Nucleobase-Bearing Amino Acid Systems and Self-Assembly

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Nucleobase-containing molecules are compounds essential in biology due to the fundamental role of nucleic acids and, in particular, G-quadruplex DNA and RNA in life. Moreover, some molecules different from nucleic acids isolated from different vegetal sources or microorganisms show nucleobase moieties in their structure. Nucleoamino acids and peptidyl nucleosides belong to this molecular class. Closely related to the above, nucleopeptides, also known as nucleobase-bearing peptides, are chimeric derivatives of synthetic origin and more rarely isolated from plants.

Keywords: nucleobase ; nucleopeptide ; hydrogel ; self-assembly

1. Introduction

Gels are three-dimensional networks of polymers and their swollen forms that cannot be dissolved in any solvents. In fact, they form structures acting as cross-linked networks that absorb solvents and swell to a limited extent without dissolving fully. Consequently, gels exist in a state that falls between a liquid and a solid. Gels are categorized by features such as the type of cross-linking forming their three-dimensional networks, their artificial or natural origin, and the types of solvents involved in the gelation process [1][2]. Gels can have various functions within living organisms, such as filtering, establishing molecular interactions between biopolymer chains and the enclosed solvent or solute [3]. Among the other types of gels, low-molecular-weight gels are a highly significant material category currently drawing considerable scientific attention. They emerge as small molecules that spontaneously assemble into linear structures interconnecting and intertwining to establish a network that can trap the solvent in its interior. The observed characteristics of gels rely on the method used for their obtainment, which leads to the creation of diverse materials possessing different properties starting from a single gelator. Moreover, using multiple gelators provides researchers the chance to realize materials with enriched information content and a broader spectrum of properties [4]. Apart from their undiscussed role in nanotechnology, gels are highly relevant also in food production, with various gelled food products being manufactured worldwide thanks to the ability of food gels to display useful viscoelastic properties [5][6][7]. Typically, the gelling agents that find application in food technology include proteins and polysaccharides. In food gels, polymeric structures are generally not held together by covalent interactions, except for the disulfide bonds found in certain protein-based gels. Instead, these structures are based on a combination of weak intermolecular interactions, such as Van der Waals forces, electrostatic forces, hydrogen bonds, and hydrophobic interactions. Polysaccharides, including the hydrocolloids used as thickeners in foods, possess noteworthy hydration properties in aqueous environments, but they often exhibit disordered structures [5]. The process of gelation depends on the nature of the gelling agent used as well as on the conditions under which gel formation occurs, including the pH, the presence of ions, the temperature, and the concentration of gelling agents. The characterization of gels is a process that involves various techniques, with rheological measurements being a routinely employed method. The development of mixed or multi-component gel systems is a noteworthy area of research, involving the simultaneous use of multiple gelling components in order to achieve specific structural and functional characteristics [5]. Polymeric gels are typically semisolid systems constituted by polymers, endowed with a three-dimensional network structure formed through either covalent or noncovalent bonding within a liquid medium, which accounts for the elastic properties of the resulting material. Among the key attributes of polymeric gels, it is worth mentioning their non-Newtonian (pseudoplastic) rheological behavior, as well as their properties of swelling, aging, syneresis, electrical oscillation, electrostatic potential distribution, electrical contraction, mechanoelectrical effects, and interaction with surfactants of opposite charge. In recent years, various types of polymeric gels (such as physical gels, cryogels, microgels, macrogels, nanogels, hydrogels, organogels, aerogels, emulgels, and xerogels) have been studied, developed, and utilized across diverse industrial strategies [8]. While the terms “gel” and “hydrogel” both refer to materials with gel-like characteristics, they differ in their composition and properties, particularly owing to their interaction with water. Gels can be composed of diverse substances, including polymers or small molecules, whose cross-linking forms a network immobilizing liquid within its structure. They exhibit properties of both liquids and solids, having an overall semi-solid consistency together with the

ability to hold a significant amount of liquid within their structure without dissolving. Gels can be formed by chemical or physical cross-linking of their components, which leads to different behaviors and characteristics. As for hydrogels, this is a specific class of gels with a strong affinity for water or, in other terms, high hydrophilicity, as indicated by the word “hydro” in hydrogel, which refers to the high water content effectively found in these substances. In fact, hydrogels are mainly composed of a network of hydrophilic polymer chains and water. The usually cross-linked networks of polymers can absorb and retain a significant amount of water without losing their structural integrity. Hydrogels find application in numerous fields including drug delivery, tissue engineering, wound dressings, contact lenses, and personal care products. Moreover, they can be engineered to acquire specific characteristics linked to their high water content, their ability to respond to stimuli like the pH and temperature, but also their biocompatibility. Hydrogels may retain a specific three-dimensional framework and were initially investigated as biomaterials for human body applications [9][10][11]. Techniques routinely used in biomaterial synthesis involving reactive polymer precursor crosslinking, polymer–polymer reactions, and crosslinking copolymerization were successfully applied to create hydrogels. However, it was not easy achieving precise structural control due to side reactions resulting in network complexities like unreacted groups and intertwinements. Additionally, conventional hydrogels suffered from limits such as suboptimal mechanical characteristics and weak responsiveness to external stimuli. This called for a significant innovation in hydrogel formulation aimed at reinvigorating the research on these materials with contemporary attempts to create hydrogels endowed with enhanced mechanical strength as well as convenient superporous characteristics and graft structures able to respond promptly to external stimuli. In this regard, interesting examples employing self-assembling hydrogels derived from hybrid graft copolymers containing protein domains and others realized from genetically engineered triblock copolymers were reported in the scientific literature, shaping a promising future for smart hydrogel biomaterials [12].

2. Nucleobase-Bearing Amino Acid Systems and Self-Assembly

Nucleobase-containing amino acids, also known as nucleoamino acids [13], consist of amino acid residues linked to DNA or RNA bases through diverse connecting moieties referred to as linkers. These hybrid derivatives, featuring heteroaromatic rings fused with amino acid-based structures, are sourced from nature or synthesized by means of chemical procedures in a laboratory. Notably, among the several natural nucleoamino acids worth mentioning is willardiine, which can be described as a uracil-bearing alanine, as well as amino nucleosides like cystocin and puromycin, recognized for their antimicrobial properties. These chimeric compounds can be seen as a bridge between the families of nucleobases and amino acids playing a significant biological role and, from a synthetic perspective, serve as fundamental components for building up peptides with intriguing DNA-binding capabilities. In the class of natural nucleoamino acids, willardiine and its analogs stimulate AMPA or kainate receptors [14], while discadenine [15], derived from *Dictyostelium discoideum*, impedes self-spore germination and acts as a plant cytokinin. Also, lathyrine, a non-proteinogenic amino acid found in several *Lathyrus* species and identified as a potential food allergen, belongs to the nucleoamino acid family [16]. Additionally, synthetic nucleoamino acid monomers serve as building blocks for fabricating the nucleobase-incorporating peptides often referred to as nucleopeptides [17][18][19][20][21][22][23][24]. Further examples of natural nucleobase–amino acid conjugates are evident in antimicrobial peptidyl nucleosides [25] and in the aminoacyl nucleoside *N*6-threonylcarbamoyl adenosine [26], present in transfer RNA and involved in the protein synthesis process. Both naturally occurring and artificially synthesized nucleobase-containing amino acids are obtainable through chemical synthesis. Similar to their nucleoamino acid constituents, nucleobase-decorated peptides also constitute a promising class of molecules with significant scientific value proving beneficial in biotechnology and medicine, as they merge a peptide-like structure with DNA or RNA nucleobases connected through diverse linkers. Interestingly, among these chimeric compounds, only a limited number were shown to occur naturally, such as willardiine-containing short nucleopeptides and peptidyl nucleosides, also referred to as nucleoside peptides, renowned for their antimicrobial and anti-tumoral properties [27]. Conversely, several research groups have prepared and investigated artificial nucleobase-containing peptides, or pseudopeptides, paying particular attention to the study of their binding characteristics. Notably, certain nucleobase-containing peptides demonstrated a capability to bind complementary DNA and RNA, with intriguing prospects for biomedical applications in antigen and antisense strategies. Gel polymer systems rely on the assembly of one or multiple types of monomeric subunits held together through non-covalent interactions. Within the various range of molecules capable of constructing supramolecular networks, particular attention is directed towards nucleobase-containing molecules. These include not only nucleic acids, which are widely used in nanomedicine, but also the above-mentioned nucleobase-containing peptides and systems composed of individual monomeric units carrying single nucleobases. Supramolecular materials based on nucleopeptides have also attracted significant interest, particularly due to their versatility in forming various nanostructures.

On the other hand, peptides, especially those based on aromatic moieties such as the tripeptide l-His–d-Phe–d-Phe, and amino acid derivatives, both extensively utilized structures, possess inherent properties suitable for nanotechnological,

biological, and medical applications, which are, at least in part, attributed to their remarkable self-assembly properties, leading to useful applications including in the formation of hydrogels [28].

For gel formation, the mono-component approach involving a single molecule is widely used due to its simplicity, but this strategy is not exempt from drawbacks in terms of the resulting properties of the obtained gel system. To overcome these limitations, the multi-component approach emerged as a promising strategy employable when realizing gels starting from low-molecular-weight molecules that interact with each other to generate novel assemblies and properties unattainable with a single gelator. In this context, a series of nucleopeptides, integrating both peptide and DNA-nucleobase components covalently linked with each other, was investigated for gel formation. Both hydrogels and soluble supramolecular networks hold promise for delivering genes and drugs to cells. These nucleopeptide-based systems were found to be able to originate gels through a cooperative effect facilitated by the presence of nucleobases [29]. Famously, phenylalanine (Phe) is an aromatic amino acid, present in proteins, that forms a dipeptide (Phe–Phe) capable of self-assembling into innovative nanomaterials [30][31]. In particular, when in a solution the dipeptide self-assembles into fibers, reliant on π–π interactions, in turn responsible for generating tubular structures, serving as templates for the formation of nanotubes. In this context, investigating the chemical modification of Phe–Phe and, in particular, the substitution of one phenyl ring in the dipeptide with different aromatic or heteroaromatic groups is an intriguing possibility, potentially influencing both aggregation and biomolecular interactions with natural targets. In this context, an experimental study aimed to synthesize and investigate the properties of PheT, an aromatic nucleobase amino acid derived from Phe and thymine nucleobase, mimicking Phe–Phe but with one aryl group substituted by the T nucleobase [32]. In the design of PheT, the Phe component contributed aromatic and hydrophobic interactions, crucial for the self-assembly properties of the construct, while the nucleobase segment potentially enabled interactions with biomolecules. The arrangement of the two aromatic rings disfavors direct stacking, allowing both phenyl and thymine units to interact with residues from other molecules (e.g., nucleic acids, proteins, or other PheT units). The effective occurrence of such interactions through circular dichroism (CD) and UV spectroscopies is demonstrated, along with binding assays involving DNA, RNA, and protein models [32]. Furthermore, another work reported on the design, synthesis, and characterization of a nucleoamino acid derivative named TrpT, based on L-tryptophanamide functionalized with a thymine nucleobase [33]. Researcher can see TrpT as another analog of the Phe–Phe in analogy to PheT with tryptophan and thymine being in place of the two Phe residues. The TrpT molecule demonstrated a clear propensity to self-assemble into supramolecular networks in aqueous solutions, as verified through dynamic light scattering (DLS), CD, fluorescence, and UV spectroscopy. The nucleoamino acid underwent self-assembly into nanoaggregates, driven mainly by thymine–thymine π−π stacking, likely exposing the tryptophan moieties of the outer layers to the aqueous environment and forming hydrophobic pockets which include other tryptophan units within the particle interior. The ability of TrpT to create nanostructures in a solution was validated through different in silico and experimental analyses, revealing predominantly spherical species with good stability. Notably, a confocal fluorescence microscopy analysis demonstrated that the supramolecular TrpT network had the capacity to accommodate and subsequently release hydrophobic drugs gradually, suggesting its potential as a drug delivery system. These nanoaggregates exhibited stability for up to 5 h at a concentration of 140 μM, displaying a mean hydrodynamic diameter of 330 nm and uniform size distribution, as observed via scanning electron microscopy (SEM). Moreover, the ability of TrpT nanoaggregates to bind to the natural anti-cancer curcumin, serving as a model drug, was assessed in the same study, and the natural drug release properties of the non-covalent polymer were eventually demonstrated using confocal microscopy [34]. Molecular docking studies indicated the accommodation of curcumin within the interior of the TrpT nanoaggregates in which the curcumin was bound through hydrophobic interactions. Additionally, the stability of the TrpT nanoassemblies in human serum and their minimal toxic effects on human model cells were revealed in the same study. Quite surprisingly, despite its thymine-based structure, TrpT did not exhibit any appreciable binding towards adenine-rich nucleic acids, suggesting a preference for self-assembly over A-T base pairings. Moreover, TrpT did interact with a serum protein, bovine serum albumin (BSA), known for enhancing the transport and bioavailability of its biomolecular cargos in the bloodstream. Overall, these findings suggested the potential utility of TrpT nanosystems in the development of new drug delivery systems [33]. Apart from these studies on non-covalent polymers based on nucleobase–amino acids conjugates, more applicative works were conducted on hydrogelators obtained through the fusion of nucleobases and short peptides, which showed that nucleopeptides, thanks to their self-assembly in aqueous environments, are able to form supramolecular hydrogels upon stimulation by enzymatic action or pH variations [35]. Notably, nucleopeptides may offer easy and broadly applicable methods for producing biocompatible structures, and the simplicity of incorporating various bioactive peptides or molecular recognition elements together with nucleobases opens new avenues for exploring innovative applications of nucleopeptides as functional biomaterials [35]. Short peptide sequences sourced from the interface of a known heterodimeric protein were combined with nucleobases, thus forming nucleopeptides which were found to predominantly self-assemble via hydrogen bonds, leading to the creation of nanofibers, ultimately leading to supramolecular hydrogels obtained by merely mixing two nucleopeptide samples in water [36]. Additionally, apart from demonstrating its biocompatibility with mammalian cells, the nucleopeptide heterodimer

exhibited a noteworthy resistance against proteinase K proteolysis, which is a favorable characteristic in view of the biomedical applications of biomaterials obtained via the supramolecular hydrogelation of heterodimeric nucleopeptides [36]. Self-assembled nucleopeptide hydrogels were shown to form nanofibril architectures by means of noncovalent interactions, including Watson–Crick interactions and π–π stacking, the former being facilitated by the presence of complementary nucleobases in the structures. These hydrogels are envisaged to offer specific advantages for biomedical applications, combining the easily modulable DNA-interacting mode [37] to the well-established advantages of peptide biomaterials, such as their customizable design, biocompatibility, and extracellular matrix-like structure. Drawing inspiration from their nucleobase-stacking structure, the capability of nucleopeptides to provide sustained delivery of the DNA-intercalating chemotherapy drug doxorubicin when locally administered to a solid tumor was assessed using an in vivo tumor-bearing mouse model [38]. This demonstrated that an adenine-bearing triphenylalanine (Ade–FFF) nucleopeptide was able to form hydrogels with a high loading capacity for doxorubicin at a 1 mM concentration, exhibiting the drug's continuous release under in vitro degradation conditions [38]. Doxorubicin-loaded Ade–FFF hydrogels decreased the tumor growth levels and enhanced apoptosis-mediated cell death within the tumor, as indicated by caspase-3 expression. Biodistribution and pharmacokinetic analyses further supported the observation that delivering the drug through the nucleopeptide hydrogel increased the levels of sustained release specifically at the local tumor site in the animal model. This investigation highlights the potential of self-assembled nucleopeptides for various biomedical applications by taking advantage of their unique dual DNA-like and peptide structural features [38]. Over the past few decades, there has been a considerable focus on peptide-based hydrogels as versatile supramolecular materials, offering novel possibilities for various biomedical applications. To gain deeper insights into their self-assembly properties and enhance their characteristics, researchers have several strategies including modifying the amino acid chains by incorporating halogenated amino acids, pseudopeptide bonds, or other chemical moieties into the peptide backbone. In this respect, integrating DNA-nucleobases into peptide scaffolds leads to the development of new examples of nucleopeptides. In previous studies, some of these hybrid molecules led to the formation of nucleopeptide hydrogels whose physicochemical and mechanical properties strongly relied on the specific nucleobase introduced in the structure (whether cytosine, thymine, guanine, or adenine) [39]. The hydrogel properties resulting from this process can be improved and precisely adjusted, leading to an enhanced resistance to external stress, significant increases in gel stiffness, and the emergence of distinctive thermo-reversible and red-edge excitation shift properties. The precise contributions of each nucleopeptide component in the self-assembly processes can be proven using an array of analytical techniques such as NMR relaxometry, rheology, TEM, fluorescence, CD, FTIR, NMR chemical shift index, and thioflavin T assays, ultimately demonstrating that nucleopeptide hydrogels offer novel opportunities for tailoring hydrogel properties according to specific requirements [39]. Nucleopeptides can be seen as innovative chimeric compounds resulting from the fusion of nucleobases and peptides [40], typically self-assembling into nanofibers driven primarily by hydrogen bonds and other weak forces. Their unique characteristic involves the potential ability to bind complementary nucleic acids due to the presence of nucleobases in the nucleopeptide structure. Thus, nucleopeptides are the subject of research as building blocks capable of self-assembling in water and as artificial oligonucleotides able to target single-stranded DNAs or RNAs. Towards DNA, specific nucleopeptide structures have been demonstrated to be able to bind with good affinity to single-stranded DNAs, which reciprocally influenced each other's self-assembly abilities [41]. Not less importantly, certain nucleopeptides have been found able to interact with plasmid DNAs and facilitate the delivery of hairpin DNA to cells [41]. In other works, other nucleopeptide analogs resembling TpT dinucleoside monophosphate generated supramolecular networks interconnected via noncovalent interactions. These non-covalent polymers exhibit the capacity to accommodate organic molecules, demonstrating potential applications in drug and gene delivery [42]. Considering the significance of these molecular systems in the field of biomedicine, nucleopeptide analogs of mononucleosides were also investigated in studies aimed to ascertain their capacity to construct supramolecular networks using these short nucleopeptides, intending to develop innovative drug delivery strategies. More in detail, thymine, adenine, cytosine, and guanine nucleoside analogues were synthesized and subjected to investigation with respect to their supramolecular assembly properties. Chemically, the four nucleopeptides originated from a diserine (Ser–Ser) peptide conjugated to a DNA nucleobase present at its *N*-terminus. These structures were investigated by light scattering and CD studies evaluating their interactions with natural nucleic acids and assessing the formation of supramolecular networks based on nucleobase recognition. The results revealed the formation of molecular networks held via weak interactions (such as hydrophobic, hydrogen-bonding, and aromatic interactions), with structural changes being influenced by temperature fluctuations. The same peptidyl nucleoside analogues demonstrated good biodegradability properties and led to the formation of supramolecular complexes involving multiple nucleopeptide units alongside with nucleic acid molecules [43]. Due to their inherent properties and flexibility in terms of structure, low-molecular-weight gelators, particularly peptide-based hydrogelators, have great importance, as the resulting supramolecular hydrogels are readily obtained from specific self-assembly of the peptide constituents, modulable through tailored chemical modifications applied to the peptide structure. Among these chemical modifications, the introduction of nucleobases, in turn constituting an additional family of biomolecules renowned for their self-assembling properties, has emerged as an attractive strategy employed to design

supramolecular hydrogels based on low-molecular-weight nucleopeptides [44]. Thanks to their dual nucleic acid–peptide properties, the nucleopeptides were often found able to co-assembly, with complementary nucleobase segments interacting through π-stacking interactions and hydrogen bonding, which resulted in synergistic effects, as proven by nucleopeptide structures in which a tetrapeptide moiety was conjugated with a two-bases-long nucleopeptide. These effects enhanced the mechanical properties of the resulting hydrogels by over 250%, with stiffness levels over 700 kPa, and elevated their self-assembling abilities by approximately 280%. The structure-to-property correlations for the above-mentioned systems were investigated through a comprehensive analysis based on multiple techniques, such as fluorescence, rheology, transmission electron microscopy (TEM), cryo-SEM, FTIR, NMR, CD, and high-resolution magic angle spinning. This in-depth analysis indicated the influence of nucleobases on the supramolecular assembly process, on the consequent formation of nanostructures, and on the three-dimensional structure of the hydrogel scaffold as well as on the resulting physical and mechanical characteristics of these synergistic nucleopeptide assemblies. Different from most hydrogels derived from single gelators through mono-component strategies, often associated with several limitations, the nucleopeptide co-assembling system demonstrated a high efficiency, yielding high storage moduli (about 720 kPa), and noteworthy synergistic physical and mechanical nanomaterial properties. Specifically, the nucleopeptides presented cooperative effects, especially when combining two complementary sequences, TG–FEFK and AC–FEFK, where TG and AC are the dibasic nucleopeptide moieties linked to the FEFK tetrapeptide. These nucleopeptide sequences resulted clearly complementary thanks to the complementary nucleobase-bearing segments. This nucleopeptide combination significantly improved hydrogel stiffness and resistance to external stress, respectively, with their resulting properties greatly overcoming those associated with the individual nucleopeptides or the systems obtained mixing samples of nucleopeptides with mismatched nucleobases. The observed synergy stems from specific interactions occurring between the peptide segments (evidenced by π-stacking interactions and β-sheet formation) and between the base-containing moieties via hydrogen bonding through complementary nucleobase pairing and additional π-stacking. The resulting supramolecular interactions led to a fibrillary network capable of self-organizing into a porous hydrogel scaffold endowed with uniform alveoli, efficaciously entrapping water molecules in their interior, as demonstrated through TEM and cryo-SEM. Taken together the above findings suggested the significant potential of nucleopeptides as a highly efficacious element in forming low-molecular-weight hydrogels. Remarkably, owing to their unique dual nucleic acid–peptide nature, nucleopeptides offer new opportunities for designing multi-component hydrogels with remarkable synergistic effects providing significantly improved mechanical and physicochemical properties that would otherwise be challenging to achieve through a traditional mono-component approach [22]. Numerous are the applications that self-assemblies and gels of nucleopeptides can provide [45]. The capability of nucleopeptides and PNA to create well-organized architectures was proven and the obtained supramolecular tools, including nanovesicles, nanotubes, nanospheres, nanofibers, or micelles (including cylindrical, spherical, or worm-like structures), exhibited applications in biomedicine, nanotechnology, or materials science due to their favorable properties of biocompatibility and biodegradability. For instance, by taking advantage of the non-covalent interactions between nucleic acids and nucleopeptides, different researchers developed nucleopeptide-based supramolecular assemblies designed for gene delivery therapy [46] or for the selective sequestration of ATP in cancer cells. Displaying minimal cytotoxicity by themselves, the nucleopeptide systems were found to significantly increase the cytotoxicity of doxorubicin used as anti-cancer drug against human uterine sarcoma cells in a dose-dependent manner [47]. This approach enhances the effectiveness of anti-cancer drugs such as doxorubicin and offers unique advantages, including reversible interactions between assemblies and nucleic acids, minimal immunogenicity, and biocompatibility.

Other multi-component self-assembling hydrogels disclosed new scenarios in creating materials with various properties that would be challenging to achieve using individual components alone. Consequently, these multi-component-derived hydrogels are envisaged to serve for wide-ranging applications in biomedicine, and the numbers of examples with such systems will probably continue to grow. Multi-component self-assembly strategies were applied to develop a biomimetic, low-molecular-weight guanosine quartet-based hydrogel under physiological conditions. The introduction in the nucleopeptide structure of the mononucleoside guanosine and the use of 4-formylphenylboronic acid and a cytosine-functionalized nucleopeptide are paramount to creating dynamic imino–boronate ester-mediated G-quartet-based hydrogels. The effective formation of a G-quartet structure, a crucial factor leading to nanofibrillar hydrogels, was demonstrated through CD, powder X-ray diffraction, and thioflavin T fluorescence assay. The multi-component self-assembled G-quartet-based hydrogel exhibited remarkable antibacterial activity against a number of bacterial species. The in vitro cytocompatibility of the hydrogel was proven on HEK 293T and MCF-7 cell lines, revealing the biocompatibility of such G-quartet-based hydrogels which, overall, were demonstrated to be injectable, biocompatible, and intrinsically antibacterial materials holding promise for preventing localized microbial infections [48]. In another experimental work, guanosine-containing self-assembling nucleopeptides were able to give rise to nanofibers and nanosheets. By taking advantage of spectroscopy and microscopy techniques, the assembly into β-sheet structures of such G-based nucleopeptides was proven, which primarily occurred thanks to the peptide moiety present in the nucleopeptide structure,

whereas the hydrogen-bonded guanosine elements contributed to the formation of additional secondary structures, cooperatively embedded within the peptide structure. Notably, the observed supramolecular nucleopeptide morphologies were not dependent on the metal cations, whose responsiveness is typically observed in guanine-based nanomaterials, but rather on the influence of the peptide moiety present at the nucleopeptide's *C*-terminus. Overall, the presented research underscores the structural diversity shown by self-assembling nucleopeptides and suggests new progresses on applications associated with these supramolecular G-containing nucleopeptides [24]. With all the above being said, hydrogels formed by nucleopeptides able to self-assemble offer substantial advantages in different biomedically relevant fields owing to their biocompatibility and broad spectrum of molecular possibilities can be concluded. The short peptide moieties present in nucleopeptides, in particular, offer remarkable advantages, including their easy synthesis and the favorable self-assembly properties conferred to the resulting nucleopeptide. While the biomedical applications of classical peptides are currently limited due to challenges such as the potential toxicity resulting from the chemical modifications of natural peptides required for their self-assembly as well as from the experimental conditions required for gelation, one possibility at researchers disposal to mitigate their cytotoxicity involves the conjugation of peptides to nucleobases, which leads to nucleopeptide structures. Nucleopeptide hydrogel formation can be achieved under specific conditions and can be easily modulated using salts and biological buffers. In this regard, the self-assembly of nucleopeptides relies on the experimental conditions adopted and can be regulated by their formulation and pKa. In solutions adjusted to physiological values of osmolarity and pH which are compatible with cell culture, hydrogel formation is often favored. In silico and analytical methods can be employed to explore the effects of salts and pH conditions on nucleopeptides at the molecular and structural levels. Thanks to the specific mechanisms governing the self-assembly of nucleopeptides, one can modulate nucleopeptides' mechanical properties through the addition of divalent cations, which leads to an increased hydrogel storage modulus. The stability of nucleopeptide hydrogel constructs offers a potential for long-term cell culture, with the survival and proliferation of fibroblasts having been shown on the surfaces of these hydrogels. The nucleopeptide hydrogelation methodology mediated using biological buffers makes way to tissue-engineering applications involving nucleopeptides [49]. In this respect, self-assembling nucleopeptides offer a methodical strategy for building hydrogels resembling the extracellular matrix in both function and structure, as shown with certain nucleo-tripeptides capable of forming hydrogels under physiological conditions. Combining experimental and in silico methods, their self-assembled structures were examined using CD spectroscopy, TEM, and rheometry methods employed to validate and complement the computational results obtained with molecular dynamics simulations. The nucleo-tripeptides were shown to form hydrogels based on nanofibers held via interactions including π–π stacking and Watson–Crick complementary base pairing. The conditions for self-assembly were modulated thanks to the hydrophobic and amphiphilic moieties present in the structures of the nucleo-tripeptides, with new possibilities offered for deliberate control using a rational molecular design. Overall, structures arising from nucleobase-containing peptides and their combinations are capable of forming hydrogels under physiological conditions, highlighting them as promising candidates for innovative biomedical applications [50].

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