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Review

Current Research on Polyelectrolyte Nanostructures: From Molecular Interactions to Biomedical Applications

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Abstract: Polyelectrolytes have been at the center of interdisciplinary research for many decades. In the field of polymer science and soft matter, they have provided the dimensions of electrostatic interactions, which opens a vast variety of opportunities for new physical properties and applications. In biological matter, polyelectrolytes are present in many forms, from extracellular polysaccharides to complex DNA molecules and proteins. This review discusses the recent research on polyelectrolytes covering the fundamental level of their conformations and nanostructures, their molecular interactions with materials that have close relevance to bioapplications and their applications in the biomedical field. This approach is motivated by the fact that the polyelectrolyte research is constantly active in all the aforementioned levels and continually affects many critical scientific areas.

Keywords: electrostatic interactions; hydrophobicity; nanoparticles; complexes; hydrogels; polysaccharides; proteins; drug delivery; tissue engineering

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1. Introduction

Polyelectrolytes are naturally connected with applications in life sciences. DNA, charged polysaccharides and proteins are characteristic examples of natural polyelectrolytes that have been the focus of research for many decades. Their fundamental role in the extracellular matrix [1] and their extended applications in biotechnology, pharmaceutics [2] and food science [3,4] are inextricably linked with their molecular structure. Solubilization of hydrophobic compounds [5], binding of proteins [6] and cell internalization [7] are subjects of undiminished interest where synthetic polyelectrolytes unambiguously play a key role. Therefore, the field of polyelectrolytes is always in the center of scientific interest, not only in relation to fundamental understanding of their interactions and nanostructure, but also in applied research.

Polyelectrolytes may be schematically classified by increasing variety of interactive groups (charge and hydropathy) as in the simplified example of Figure 1. These molecular properties are responsible for a big number of possible nanostructures which in many cases are self-organized. From these nanostructures many applications may originate. Characteristic examples of nanostructures are the multi-scale organization of triblock polyelectrolyte hydrogels [8], the self-organized protein-polyelectrolyte complexes [9] and the hierarchical structure in thermoresponsive triblock polyelectrolytes [10]. The interactions of star-like micelles with hydrophobic counterions [11], polyelectrolytes with nanoparticles (NPs) of patchy surface charge [12] and anionic and cationic polyelectrolytes with cells [13] are intriguing new research works. Hydrogels for wound healing [14] and cartilage regeneration [15] are a couple of indicative possibilities offered by polyelectrolyte nanostructures.

In this review the recent literature on the use of polyelectrolytes for biomedical applications is presented in connection to the current interest on polyelectrolyte interactions and organization at the nanoscale. The polyelectrolyte intermolecular electrostatic interactions as a function of solution pH and salt content are a field of intense research especially in the case of polyelectrolyte/protein interactions. In addition, the role of hydrophobic, thermoresponsive and helical units and the ability of polyelectrolytes to create a great variety

of nanostructures are presented. The interactions of polyelectrolytes with drugs, proteins and cells are discussed as they are crucial for applications in drug delivery and tissue engineering and the current progress on experimental and simulation works is presented. The fascinating properties of polyelectrolyte-based systems is a multi-dimensional and multi-disciplinary subject which will keep attracting the focus of research in the future.

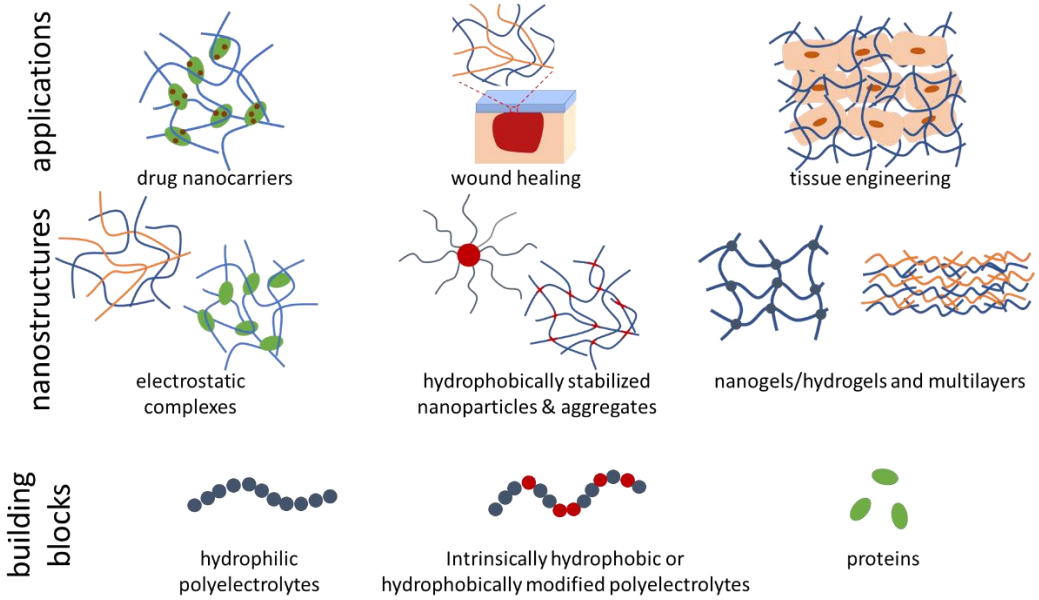

Figure 1. Illustrated examples of polyelectrolytes as building blocks, nanostructures and applications in life sciences.

2. Polyelectrolyte Nanostructures

2.1. Universal and Intrinsic Properties

Polyelectrolytes are macromolecules that contain groups that can dissociate in water and other polar solvents creating charged monomers on the macromolecular chain and free counterions in the solution [16]. Their conformation in dilute solution and in the absence of any salt is fully extended i.e., their size R (e.g., end-to-end distance or radius of gyration) scales with the degree of polymerization N as $R \sim N$ because of the long-range of the Coulomb potential [17–19]. Addition of salt ions leads to screening of the electrostatic interactions. Under the Debye– Hückel theory, the Coulomb potential is modified by an exponential term which introduces a finite range to the electrostatic interactions, the so called Debye screening length r_D [20] This allows for tuning the size of a linear polyelectrolyte chain by the ionic strength I as $r_D \sim I^{-1/2}$ and consequently the fully extended conformation $R \sim N$ at no or low added salt may change to the excluded volume chain conformation $R \sim N^{3/5}$ at high added salt. In weak polyelectrolytes, where the degree of ionization depends on the solution pH, contrary to the strong polyelectrolytes, pH is another external trigger that can tune the chain conformation as it defines the number of charges along the chain. Current investigations focus on the charge regulation mechanism [21] and ion solvation effects [22].

The conformation of polyelectrolytes and their interactions with other small and large molecules are not determined solely by the distribution of charges and the electrostatic effects. Their intrinsic characteristics i.e., their charge-independent properties differ greatly and allow wide variations from universal scaling laws in conformation and dynamics. Charged macromolecular chains may contain hydrophobic units either as comonomers, in the case of hydrophobically-modified polyelectrolytes [23,24], or intrinsically hydrophobic chargeable monomers in the case of intrinsically hydrophobic polyelectrolytes [25], which introduce another tuning parameter to harness their interactions and enhance their functionality. The combination of hydrophobic interactions with other short-ranged in-

teractions such as hydrogen bond may lead to stabilization of complex structures with the characteristic example of globular proteins. Another important intrinsic property of polyelectrolytes is the intrinsic persistence length l_p [26,27]. Polymer chains are divided in flexible for $l_p \approx a$, semi-flexible for $l_p \approx L$ and rigid for $l_p \ll L$, where a and L are the monomer length and contour length respectively [28,29]. In semi-flexible polyelectrolytes the persistence length can be decoupled into intrinsic and electrostatic persistence length, where the latter is a decreasing function of ionic strength [30].

2.2. Nanoscopic Assemblies

Amphiphilic copolymers of charged and hydrophobic monomers combine the responsiveness of the charged polymers to solution conditions and the tendency of hydrophobic groups to associate with each other in aqueous media. Block polyelectrolytes with one charged and one hydrophobic block lead to the formation of core-shell micelles where a hydrophobic core is surrounded by a charged polymer corona. This kind of NP allows for the solubilization of hydrophobic molecules, e.g., drugs, in their core while the external shell not only stabilizes them in aqueous media [31,32] but also can bind oppositely charged molecules, e.g., proteins [33–35]. On the other hand, random amphiphilic copolymers of polyelectrolyte and hydrophobic monomers, the so called hydrophobically-associating polyelectrolytes, are able to tune viscoelastic properties in aqueous media via a balance between electrostatic repulsions and hydrophobic associative attractions [36,37]. Except from the response to the pH and salt stimuli, polyelectrolyte-containing copolymers can response to the temperature of the solution by the incorporation of thermo-responsive blocks, the most often used being poly(N-isopropylacrylamide) (PNIPAM) [38]. The reason for PNIPAM's popularity is its lower critical solution temperature (LCST) at about 32 °C, which is between room and human body temperature. The interactions of proteins with thermo-responsive amphiphilic triblock polyelectrolyte micelles [39,40] and doubly hydrophilic thermo-responsive triblock polyelectrolytes [10] have been investigated by small angle neutron scattering and light scattering. It was shown that temperature response of the polyelectrolyte nano-assemblies was length-scale dependent and that salt content could determine protein–polyelectrolyte interactions.

Charged polysaccharides are a special class of polyelectrolytes that are very important for health and are constantly used in life sciences [41,42]. Their hydrophilic backbone distinguishes them from many synthetic polyelectrolytes that are intrinsically hydrophobic. As they are of natural origin, they are biocompatible, nontoxic and safe, and, therefore, they are very often used in biomaterials and biomedicine [43,44]. Hyaluronic acid is a flexible polyelectrolyte of the extracellular matrix with many applications in tissue regeneration and engineering [45]. Chitosan is a very common polysaccharide in applications as it is a natural cationic polyelectrolyte [46,47]. Xanthan gum forms a very interesting secondary structure at room temperature and moderate ionic strength i.e., its charged side-chains stabilize single or double helices that induce rigidity to xanthan macromolecules [48]. Carrageenans are sulfated gel-forming polysaccharides. κ- and ι-carrageenans have a coil conformation which may transform into single helix conformation in suitable ionic strength [49].

2.3. Network and Film Nanostructures

Polyelectrolyte hydrogels and nanogels are fascinating nanomaterials with tremendous ability for swelling–de-swelling transitions under the external triggers of salt and pH. They provide a highly hydrophilic environment for accommodation of hydrophilic molecules and they can interact via electrostatic interactions with charged molecules. Fascinating self-assembled hydrogels were formed by triblock copolymers whose end-blocks consisted of either an anionic or a cationic polyelectrolyte. The poly(allyl glycidyl ether)−poly(ethylene oxide)−poly(allyl glycidyl ether) end-blocks were functionalized either by sodium sulfonate or guanidinium. Increasing polymer concentration, the electrostatically stabilized domains of the oppositely charged interconnected polyelectrolyte

end-blocks led to disordered spheres, BCC spheres, HCP cylinders, gyroids and parallel lamellae (Figure 2). These features were proven by small angle neutron scattering. The viscoelasticity of these unique hydrogels was greatly affected by the morphology of their nanostructure [8].

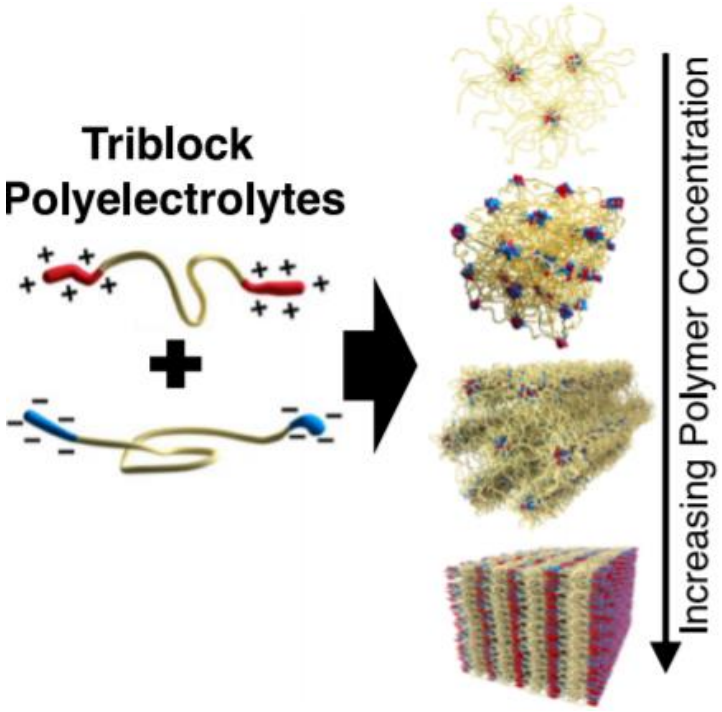

Figure 2. Illustration of spatial organization of electrostatic complexes between end-functionalized oppositely charged polyelectrolytes as a function of concentration. Reprinted with permission from [8]. Copyright 2020 American Chemical Society.

Gelatin–polyacrylamide interpenetrating networks were obtained in two steps. First, a gelatin–acrylamide aqueous solution was heated and re-cooled in order to obtain the three-strand spiral structure of gelatin. Subsequently, acrylamide was polymerized at a higher temperature. The hydrogels were characterized as super-compressible, and they had very good compression resistance and dissipation energy. The mechanical properties were attributed to the triple-helix association of gelatin [50]. Nanogels (NGs) of a size of about 400 nm that were able to respond to the external pH and temperature were realized by interpenetrating networks of sodium alginate and PNIPAM. Ethylenediamine and glutamic acid groups were added to alginate resulting to an isoelectric point of the NPs at 5–5.5. The LCST of the NGs was 31 °C. Therefore, two volume phase transitions were possible in this system [51].

2.4. Electrostatic Complexation

Polyelectrolyte macromolecules can associate with other charged molecules with noncovalent interactions to prepare interconnected structures. Alginate crosslinking by the divalent cations Ca^{2+} and Zn^{2+} can be used for the preparation of NPs and hydrogels [52]. Crosslinking using salt ions has been also reported in carboxymethyl xanthan [53] and carrageenan [54,55]. Complexes between oppositely charged polyelectrolytes is an often-used strategy to prepare nanostructures in aqueous solutions [56]. Recent examples include electrostatic associations between diethylaminoethyl chitosan and hyaluronic acid [57], poly(maleic acid-alt-octadecene) sodium salt and Eudragit E-100 chloride salt [58] and chitosan and chondroitin sulfate [59]. A versatile methodology was proposed in the latter where a low viscosity chondroitin sulfate (low molar mass) solution was poured on top of a viscous chitosan (high molar mass) solution to form the complex (Figure 3). Recently,

the electrostatic interaction of polyelectrolytes with proteins has been considered as a biocompatible method to prepare multifunctional nanostructures [9] (Section 3.2).

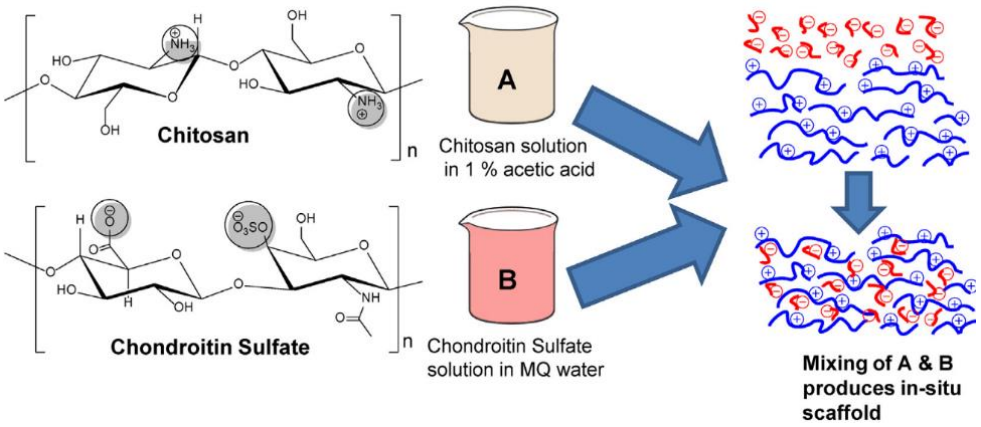

Figure 3. Formation of chitosan–chondroitin sulfate polyelectrolyte complex scaffold for chronic wound management. Reprinted from [59], copyright 2021, with permission from Elsevier.

3. Interactions of Polyelectrolytes with Pharmaceutical and Biological Matter

3.1. Interactions with Molecular Drugs

Significant current research focuses on the understanding and manipulation of the interaction mechanisms between molecular drugs and polyelectrolyte nanostructures. These investigations are important because they lay the ground for more systematic and effective use of polyelectrolyte-based materials for drug delivery and release. The electrostatic association of insulin (INS) with the cationic polyelectrolyte poly(2-acrylamidoethyl -co-phenylboronic acid) resulted in stable complexed NPs containing the diabetes drug. The charge of the polyelectrolyte was decreased and reversibly regulated in the presence of glucose avoiding the risks of hyperglycemia and hypoglycemia. Glucose binding to phenylboronic acid causes the decrease of its charge density. This is due to the formation of a reversible covalent bond of phenyl borate with glucose that stabilizes its negatively charged form [60].

Interactions of hydrophobic drugs with amphiphilic neutral block copolymer core-shell micelles have been predicted by docking studies. These works can be potentially applied on polyelectrolyte core-shell micelles to estimate the possibilities for drug solubilization in the core of the micelles for systems of interest. In more detail the affinity of the hydrophobic core of micelles from the di-block copolymer poly-[D,L-lactic acid]-b-poly-(ethylene glycol) (PLA-b-PEG) has been investigated by the atomistic simulations. The adsorption free energies and binding constants on modelled PLGA surfaces were calculated and correlated well with experimental studies [61].

The structure of layer-by-layer constructed nanocapsules from the oppositely charged polyelectrolytes poly(styrene sulfonate) and poly(diallyldimethylammonium) was investigated by coarse-grained all-atomistic molecular dynamics simulations using a modified MARTINI force field. This work demonstrated that the incorporation of the dexamethasone drug in multilayered nanocapsules could be modelled and the methodology could be applied to other polyelectrolyte/drug systems. Remarkably, it was observed that as salt content increased the single or multiple layers became denser and thicker due to the relative enhancement of Van der Waals attractions in screened electrostatic interactions [62].

An interesting simulation work on the effect of hydrophobicity in polyelectrolyte systems was performed by Fernandez-Alvarez et al. Polystyrene-block-poly(2-vinyl pyridine) (PS-b-P2VP) of star-like (micellar) morphology with the model cobalt bis(1,2-dicarbollide) (COSAN) as model hydrophobic counterion [11]. Their findings were supported by light scattering, microscopy and NMR experiments. The Hamiltonian Monte Carlo approach contained steric repulsions and hydrophobic attractions of the same short-range form

(different magnitude and sign) and Coulomb interactions. Additionally, the weak character of the polyelectrolyte was taken into account by the monomer dissociation equilibria. An example of the simulation results is illustrated in Figure 4. A star with dissociation constant $pK_A = 4$ contains some collapsed chains on the uncharged hydrophobic core together with charged extended chains even at high salt content when the counterions are not hydrophobic. The situation is very different when counterions are considered to be hydrophobic. The arms of the micelle gradually collapse leading to a globular morphology with no ionized shell at high COSAN/P2VP molar ratios (ξ). Experimentally, this effect leads to aggregation and precipitation of the micelles.

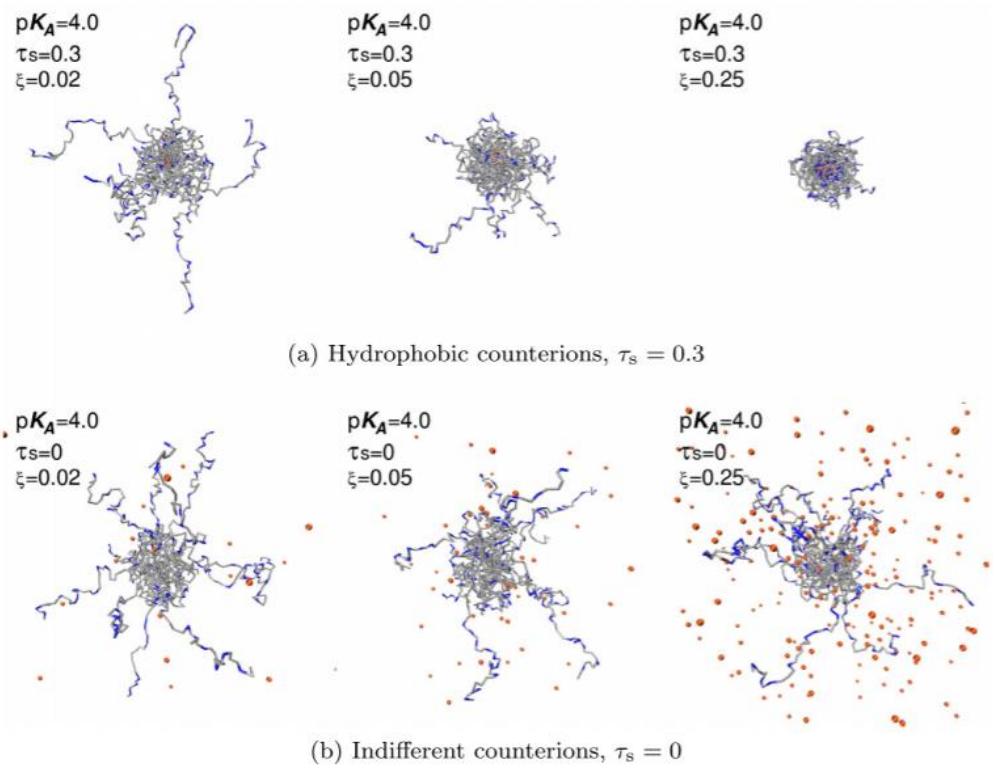

Figure 4. Snapshots from simulation of a star polyelectrolyte with 20 arms with 50 segments each and hydrophobic (top) or hydrophilic (bottom) counterions. Reprinted from [11], copyright 2019, with permission from Elsevier.

3.2. Interactions Involving Proteins

Proteins can be viewed as polyelectrolytes of high complexity as they are polyampholytes with pH-dependent surface charge distribution. In addition, they can be considered as building blocks of polyelectrolyte-based multifunctional NPs. In that sense it is very useful to explore advanced studies on proteins that are related to applications in drug delivery. Casein micelles were stabilized by Ca^{2+} together with the anticancer drug doxorubicin (DOX) to produce orally administered NPs for controlled release of DOX at the acidic environment of the stomach [63]. Fluorescence quenching experiments were used to quantify the interaction of DOX with the protein's tryptophan residue [64]. The resulting binding constants revealed static binding of the drug.

Albumin is a plasma protein that has served as a carrier of molecular drugs [65,66]. In its unmodified form it contains two main drug-binding sites the Sudlow site I and II which have been used to encapsulate the anticoagulant warfarin and the anxiolytic diazepam respectively. Natural albumin has been modified with isothiocyanate conjugates to increase its high-affinity sites and improve the antitumor activity of paclitaxel (PTX) loaded in BSA and in Abraxane. The study was supported by molecular docking and molecular dynamics simulations in combination with molecular mechanics Poisson–Boltzmann surface area to

investigate the mechanism and calculate the energies of the drug binding to the protein [65]. It was shown that phenethyl isothiocyanate (PEITC) could significantly lower the binding free energy of PTX due to the exposure of hydrophobic domains in BSA-PEITC leading to a more sustainable drug release (Figure 5). Weakening of hydrogen bonds at acidic pH led to an increase in the release of the drug which is extremely important for delivery in the acidic tumor environment.

Interactions of polyelectrolytes with proteins is a complex phenomenon with intense research interest because of its practical applications. Complexation between the two kinds of macromolecules may occur even when the overall charge of the protein is of the same sign as the one of the polyelectrolyte [67–69]. There are two possible mechanisms under this effect, namely the surface charge regulation and the charge anisotropy. In addition, the release of counterions has been proposed as a mechanism where the increase in entropy favors complexation [70]. Interactions of the dendritic polyelectrolyte polyamidoamine with the proteins human serum albumin and immunoglobulin have been studied with atomistic discrete molecular dynamics [71]. It was shown that electrostatic attraction between the polyelectrolyte and the negatively charged moieties on the protein surfaces was the main driving force for complexation while they were assisted by hydrophobic interactions.

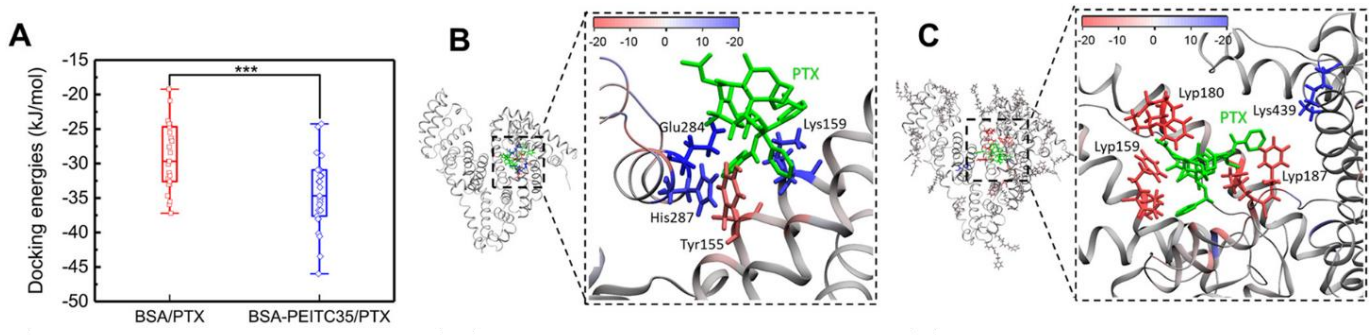

Figure 5. Binding mechanisms described by BSA/PTX (blue) and BSA-PEITC35/PTX (red) lowest binding energies (**A**) and respective snapshots (**B**,**C**). Reprinted from [65], copyright 2019, with permission from Elsevier.

A patchiness parameter for the proteins' surface has been used to quantify the charge anisotropy on the surface of mutated green fluorescence proteins. It was shown that increased patchiness significantly increased the possibility for complexation with a polyelectrolyte [69]. For patchiness greater than about 0.6, insoluble proteins were observed in the absence of salt. In the case of qP4VP, complex coacervates were found for 0.45–0.55 and solid precipitates at 0.2–0.35. At high salt content screening of electrostatic interactions prevented complexation (Figure 6). Charged NPs with heterogeneous surface charge distribution were employed in mean field simulations as models for proteins. Interactions with anionic polyelectrolyte chains revealed that the increase in the number of charge patches keeping the total positive and negative charge was equivalent to reducing the overall positive charge [12].

3.3. Polyelectrolyte-Cell Interactions

Polyethylenimine was combined with several polyanions in polyelectrolyte multilayers in order to test the effect of surface charge and wettability on cell adhesion and proliferation [72]. This effect was strong in the case of un-crosslinked multilayers whereas it did not play significant role when crosslinking was performed. It was observed that when the surfaces where positively charged, fibroblast adhesion was more pronounced, due to the electrostatic attraction with the cell surface and the possible interaction with the negative serum proteins. However, cells demonstrated better proliferation in negative surfaces due to the documented cytotoxicity of polyethylenimine. Very interestingly, cells adhered and proliferated very well on crosslinked surfaces regardless of the charge sign. This revealed that the mechanical stiffness of the layer is a defining parameter and that the

restriction in mobility of polycationic chains prevents toxicity. Hydrophilic and negatively charged surfaces were more effective in preventing bacterial adhesion [72].

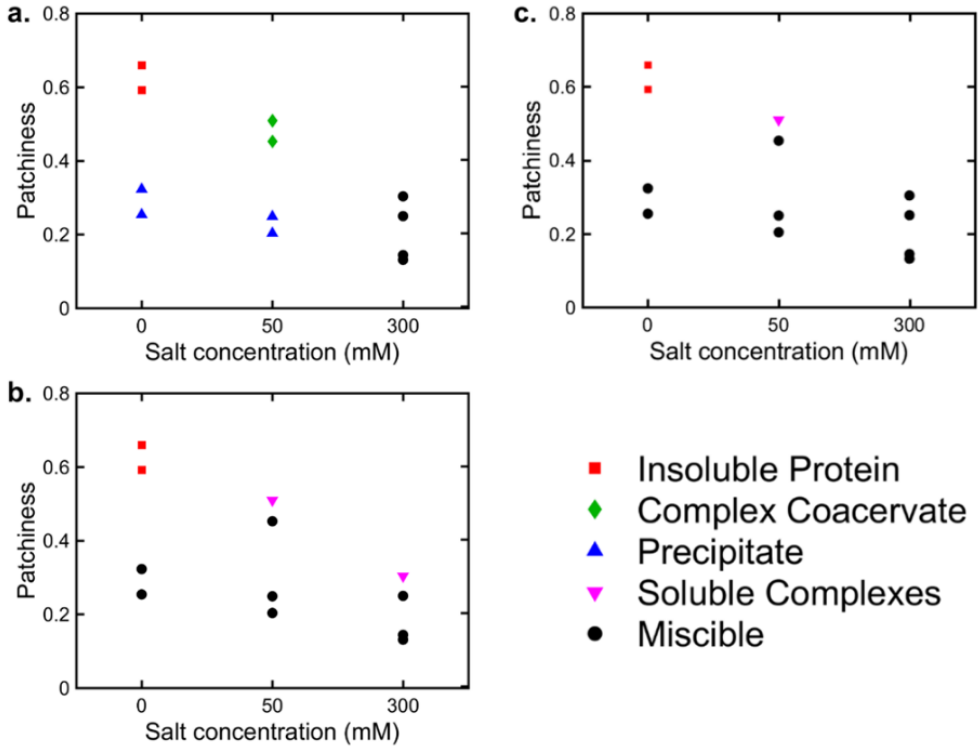

Figure 6. Phase diagram of GFP variants with (**a**) quaternized poly(4-vinylpyidine, (**b**) polyacrylic acid, and (**c**) hyaluronic acid at maximal complexation for different patchiness values. Reprinted with permission from [69]. Copyright 2020 American Chemical Society.

The adhesion of polymers to epithelial cell monolayers and soft tissues has been quantified by attaching macromolecular brushes on a silica NPs on the edge of an AFM tip [13]. The experiments were supported by measurements on self-assembled monolayers with different properties such as hydrophilicity, charge and hydrogen bonding. The interaction of human keratinocyte cells and human primary epidermal keratinocytes monolayers with the cationic polyelectrolyte poly(dimethylaminoethyl methacrylate) (PDMAEMA), the anionic polyelectrolyte poly(acrylic acid) (PAA) and the neutral hydrophilic polymer poly(oligoethylene glycol methacrylate) (POEGMA) were studied (Figure 7). The length and the high density of POEGMA side-chains resulted in low adhesion forces in agreement with the known resistance of POEGMA brushes to proteins and resistance. PDMAEMA brushes showed higher adhesion, which was, however, rather weak. This was attributed to the possibility of rapid protein adsorption and masking of PDMAEMA. PAA showed strong adhesion to primary keratinocytes and weak adhesion to human keratinocytes. The difference was attributed to the differences of the glycocalyx layers between the two cell cultures. Deeper insights to the physical interactions between the brushes and the cells were obtained by enzymatic cleavage of the glycocalyx components [13].

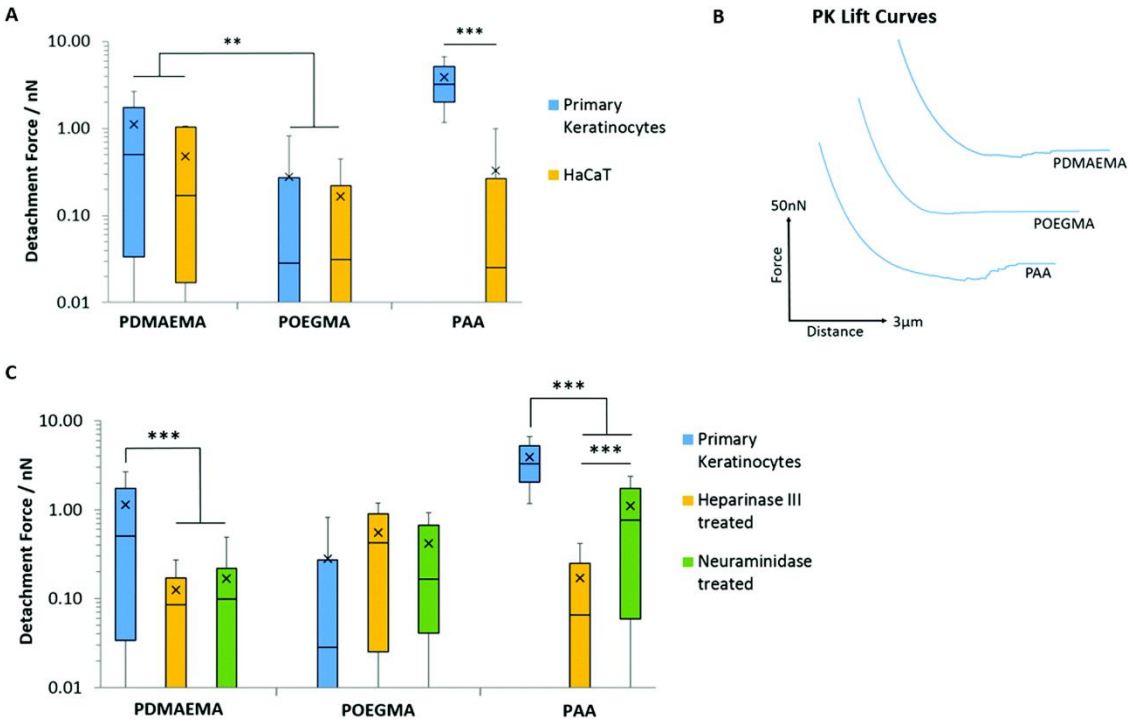

Figure 7. Detachment forces of polymeric brushes from cell monolayers (**A**), AFM lift curves (**B**) and detachment forces with and without treatment with enzymes (**C**) [13]. Published by The Royal Society of Chemistry.

4. Polyelectrolytes in Drug and Protein Delivery

4.1. Self-Assembled Nanoformulations

Self-organization in polyelectrolyte systems can be used to prepare pH-responsive and biocompatible drug carriers. The combination of an o-carboxymethyl chitosan gel with folic (conjugated) and bisdemethoxycurcumin (encapsulated) with aminated mesoporous silica NPs loaded with the hydrophilic drug 5-Fluorouracil (5-FU) and coated with alginate [73]. The electrostatic interactions between the two polysaccharides and the silica NPs played the main role on the formation of the final multifunctional NPs. The release of the two anticancer drugs was enhanced at physiological pH driven by the swelling of the polyelectrolyte matrix. The dual drug system was proposed for an improved therapy of colorectal cancer. Double water-in-oil-in-water emulsions with internal aqueous phase of monomethoxy polyethylene glycol-poly (lactic-co-glycolic acid) (mPEG-b-PLGA) with solubilized insulin were prepared for oral delivery and showed pH-responsive release and non-toxicity [74]. The emulsion NPs were coated by either alginate or chitosan and upon mixing resulted in electrostatic complexes with a size of 200–300 nm.

The combination of electrostatic, hydrophobic and specific interactions may be used to tune self-assembly, drug loading and release and targeting. Anionic and cationic polypeptides were patterned with different charge and hydrophobicity [75]. Complexes between oppositely charged polypeptides were found more stable for increased charged density and hydrophobicity, while hydrophobic content enhanced the encapsulation of hydrophobic molecules. Electrostatic complexes of the amphiphilic polyelectrolytes, cationic polysuccinimide with secondary hydrophobic amine groups and anionic sodium polyaspartate, showed an increased ability to encapsulate curcumin, rapid cell internalization and reduced cytotoxicity in comparison to the individual polyelectrolytes [76]. Incorporation of carbamoylmannose in polyelectrolyte NPs illustrated specific uptake from cancer cells through receptor-mediated endocytosis [77]. Interestingly, this bleomycin monosaccharide was loaded on NPs formed by host–guest interactions between β-cyclodextrin-terminated poly(N-vinylpyrrolidone) and adamantyl-ended poly(aspartic acid). Core-shell micelles of poly(aspartic acid-graft-imidazole)-poly(ethylene glycol) block copolymers assembled

and disassembled upon pH trigger [78]. They could release indole-3-acetic acid and be toxic at the acidic tumor environment due to the protonation of imidazole groups. Finally, a poly(l-glutamic acid) and histidine-glutamic acid copolymer was combined with poly(l-lysine) to make complexes with tunable pH-induced dissociation and controlled release of daunomycin [79].

4.2. Macro- and Nano-Hydrogels from Polyelectrolytes

Polymerization methods play a central role in the synthesis of polyelectrolyte networks for nanodelivery applications. A promising method for NG synthesis has been recently proposed where the building block of the NG is an ionic monomer that is polymerized and cross-lined in the presence of a template di-block hydrophilic polyelectrolyte of opposite charge [80,81]. The template di-block polyelectrolyte directs the formation of complex micelles and can be reused after its removal by addition of salt. This electrostatic assembly directed polymerization was applied to synthesize cationic and pH responsive NGs of Poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) by the aid of poly (acrylic acid)-b-poly(ethylene oxide) (PAA-b-PEO). The NGs were able to encapsulate and release the anionic dye new coccine. NGs of size about 250 nm and a good size distribution were obtained by copolymerization of methyl methacrylate (MMA) and itaconic acid (IA) in the presence of the ethylene glycol dimethacrylate (EGDMA) crosslinker [82]. In vitro experiments on Culture of Caco-2 cells and in vivo studies on female Sprague Dawly rats did not show any toxicity. NGs based on MMA and IA have been also employed for the encapsulation of insulin (INS) for enhanced absorption in oral administration [83].

Different kinds of drugs, proteins and growth factors are delivered by NGs that are based on polysaccharides and other biocompatible polyelectrolytes. NGs of maleic anhydride-modified chitosan and poly(asparctic acid) were formed in water by electrostatic interactions between the amino groups of chitosan and the carboxyl groups of poly(asparctic acid) and the hydrogen bonding between the hydroxyl hydrogens of chitosan and the carbonyl oxygens of poly(asparctic acid) [84]. Release of amoxicillin from the NGs was higher at pH 7.4 in comparison to pH 5.4, which was considered promising for treatment of skin infection and was caused by the ionization of the -COOH groups of both polyelectrolytes and the resulting enhancement of electrostatic repulsions. The drug-loaded NGs had antibacterial properties and were fairly biocompatible. NGs based on poly (acrylamide-co-diallyldimethylammoniumchloride) [85], alginate [86], hyaluronic acid [87] and gum arabic [88] have been used for the delivery of anticancer drugs and imaging agents. Microgel beads based on gelatin and crosslinked with glutaraldehyde were developed for the delivery of bone morphogenic protein 4 and basic fibroblast growth factor [89]. The crosslinking density could be tuned to achieve complete release of the growth factor. Injectable and self-healing hydrogels based on DNA were made by combining with oxidized alginate and the formation of reversible imine crosslinks [90]. They were applied as carriers for simvastatin to avoid any cytotoxic effects. Sulfonated poly(vinyl alcohol)-based hydrogel beads were decorated by electrostatically stabilized alternating multilayers of chondroitin sulfate and hydrolytically degradable poly β-aminoester in order to prevent the burst release and prolong the discharge of the model drug protein lysozyme [91].

4.3. Polyelectrolyte Nano-Assemblies with Proteins as Building Blocks

Polyelectrolyte–protein electrostatic complexation can be both employed for protein delivery and creation of multifunctional NPs where proteins are actual building blocks. Encapsulation of enhanced green fluorescent protein in poly(2-vinyl-pyridinium)-b-poly(ethylene-oxide) was achieved by the formation of electrostatic coacervate-core PEO-shell micelles which release the protein at high salt content [92]. Similarly, electrostatically stabilized core-shell micellar structures were stabilized from the interaction between poly(ethylene glycol)-b-poly(l-glutamic acid) and brain-derived neurotrophic fac-

tor and were used for intranasal delivery to the mouse brain with increased neuroprotective action [93].

Protein–polysaccharide complexes thermally stabilized against pH changes with ability to encapsulate nutraceuticals have been realized by the pairs of chondroitin sulfate-BSA [94] and hyaluronic acid-fibrinogen [95]. Recently, their ability to encapsulate and preserve curcumin was demonstrated by the xanthan-BSA system [96]. The degradation of curcumin's structure at neutral pH was prevented by its loading on the NPs (Figure 8). Curcumin's structural stability is very important for its physiological activity. However, it degrades rapidly at neutral and basic pH because of a compromise in its conjugated diene structure. The kinetics of curcumin's degradation was investigated by monitoring its UV-Vis absorbance at maximum (425 nm) (Figure 8). The structure was fairly stable either if the xanthan-BSA NPs were present or not at pH 5. At pH 7 the NPs sustained more than 85% of the absorbance while in the absence of NPs the absorbance droped to less than 60% within 250 min. This investigation shows the possibilities that may arise by combining hydrophilic polyelectrolytes with globular proteins. The resulting NPS inherit the hydrophobic and pH-tunable surface charge of the protein and therefore can interact with other molecules both by hydrophobic and electrostatic interactions. In addition, the aforementioned methodology is based on biocompatible components and does not involve any chemical reactions or toxic organic solvents.

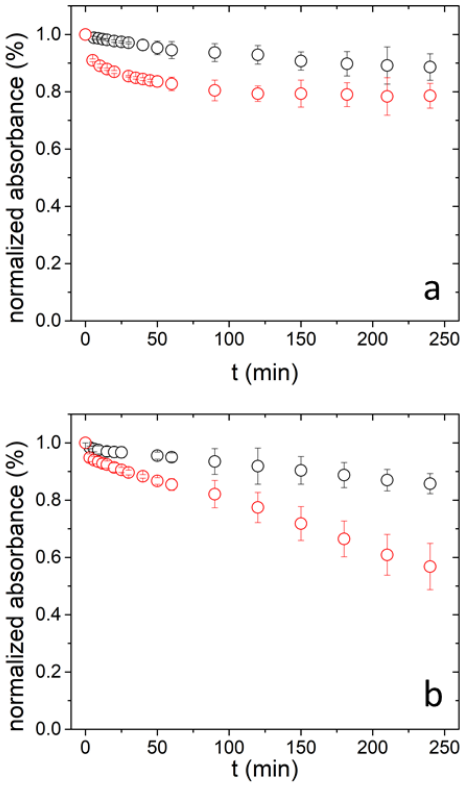

Figure 8. Curcumin's absorbance at 425 nm in free solution (black) and loaded on xanthan -BSA NPs at pH 5 (**a**) and pH 7 (**b**). Reprinted from [96], copyright 2021, with permission from Elsevier.

4.4. Polyelectrolyte-Based Films for Sustained Drug Release

Polyelectrolytes have been successfully used in films for delivery applications. Aqueous solutions of chitosan mixed either with pectin or with hydroxypropylmethyl cellulose and plasticizers were prepared by casting and drying [97]. The films had good mechanical and mucoadhesion properties, they could controllably release miconazole nitrate and they proved promising as buccal films against oral candidiasis. Chitosan combined with alginate was used to prepare films where the molar mass of chitosan and the amount of alginate

could tune the adhesiveness and release kinetics of clindamycin phosphate for periodontal therapy [98].

Polyelectrolyte films can also be used for food packaging applications as for example in antibacterial sodium alginate and cationic starch mixtures [99]. These materials had adequate thermal properties and a glass transition temperature that could be increased by the content of sodium alginate. Highly hydrophilic hyaluronic acid/poly-l-lysine multilayer constructions could create free standing films for the delivery of platelet lycates in healing of cutaneous injuries [100]. In vitro studies on primary human umbilical vein endothelial cells and fibroblasts showed that they were effective substrates for angiogenesis and fibrogenesis. The release of the proteins was sustained for many days while crosslinked films showed slower kinetics [100].

5. Polyelectrolytes in Tissue Regeneration

5.1. Wound Treatment

Polyelectrolytes are extensively used in tissue engineering as they can form highly hydrophilic networks able to accommodate cells and are biocompatible, in particular when they belong to the class of polysaccharides or polypeptides. Ethylene glycol oligomers acting as crosslinkers were successfully used to tune the mechanical properties of methacrylated hyaluronic acid hydrogels that were promising for cell encapsulation [101].

Biocompatible hydrogels of heparin in combination with polyethylene glycol (PEG) with encapsulated human epidermic growth factor showed very good histological results on granulation tissue formation and epithelization on full thickness skin wounds on mice [102]. Networks of heparin were electrostatically stabilized and tuned in regard to their viscoelasticity by linear and star poly-l-lysine and loaded with vascular endothelial growth factor had good cell compatibility, antimicrobial activity and were applied on in vivo animal models for wound healing [14].

Click chemistry was successfully applied to synthesize pectin conjugated with a cell adhesive peptide and crosslinked with a biodegradable peptide [103]. These cell-instructive hydrogels could be formed or remodeled in the presence of fibroblasts and facilitated cell attachment showing promising behavior for treatment of skin wounds. κ-Carrageenan shows good potential for tissue repair as it has been tested in sprayable hydrogels [104], in ionic-covalent crosslinked interpenetrating bio-printed hydrogels [105] and in nanocomposites with nanosilicates that reduce blood clotting time and can sustainably deliver active molecules [106]. Treatment of nasal epithelium wounds could be achieved by spraying a tranexamic acid-containing chitosan solution that could rapidly gel in the nasal cavity environment by the aid of glycerol-2-phosphate disodium salt hydrate [107].

5.2. Bone and Cartilage Regeneration

Polyelectrolyte hydrogels may be used in bone and cartilage regeneration by providing a medium for cell accommodation and by delivering growth factors and proteins. Hyaluronic acid is very hydrophilic, biocompatible and biodegradable and its hydrated networks can simulate the extracellular matrix [108]. Hyaluronic acid hydrogels with embedded platelet-rich plasma were applied on full-thickness cartilage defects on a porcine model [15]. The successful regeneration of the bone tissue was attributed to the hydrogels' ability for cell adhesion and their good integration with the surrounding tissue. Simvastatin-loaded hyaluronic acid hydrogels have shown enhanced osteogenesis. Neocartilage tissue engineering was achieved in bio-printed 3D networks of methacrylated hyaluronic acid, with chondroitin sulfate or gelatin acting as a scaffold for the adhesion and proliferation of bone marrow-derived human mesenchymal stem cells [109].

Chondroitin sulfate is another good material for bone regeneration as it is the main constituent of glycosaminoglycan in cartilage [110]. It is involved in bone homeostasis and in the coordination of osteoblastic cell attachment. Kim et al. investigated the role of chondroitin sulfate's negative charge on the binding of cations (e.g., calcium and phosphate) and showed the hydroxyapatite crystal formation was enhanced accelerating osteogenesis.

In another study adipose derived stem cells and neonatal chondrocytes were co-encapsulated in hydrogels to investigate which cartilage phenotype is favorable depending on the matrix composition [111]. It was shown that hyaluronic acid and chondroitin sulfate matrices favored hyaline cartilage whereas in heparin sulfate matrices cells produced the undesirable fibrocartilage. It was additionally shown that an optimal hydrogel stiffness 7–33 kPa was needed for structural integrity and adequate deposition of neocartilage.

5.3. Cardiac Muscle and Vitreous Humor Tissue Repair

Promising advances in cardiac muscle regeneration have been made using bio-polyelectrolyte hydrogel matrices. Treatment of myocardiac infraction with stem cell implantation and limitation of reactive oxygen species has been addressed by introducing fullenerol NPs in injectable alginate hydrogels. The antioxidant properties of fullenerol NPs decreased the generation of reactive oxygen species in the intracellular environment of brown adipose derived stem cells reducing that were delivered by the ionically crosslinked alginate scaffolds [112]. Chitosan has been incorporated in de-cellularized extracellular matrix from porcine heart in order to optimize the mechanical properties of the injectable matrices [113].

Awada et al. followed a multi-component treatment strategy to design a system for myocardial regeneration after infraction [114]. A fibrin hydrogel was enriched with tissue inhibitor of metalloproteinases-3 to signal the initial treatment phase by its early release. Basic fibroblast growth factor and stromal-cell derived factor 1-alpha were incorporated in electrostatic coacervates between the anionic polysaccharide heparin and the cationic synthetic polyelectrolyte poly(ethylene argininylaspartate diglyceride). Loading the proteins in coacervates for the encapsulation into the fibrin hydrogels was a means to sustain the long-term healing process (Figure 9).

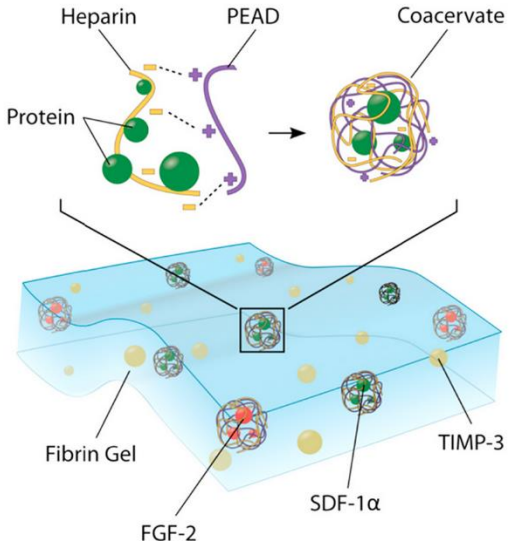

Figure 9. Fibrin hydrogels with protein-loaded coacervates. Reprinted from [114], copyright 2021, with permission from Elsevier.

Visible light photo-crosslinked gelatin-based injectable hydrogels have been proposed as a safe alternative to UV-crosslinked to avoid biosafety issues. The hydrogels had a compressive modulus in the order 5–60 kPa which makes them suitable for myocardium tissue regeneration [115]. New chitosan scaffolds with the ability to maintain high compression in order to be introduced in surgical lesions and with high elasticity to restore their shape afterwards could were prepared using ammonia instead of sodium hydroxide for neutralization [116].

Vitreous humor is a hydrogel matrix composed of hyaluronan and collagen that supports the ocular volume, protects retina and facilitates the diffusion of metabolites. Hyaluronic acid and silk-fibroin were crosslinked by horseradish peroxidase in order to

prepare matrices for replacement of vitreous humor tissue [117]. These materials were good candidates to fulfil the role of the multifunctional vitreous tissue regarding, mechanical stability of ocular volume, support and protection of retina and assisting diffusion of metabolites.

6. Conclusions

In this review, polyelectrolyte research was presented as a multifaceted area originating from fundamental molecular properties to advanced biomedical applications, as there are still many open questions and challenges. Polyelectrolytes present a very broad range of structures that stretch from simple hydrophilic homopolyelectrolytes to proteins with pH-dependent charge patch and hydropathic heterogeneity. This rich variety allows for the creation of multifunctional nanostructures with tremendous possibilities for biomedical applications. This field is unambiguously going to keep its momentum in every level for the next few decades as there are still many opportunities for development in pharmaceutical science and tissue engineering, and there is continuous feedback from the materials science perspective. Understanding the interactions and nanostructure at the molecular level makes significant advances based on powerful experimental techniques and simulation methodologies and opens a very promising way for more effective drug delivery and tissue regeneration in the near future.

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