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POLYELECTROLYTE COMPLEX COACERVATES: ATTRACTIVE SOFT MATTER: DETAILED INSIGHTS AND APPLICATION IN ADHESIVE TECHNOLOGY

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ABSTRACT

Underwater adhesion is a great challenge for the development of adhesives as the attractive interfacial intermolecular interactions are usually weakened by the surface hydration layer. Complex coacervation is a liquid–liquid phase separation driven by complexation of oppositely charged macromolecules. Coacervates have been widely employed in many fields including the pharmaceutical, cosmetic, and food industries due to their intriguing interfacial and bulk material properties. More recently, the coacervation process of sessile organisms like marine mussels and sandcastle worms has inspired substantial research interest in the fabrication of long-lasting underwater adhesives. Here, this review provides an overview of complex coacervations in adhesive technology, with an emphasis on their characterization and the uses of such materials for applications in the fields of biomedicine and tissue engineering.

1. What are complex coacervates?

The word "coacervation" was introduced by the pioneering work of Bungenberg de Jong and Kruyt[1] and derives from the Latin "acervus", which means aggregation (a heap), and the prefix "co" (together) to signify the preceding union of the colloidal particles. The process of coacervation leads to the formation of a dense macroion-rich phase (the coacervate) in equilibrium with a dilute macroion-poor phase (continuous phase or supernatant).[2-4] This process was initially reported by Tiebackx in 1911 without using the word;[5] he found that the addition of an acid to a mixed solution of Arabic gum and gelatin results in phase separation. It was almost two decades later that the term "coacervation" was coined by Bungenberg de Jong and Kruyt, who studied the phase behavior of several binary mixtures by optical microscopy. IUPAC defines coacervation as the separation into two liquid phases in colloidal systems (the phase more concentrated in colloid component is the coacervate, and the other phase is the equilibrium solution).[6] H. G. Bungenberg de Jong, one of the early leaders in colloid chemistry at Utrecht University in The Netherlands, defined and described the phenomenology of complex coacervation in several superb chapters in a book, Colloid Science II, edited by H.R. Kruyt,[7] written over several years and finally published in 1949. According to Bungenberg de Jong and Kruyt[7,8] two types of coacervation are observed: (i) simple and (ii) complex. In simple coacervation, phase separation is induced by the addition of incompatible, and poor solvents (like acetone, alcohols etc.) to the aqueous polymer solution, while complex coacervation is commonly described as a phase separation of polymer-poor supernatant phase and a dense, polymer-rich coacervate phase which results from the association / electrostatic interactions 9 between oppositely charged macro-ions when such associates approach electroneutrality.[10-13]

The principal requirements for such behavior appear to be the formation of soluble complexes with near-neutral charge and noncomplementarity between macroions, including gelatins of different isoelectric points,[14-16] proteins with either synthetic[17,18] or natural polyelectrolytes (PEs),[19,20] PEs with dendrimers[21] and PEs with oppositely charged surfactant micelles.[22] If ion- pairing is too efficient, precipitation will occur. An advantage of the system consisting of PEs with oppositely charged surfactant micelles is that micelle surface charge density (σ, C nm-2) can be varied continuously in a given sample via pH[23] or mixed surfactant stoichiometry.[24,25] Tuning σ allows construction of phase boundaries, which describe the formation of soluble complexes and coacervates at values of σ that depend on key electrostatic variables, that is, ionic strength, I, and polyelectrolyte linear charge density, ξ. The observed interdependence of σc, I, and ξ facilitate comparison of experiment with theories of polyelectrolyte-colloid interaction [26,27] and intermacroion condensation.[28,29]

Coacervation is considered to be an eco-friendly process as it usually takes place in water and under relatively mild conditions of pH and temperature. Additionally, it is also a cost- effective technique since neither a special device nor extensive production steps are required. The obtained structures induced by demixing are considered to be among the more intriguing systems in colloid chemistry.[30-32]

Theoretical treatment of complex coacervation of solutions of biocolloids and of synthetic polyelectrolytes followed shortly[33] after Bungenberg de Jong's pioneering studies.[8] These early approaches and extensions coupled Flory-Huggins theory with an additive Debye-Huckel electrostatic term, implicitly assuming that the macroions could be treated as well-separated, high- valence, pointlike particles. Such an assumption is clearly invalid in the coacervate phase, which is locally of high polymer concentration beyond c*. The earliest theories of complex coacervation, notably that due to Voorn and Overbeek,[33-35] attempted to augment the veritable Flory-Huggins description of polymer solution thermodynamics with a term to account for the screened electrostatic interactions among polyions, and following theoretical models were developed by Veis et al., 36 Nakajima and Sato,[37] and Tainaka.[38] The Veis-Aranyi theory 39 described the complex coacervation in gelatin systems as a two-step phase separation. Charged molecules interact first through electrostatic interactions and then aggregate. The neo- formed aggregates (identified later as soluble intrapolymeric complexes) slowly rearrange in time to form droplets called coacervate. This theory was subsequently confirmed and completed by an intermediate step. The primary soluble complexes interact to form electrostatically neutral interpolymeric complexes. These insoluble complexes ultimately form coacervates, which coarsen with time and sediment to form the so-called coacervated phase.

A more modern approach, based on the random phase approximation (RPA), accounts for the extended nature of polyelectrolytes and the coupling between conformational and electrostatic degrees of freedom in a mixed polyelectrolyte solution.[40-42] These theories are, however, restricted to weakly charged polyelectrolytes at modest salt concentrations where local details of ion size and hydration are relatively unimportant. Similar approaches have been used to study pH effects on charged colloids.43 Most recently, “field- theoretic” simulations have been used to examine the conditions under which oppositely charged polymers produce complex coacervates.[44,45]

2. Complex coacervation in adhesive technology

Adhesive technology, despite being known and exploited since the middle Pleistocene is rarely applied when dealing with adverse environments.[46] Most commercially available adhesives fail to offer a proper performance in wet and dynamic environments and do not achieve the required bonding strength.[47,48] Many surgical procedures are performed worldwide and the number continues to grow every year. In a recent study, over 300 million surgeries were performed in 2012; an increase of 33.6% over the last 8 years.[49,50] Currently, no tissue adhesive has been approved for clinical use that complies with all the requirements, including: easy delivery, fast setting time, strong adhesive and cohesive properties, and biocompatibility.[51,52] Due to these difficulties, in medicine, adhesive technology has been applied primarily for stopping bleeding and gluing skin externally, while current surgical closure techniques involve the use of invasive techniques like sutures, staples, or clips, which often result in secondary tissue damage, microbial infection, fluid or air leakage, and poor cosmetic outcome.[53] The development of effective surgical glues would dramatically reduce the incidence of such complications. A different, largely unexplored, strategy for the development of surgical glues is based on complex coacervation,[54-60] which is involved in the processing of natural adhesives employed by several organisms to attach to different surfaces underwater. 61-63 Coacervate-based adhesives were first observed in natural systems, such as the sandcastle worm and mussels. In the case of sandcastle worms and mussels, the coacervation phenomenon was found to play an important role in the formation of adhesive in a wet environment.[64-67]

Complex coacervates as being polymer-rich, water-insoluble complexes of oppositely charged polyelectrolytes with a low surface tension that makes them compliant with surfaces. After delivery, additional interactions need to be introduced to transition the viscous liquid into a strong and tough material to prevent flow under an applied stress.[68] Work on biomacromolecule-based complex coacervates has revealed that they have great potential as wet adhesives. Several research groups have employed the electrostatic interactions of complex coacervation to fabricate viscous glues, most of which set underwater due to covalent cross-linking reactions, providing the strength required to oppose detachment.[69-74] The higher water content of coacervates is due to the hydration of excess small ions. For all water-containing adhesives, the adhesive performance and the mechanical properties heavily depend on water content. The optimization of the water content and, consequently, of the polymer concentration is necessary to enhance the adhesion performance. For instance, commercial poly (ethylene glycol) (PEG)-based glues, such as DuraSeal and CoSeal, bind to tissues with a low adhesive strength, which is mainly attributable to the high water content, ranging from 90% to 99% w/v.[75] Water may also act as plasticizer, which can improve the adhesion performance.[76] Feldstein et al. studied how water content affects the adhesive properties of poly(N-vinylpyrrolidone)−poly(ethylene glycol) (PVP−PEG) blends.[77,78] By increasing the content of PEG, the water content increased and adhesion was enhanced, enabling the material to sustain higher deformations without considerably affecting the ultimate tensile strength. The optimal performance, in terms of peel force, was observed when the PEG concentration was increased to

36%, with the mode of failure transitioning from adhesive to cohesive, allowing fibrillation within the material.

More recently, attempts to develop an effective underwater adhesive have been made using complex coacervates that are based on recombinant mussel adhesive proteins (MAPs) due to the water immiscibility of complex coacervates and the adhesiveness of MAPs.[79] Lim and co-workers developed MAP-based encapsulated coacervates as smart tissue adhesives with drug carrier ability. In this study, an adhesive was formed by complex coacervation between cationic recombinant hybrid MAPs (fp-131 or fp-151) and the anionic hyaluronic acid (HA). The bulk adhesive strengths of coacervates were twice as strong compared to the protein itself on aluminum substrates.80 Cha and co-workers used the recombinant expression method to obtain rfp-1 MAP (AKP- SYPPTYK) for hydrogel formation by coordination (Fe3+) or covalent cross-linking (NaIO4). The hydrogel system showed maximum adhesion strengths of ∼130 and ∼200 kPa when cross-linked with Fe3+ and NaIO4 respectively. The difficulty in synthesizing the rfps in bulk limit the clinical application of these adhesive hydrogels.81 In another study, they engineered a residue-specific DOPA-incorporated recombinant mussel adhesive protein (dfp-3 and dfp-5) with DOPA content up to 23 mol%. The recombinant protein showed strong dry and underwater adhesion along with significant water resistance.[82] They also developed a light-activated, mussel protein-based bioadhesive (LAMBA) hydrogel using a photo-oxidative reaction in the presence of blue light involving Ru(II)bpy32+ as the activator and sodium persulfate (SPS) as the oxidizing agent with recombinant MAP. LAMBA demonstrated strong adhesion to wet porcine skin and also promoted wound healing in addition to wound closure in a rat model.[83] Lu and co- workers developed a hybrid molecular adhesive by fusing Mfps found in DOPA from mussel adhesives with the CsgA proteins found in the amyloid-based adhesives in E. coli (monomeric, CsgA-Mfp3; Mfp5-CsgA and copolymer constructs, (CsgA- Mfp3)-co- (Mfp5-CsgA)). The molecular hybrid self-assembled in which the β-sheet amyloid protein formed the core, whereas the disordered Mfps were exposed on the exterior. The (CsgA-Mfp3)-co-(Mfp5-CsgA) copolymer demonstrated impressive adhesion energy of 20.9 mJ/m2, which made it a strong competitor for application in medical adhesives.[84]

Biomimetic adhesives is another class of tissue adhesives inspired from the examples of adhesion in nature and rapidly gaining momentum in the field of biological adhesives. Stewart and co-workers described an electrostatically driven coacervate formation by using alternating anionic and cationic block copolymers. As a mimic of caddisfly silk, these block copolymers are functionalized with amine, phosphate groups, divalent cations, and also dihydroxyl aromatic groups for oxidative cross-linking.[85] The main biomedical areas for such adhesives include bone,[86] cartilage,[87,88] and tissue repair,[86,88-94] as well as implants.95 For example, coacervate-based adhesives composed of poly (acrylamide-co-aminopropyl methacrylamide)-poly (ethylene glycol diacrylate) and poly (2- (methacryloyoxy) ethyl phosphate dopamine methacrylamide)-poly (ethylene glycol diacrylate) were used in vitro to seal an iatrogenic defect in a fetal membrane patch. The adhesives were able to function, as well as withstand traction and turbulence without leakage of fluid or slippage.[96] Cytotoxicity tests revealed the adhesive to be non-toxic and may help prevent iatrogenic preterm premature rupture of the membranes.[96] In another example, craniofacial reconstruction via a non-cytotoxic coacervate adhesive of gelatin and phosphodopamine in rats was conducted. The adhesive was used to attach a piece of circular bone in the skull and, after recovery from anaesthesia, were allowed to move freely. The adhesive was observed to effectively hold the bone in place despite free movement of the animals. Furthermore, as the adhesive material was resorbed by the body, it was replaced by new bone without affecting alignment. Though these and other examples have demonstrated the potential for coacervate-based adhesives, further in vivo testing and ultimately clinical experiments are still needed to fully validate their safety and efficacy.88 However, one particular advantage of bio-inspired coacervate-based adhesives would be the potential for repairing tissue and bone without the need to remove the adhesive at a later time.

3. CONCLUSION AND FUTURE PERSPECTIVES

Our knowledge of underwater adhesion is still quite limited and considerable efforts are being invested to study adhesion in natural systems. Deeper understanding of the interplay of environmental and chemical factors, chemistries, and mechanisms of natural adhesion will open numerous possibilities for further advancement in biomimetic tissue adhesives. Currently, biomimetic adhesives involve coacervate formation or functionalization with an adhesive group like DOPA, catechol, or phosphates.[97,98] Although these strategies have shown satisfactory adhesion under dry conditions, they often tend to fail under humid and/or wet conditions.[99]

Recent studies have looked at incorporating a range of cross-linking chemistries to strike a balance between the adhesive and cohesive strengths (see above). Despite such extensive research on tissue adhesives, we have been unsuccessful in developing adhesive mimics capable of rivaling natural adhesives that are scalable, nontoxic, biocompatible, easy to use and degradable. There is still a need to explore the effect of backbone chemistry, polymer hydrophilicity/hydrophobicity, combination of different amino acids, and charged side groups on adhesion strengths. It is also necessary to widen the scope of adhesives beyond tissue adhesion toward drug delivery, tissue grafts, wound healing, and tissue reconstruction via addition of peptides. In addition, long-term studies and clinical trials are essential before these adhesives can be realized in medical or surgical applications.

Another focus of future work should be to better understand processing methods used by marine creatures to store, process, and deliver the adhesive to the interface. Indeed, biomimicry of sandcastle worm complex coacervate formation is an early example of this. However, further research into coacervate formation is necessary to relate phase behavior to mechanical properties as a function of pH, concentration, temperature and ionic strength.

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Notes

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