLT2, VLTKVKTKVPPTKVEVKVLV. [a] Covalently conjugated. [b] Non-covalently conjugated.

The subcutaneous immunisation of mice with OVA-conjugated nanofibers formed using the D- or L-naphthylacetic acid-linked GFFY β sheet-forming peptide significantly enhanced the OVA-specific IgG response.[28] Based on FITC-OVA and flow cytometry, the D or L nanofiber-OVA formulations were found to exhibit elevated uptake of OVA by DCs under *in vitro* conditions. Notably, the D-nanofiber-OVA formulation showed a more significant cytosolic accumulation of FITC-OVA when compared to L-nanofiber-OVA. Furthermore, the release kinetics of D-nanofiber-OVA at a lysosomal pH of 4.5 surpassed that of L-nanofiber-OVA, highlighting the ability of the D-nanofiber to facilitate antigen escape into the cytosol. These findings suggested that variations in optical properties could significantly impact the vaccine-mediated adjuvanticity. Thus, finding bioactive sequences that maintain this potency is crucial for the nanofiber's effectiveness as a vaccine adjuvant. Enhanced and sustained retention of the introduced fluorescent OVA at lymph nodes, both proximal and distal to the subcutaneous injection site, was notably observed with the D-nanofiber variant. After administration of the D-nanofiber-OVA vaccine to mice, significant increases in cell proliferation and IFN-γ production were observed upon *ex vivo* stimulation of splenocytes. Specifically, the D-nanofiber-OVA formulation was more effective in suppressing OVA-expressing melanoma B16 cells within a subcutaneous tumour model, leading to improved mouse survival rates compared to either OVA or L-nanofiber-OVA vaccination approaches. To assess the induction of protective immunity, mice were first administered with E.G7 lymphoma cell-incorporated nanofibers and later challenged with the E.G7 tumour cells after 28 days. Remarkably, D-nanofiber-bound E.G7 tumour antigens significantly slowed tumour growth.

Li *et al.* assessed the efficacy of a vaccine formulation comprising a covalent linked peptide from the human papillomavirus oncoprotein E7(44–62) with β sheet-forming Q11 peptide-contained nanofibers.[96] Mice immunised with Q11-E744-62 exhibited a pronounced $CD8^+$ T cell response specific to the antigen, leading to inhibited growth of subcutaneous human papilloma virus oncoprotein-expressing epithelial TC-1 cells. In a related study, mice vaccinated with Q11-E744-62 *via* intravaginal or intranasal routes displayed significant delay in genital TC-1 tumour growth.[97]

The studies discussed collectively highlight the promising potential of peptide nanofibers as dynamic tools for inducing and modulating immune responses against tumours. These studies showed the capacity of peptide nanofibers to effectively deliver tumour-specific antigens, adjuvants, and immune modulatory molecules, thereby directing immune reactions that result in tumour growth retardation and prolonged survival outcomes. Moreover, the immunisation strategies involving nanofiber-mediated formulations have demonstrated significant efficiency in inducing antigen-specific immune responses, marked by heightened production of immune effectors such as antigen-specific antibodies and cytokines. Further investigations into the optimisation of nanofiber formulations, delivery methodologies, and combination therapies hold great potential for advancing these nanofiber-mediated cancer immunisation strategies towards clinical application.

3.3.3. Development of needle-free vaccination approaches. In light of the promising results from previous studies demonstrating the efficacy of nanofiber-based vaccines in eliciting immune responses, this section explores the utilisation of peptide nanofibers in needle-free immunisation strategies. Needle-free vaccination, *via* intranasal or sublingual routes, for example, offers a range of advantages compared to traditional injection-based vaccines.[98] The user-friendly nature of needle-free vaccine administration, coupled with its elimination of the need for specialised skills or training, presents a viable option for self-administration. This attribute proves particularly advantageous for global vaccination initiatives, as it reduces the demand for healthcare personnel and facilitates the implementation of contingency strategies and isolation protocols during instances of pandemic outbreaks. Furthermore, the painless nature of needle-free vaccination, as opposed to conventional injection methods, is a compelling feature that can potentially boost compliance with multi-dose vaccination regimes. Notably, needle-free approaches not only mitigate the risks associated with needle accidents and needle-stick injuries, thereby substantially diminishing the potential for blood-borne transmissions, but also contribute to enhanced patient comfort and safety.

Previous studies demonstrated that the intranasal administration of Q11-based nanofibers, covalently linked to an epitope derived from the influenza A/PR/8/34 virus, elicited specific $CD8^+$ T cell responses and provided protective immunity in C57BL/6 mice.[99] Kelly *et al.* conducted a study exploring a potential vaccination strategy using nanofiber-based vaccines delivered through an alternative route of administration.[100] Mice that were given a vaccine consisting of cholera toxin and a protein fragment linked to an OVA peptide sublingually exhibited elevated levels of OVA-specific IgG in their bloodstream over a duration of 72 weeks, in contrast to unvaccinated mice. Interestingly, the use of fluorescently labelled nanofibers, detectable subsequent to sublingual administration in mice, indicated an extended sublingual retention period attributed to the presence of polyethylene glycol within their structural composition. To further explore the needle-free vaccination approach, Kelly *et al.* employed nona-arginine-assembled Q11 peptide nanofibers complexed with a cyclic dinucleotide for sublingual delivery to mice.[101] *In vivo* assessments underscored the capacity of the peptide nanofibers to enhance the mucosal immune adjuvanticity of the cyclic dinucleotide, as evidenced by upregulation of CD80 and CD86 levels, crucial costimulatory molecules governing antigen presentation. In a separate study by the same research cohort, sublingual administration of peptide nanofibers loaded with a B-cell epitope from uropathogenic *Escherichia coli* to mice demonstrated significant protection against urinary tract infection.[102] Earlier investigations have also demonstrated that intranasal or vaginal administration of Q11 peptide nanofiber-delivered human papilloma virus-derived epitopes in mice elicited an antitumour immune response, leading to delayed growth of genital tumours.[97]

These encouraging findings highlight the prospective utility of peptide nanofibers as a promising carrier for needle-free vaccination strategies *via* mucosal immunisation that could offer more accessible and effective vaccines (Fig. 4).

4. Future perspective

Building upon the foundations laid by the previously discussed studies, this section offers a forward-looking exploration of promising avenues that could enhance the effectiveness of peptide nanofiber-based vaccines.

4.1. Comparative studies and hybrid systems for enhanced vaccine delivery

The diverse capabilities of various nanocarriers pose an important question about how they compare with peptide nanofibers. To this end, the initiation of comparative studies to evaluate how material and morphological characteristics of nanosized carriers influence the potency of incorporated vaccines could provide valuable insights into optimising therapeutic effectiveness. Furthermore, the results of these comparative studies could facilitate the development of a hybrid nanocarrier system that could, for instance, leverage the efficient cell internalisation properties of one type of nanocarrier alongside the vaccine-loading capacity and self-adjuvanticity of another.

Several tubular and spherical nanocarriers showed the capacity to improve the potency of a loaded vaccine.[29] Examples for spherical nanoparticles include the cationic chitosan biopolymer-based nanocarriers that were previously utilised as an efficient mucoadhesive vaccine delivery system.[103–105] Niosomes, another spherical non-ionic surfactant-based nanomaterial, have also showed a remarkable ability to enhance the delivery and the pharmacological action of the loaded cargo.[32,106] Previous studies demonstrated the niosomal ability, as a vaccine delivery vector, to intensify the immune response against the delivered antigens derived from infectious agents or tumour cells.[107,108] The efficient deliverability of inorganic

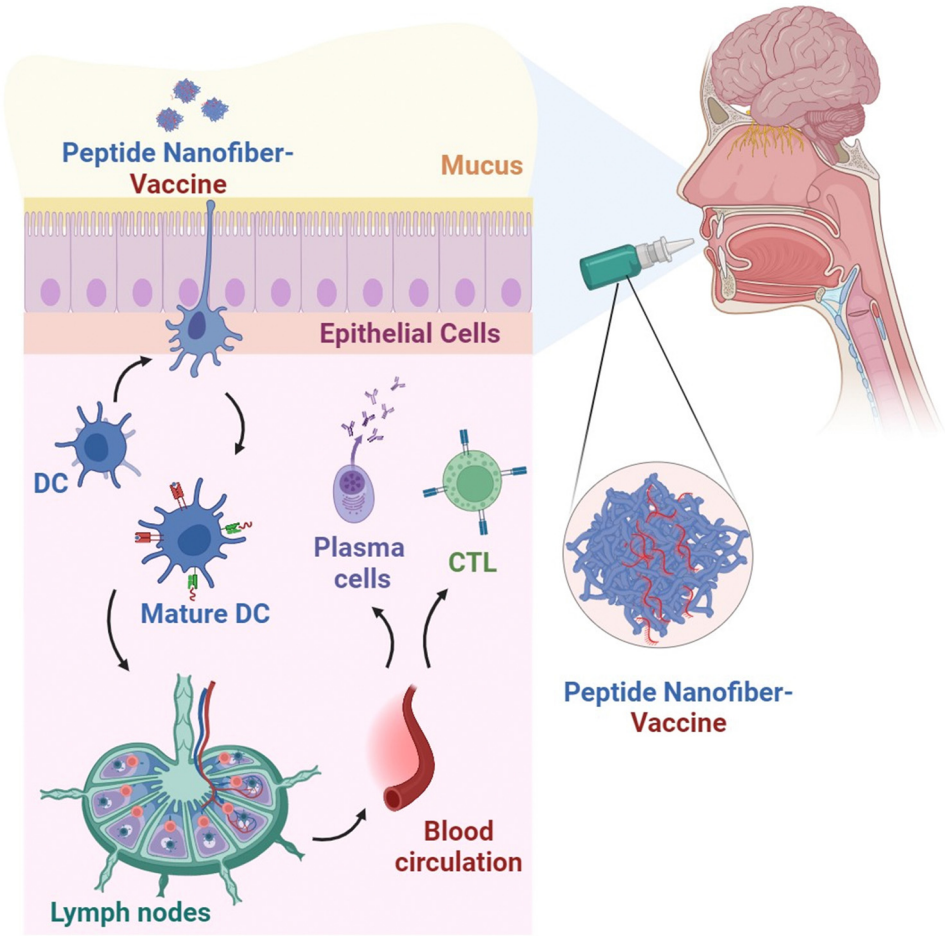

Fig. 4 Peptide nanofiber-mediated mucosal non-invasive vaccination approach. The induction of mucosal immunity that can establish a defensive barrier at the mucosal surface against invading pathogens or develop antitumour immune response against mucosal cancer could be attained through needle-free vaccination routes such as the nasal, sublingual or oral routes. Following the detection and uptake of administered vaccines by the DC at the mucosal tissues, mature DCs migrate to the mucosal lymph nodes to stimulate the resident B-cells and T-cells. The antigen-specific plasma cells and CTLs subsequently migrate to the mucosal sites to establish humoral and cell-mediated immunity, respectively.

material-based nanoparticles such as gold nanoparticles has also promoted their utilisation in vaccine delivery.[109] Additionally, tubular nanostructures such as carbon nanotubes previously showed significant enhancement in immune response potency as vaccine carriers.[110,111] While spherical and tubular carriers may possess high loading capacity and efficient cell internalisation capacity, respectively, peptide nanofiber-mediated adjuvanticity holds the potential to facilitate the development of nanocarrier-based vaccines capable of eliciting immune responses without relying on additional adjuvants.[74] The conductance of comparative studies aimed at identifying the influence of these aforementioned nanocarriers' attributes on immune response potency could guide future research endeavors towards nanocarriers with favorable properties. This could also facilitate the construction of hybrid systems that harness the full potential of existing nanocarriers to address pressing challenges.

4.2. Exploitation in mRNA-based vaccine delivery

Harnessing a diverse array of antigens expressed by cancer cells or infectious agents, as opposed to a limited selection, can potentially amplify the therapeutic or preventive outcomes of vaccination. This approach could counteract immune evasion mechanisms, often driven by the downregulation of specific antigens.[112] Providing the APCs with a comprehensive repertoire of antigens, covering both known and unidentified entities, may promote the generation of a wide range of cytotoxic T cells specific to different antigens. The construction of such a vaccine entails capturing a wide spectrum of antigens through the isolation of either DNA or mRNA.[113] A significant challenge in DNA-based vaccines pertains to their requisite delivery to the nuclei of APCs for optimal functionality.[114] Consequently, mRNA-based vaccines, which only require cytosolic delivery, present a potentially more potent approach for vaccination.

To date, the application of peptide nanofibers for the delivery of mRNA-based vaccines incorporating antigens derived from infectious agents or cancer cells remains unreported. However, prior investigations have concentrated on exploring the potential utility of peptide nanofibers in gene delivery. Leveraging the capacity of peptide nanofibers as gene delivery carriers in the development of mRNA-based vaccines could potentially help overcome challenges associated with traditional vaccines. In light of this, Mazza *et al.* utilised the palmitoyl-conjugated GGGAAAKRK peptide in the delivery of BCL2 siRNA, a modulator of apoptosis.[115] Their findings indicated that nanofibers significantly augmented the internalisation of fluorescently labelled siNEG-A546 by human neuronal SH-SY5Y cells. Moreover, administration of nanofiber-siRNA in rats resulted in notably diminished BCL2 expression levels, surpassing those induced by naked siRNA injections. In a separate study, Zhang *et al.* used a peptide containing four arginine residues, suitably modified with palmitic acid and tetraphenylethene, to facilitate the efficient delivery of plasmid DNA encoding enhanced green fluorescent protein (EGFP-N1).[75] The resulting nanofiber complexes effectively transduced HeLa, HepG2, NIH 3T3, and stem cells after 24 and 48 hours of *in vitro* incubation. These investigations highlight the suitability of peptide nanotubes as promising candidates for the development of potent mRNA-based vaccines.

4.3. Disruption of tumour-mediated immune suppression

The immune regulatory receptor programmed cell death protein 1 (PD-1), expressed by T cells, has shown elevated expression in T cells that are specific for tumour antigens.[116–118] Its ligand PD-1L is expressed not only by cancer cells but also APCs. The interaction between PD-1 and PD-L1 has been substantively elucidated as a mechanism that dampens T cell functionality, particularly by curbing their proliferative potential and tempering cytokine production. Notably, blocking this interaction with specific antibodies against either PD-1 or PD-1L can restore T cell activities.[117–119] Accordingly, anti-PD-1 antibodies such as nivolumab and pembrolizumab have successfully attained clinical approval for the treatment of malignant melanoma due to their proven efficacy.[120,121] Concurrently, various clinical trials are evaluating alternative anti-PD-1 or anti-PD-1L antibodies.[122] Previously reported studies utilised nanoparticles in disruption of the PD-1/PD-1L interaction through the application of either specific antibodies[123–125] or siRNA.[126,127] However, this strategy raises safety concerns due to potential emergence of autoimmune disorders, given that it blocks a regulatory pathway governing T cell activity.[128,129] Additionally, the efficacy of therapeutic approaches centered on targeting the PD-1/PD-L1 axis is susceptible to decline due to the eventual emergence of acquired therapeutic resistance.[130]

Emerging investigations have brought to light a fresh array of co-inhibitory receptors such as T-cell immunoglobulin mucin-3 (Tim-3) and lymphocyte-activation gene-3 (Lag-3), which contribute to the immune suppressive mechanisms. These receptors are expressed in regulatory T cells, $CD8^+$ T cells, and natural killer (NK) cells.[131] In the preclinical settings, previous studies aimed at evaluating the effects of disrupting these receptors on antitumour immune responses demonstrated promising results, encompassing both effectiveness and tolerability.[132,133] This encouraging preclinical evidence has set the stage for further clinical investigations. To this end, the prospect of development of a nanofiber-based immune therapeutic approach targeting these emerging co-inhibitory receptors represents an exciting opportunity. The rationale of this approach is rooted in its potential to harness the nuances of these novel receptors, thus holding promise as a pathway to augmenting immune therapeutic strategies.

The recent advancements in immune modulatory strategies could offer promising avenues for therapeutic interventions. Nevertheless, a significant gap remains in the development of precise delivery vectors to maximise the efficacy of these approaches. The full therapeutic potential of these immune modulators could be effectively achieved through their integration into cutting-edge nanoscale materials, with peptide nanofibers standing out as promising candidates for this purpose.

5. Conclusions

Immunologically mediated prophylactic and therapeutic strategies have attracted significant attention. The quest for safe and efficacious methods to regulate and amplify immune modulatory responses has catalysed the development and application of innovative nanomaterials, including peptide nanofibers. Beyond their deliverability attributes, peptide nanofibers exhibit unique immune adjuvanticity, potentially positioning them as optimal candidates for vaccine delivery. Nonetheless, the proposed immune therapeutic use of peptide nanofibers remains a rich domain of research, mandating thorough investigation to fully elucidate their potential merits. Future research endeavours should prioritise the optimisation of nanofiber synthesis to ensure biocompatibility, stability, and enhanced delivery efficiency. A crucial element of this pursuit is the rigorous evaluation of the clinical efficacy of peptide nanofibers, which demands the design and execution of robust clinical trials assessing therapeutic outcomes and monitoring potential toxicities. To tailor these materials for specific therapeutic applications, a deeper understanding of the molecular interactions between peptide nanofibers and immune cells is pivotal. Integrating computational modelling with experimental studies can provide valuable insights into designing nanofibers with enhanced immune modulatory properties. Furthermore, the development of multifunctional peptide nanofibers, capable of simultaneously delivering antigens, adjuvants, and immune modulators, holds promise for enhancing the efficacy of therapeutic interventions. This combinatorial approach can potentiate immune responses while minimising potential side effects. The incorporation of bio-sensing or imaging modalities into peptide nanofibers enables real-time monitoring of their distribution, degradation, and interaction with immune cells *in vivo*, offering valuable feedback for dose adjustments and therapeutic efficacy evaluations. In addition, while preliminary findings on peptide nanofibers are promising, upscaling production poses a significant challenge in the transition from bench to bedside. Addressing this challenge involves investigating scalable synthesis methods, optimising purification protocols, and ensuring batch-to-batch consistency, all of which are critical components of this endeavour. Moreover, exploring the potential synergy of peptide nanofibers with other immune modulatory agents opens up exciting avenues for combinatorial therapeutic strategies, potentially leading to therapies with augmented efficacy.

Author contributions

Hatem A. F. M. Hassan, conceptualisation, investigation, visualisation, writing – original draft, and writing – review and editing. Mohamed Haider investigation, writing – original draft, and writing – review and editing. Sherif Ashraf Fahmy, investigation, writing – original draft, and writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

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