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Coacervate delivery systems for proteins and small molecule drugs

Noah R Johnson, BS[1,2] and **Yadong Wang, PhD**[1,2,3,4,5]

[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15219

[2]McGowan Institute for Regenerative Medicine, Pittsburgh, PA 15219

[3]Department of Chemical and Petroleum Engineering, University of Pittsburgh, Pittsburgh, PA 15219

[4]Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15219

[5]Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA 15219

Abstract

Coacervates represent an exciting new class of drug delivery vehicles, developed in the past decade as carriers of small molecule drugs and proteins. This review summarizes several well-described coacervate systems, including

1. Elastin-like peptides for delivery of anti-cancer therapeutics,
2. Heparin-based coacervates with synthetic polycations for controlled growth factor delivery,
3. Carboxymethyl chitosan aggregates for oral drug delivery,
4. Mussel adhesive protein and hyaluronic acid coacervates.

Coacervates present advantages in their simple assembly and easy incorporation into tissue engineering scaffolds or as adjuncts to cell therapies. They are also amenable to functionalization such as for targeting or for enhancing the bioactivity of their cargo. These new drug carriers are anticipated to have broad applications and noteworthy impact in the near future.

Keywords

chitosan; coacervate; controlled release; doxorubicin; drug delivery; elastin; growth factor; heparin; hyaluronic acid

Corresponding Author: Dr. Yadong Wang, yaw20@pitt.edu, Phone: 1-(412) 624-7196, Fax: 1-(412) 524-3699, Postal Address: 320 Benedum Hall, 3700 O'Hara St, Pittsburgh, PA 15261 USA.

Declaration of interest

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I. Introduction

A coacervate is a liquid-liquid separation held together and apart from their surrounding liquid by hydrophobic forces. Coacervates exist as spherical droplets, often on the nanometer or micron scale in diameter, resembling an emulsion except that they do not necessarily contain stabilizing molecules such as surfactants. Coacervates have been investigated since the mid-20th century mostly within the context of food science research [1]. With the advafncement in drug delivery, the use of coacervates as controlled delivery vehicles has started only recently. One particularly attractive feature of coacervates for drug delivery is their high loading capacity and the fact that they form by self-assembly in an aqueous medium [2]. Once encapsulated within the coacervate phase, labile proteins or drugs are separated and protected from the surrounding environment, thereby preserving their bioactivity. Compared to other common vehicles such as hydrogels and microparticles, coacervates form quickly and do not require organic solvents which could reduce protein drug retention and bioactivity. Finally, their size often enables coacervates to be applied via fine gauge needles, important for minimizing damage associated with injection.

Several themes have become apparent among coacervates developed for drug delivery. One major theme is the incorporation of native extracellular matrix (ECM) proteins such as heparin, elastin, hyaluronic acid, and chondroitin sulfate. These approaches strategically employ the body's own charged molecules as one or both components of the coacervate. Secondly is the slow and sustained manner of release of loaded cargos, avoiding burst release experienced with many other types of delivery systems. Thirdly, is their existence in dynamic equilibrium with their environment, highly susceptible to disaggregation in response to changes in ionic concentration or temperature of their environment. The consequences of these characteristics are thankfully very well understood and can be anticipated and even exploited to engineer coacervate drug carriers with precise design requirements.

The purpose of this review is to exhibit several coacervate systems which have been developed for the delivery of small molecule drugs and proteins. Despite their apparent similarity with coacervates, nanogels [3], charge-based polyplexes for nucleic acid delivery [4], and lipid-based emulsion delivery systems [5] are thoroughly reviewed elsewhere and will therefore not be considered here.

2. Current Coacervate Delivery Systems

2.1 Elastin-like peptides

Elastin-like peptides (ELPs) were developed in the late 20th century [6], then further explored as drug delivery vehicles more recently [7]. ELPs are recombinant proteins designed to mimic the hydrophobic regions of tropoelastin. They consist of simple pentapeptide repeats, most commonly VPGXG, where X represents any amino acid except proline, termed the "guest residue". ELPs exhibit an inverse temperature phase transition, aggregating to form a coacervate above their transition temperature (T_t) which is tunable based on the guest residue and ELP chain length [8].

Drug delivery using ELPs has been focused on targeting and abolition of solid tumors [9]. One approach is local injection of ELPs with a T_t between room and body temperature, enabling *in situ* coacervation to form drug depots which slowly release their cargo. After intratumoral injection of radionuclide-conjugated ELPs, tumor regression was observed in more than two-thirds of the mice treated and the therapeutic effects lasted for at least 2 months [10]. A second approach is systemic injection of ELPs with a T_t between body temperature and 42 °C in combination with mild hyperthermia at the target tumor site. Local heating of the tumor, achieved clinically by methods such as ultrasound, increases extravasation of the ELPs and induces *in situ* coacervation. This method resulted in two-fold higher accumulation of doxorubicin-conjugated ELPs at the tumor site compared to non-heated tumors and even greater accumulation if multiple thermal cycles were applied [11,12]. ELP coacervate has also demonstrated usefulness in tissue engineering applications as a viscous medium for delivery and differentiation of chondrocytes or progenitor cells [13,14].

2.2 Heparin:polycation coacervate

Another coacervate delivery system utilizes heparin to first bind one or more heparin-binding proteins, including numerous growth factors, cytokines, and morphogens [15]. A synthetic polycation containing arginine [16] or lysine [17] as the positive charge-bearer is then introduced to interact with the highly-sulfated heparin by polyvalent charge attraction. A complex coacervate instantly forms with droplet sizes range from 0.1–10 μm in diameter, dependent on the loaded protein and the ionic environment. The strong, specific interaction between heparin and heparin-binding proteins results in very high loading efficiency of this coacervate, often greater than 99%. The release rate is influenced by multiple factors, including the heparin-binding affinity of the protein of interest, the net charge of the coacervate, and the polycation molecular weight, charge density, and biodegradation rate [18,19].

One significant advantage of this coacervate system is owed to pre-conjugation of the growth factors to heparin which potentiates their bioactivities by mimicking the way that ECM proteoglycans present these factors to cell receptors [18,19]. In one instance, a single injection of heparin-based coacervate delivering fibroblast growth factor-2 (FGF-2) induced stable angiogenesis subcutaneously [20]; a similar benefit was observed after injection into the infarcted myocardium which resulted in improved cardiac function [21]. A second formulation for cardiac repair contained sonic hedgehog, establishing the versatility of the heparin:polycation coacervate to deliver morphogens [22]. In another study, skin wound healing was accelerated after a single application of the heparin:polycation coacervate containing heparin-binding epidermal growth factor (HB-EGF) into the wound [23]. This coacervate system also proved useful in combination with adult stem cells, slowly releasing bone morphogenetic protein-2 (BMP-2) to stimulate their osteogenic differentiation and ectopic bone formation [24]. Another advantage of this controlled release system is its simple incorporation into tissue engineering scaffolds by adsorption. This technique was recently employed to recruit progenitor cells into a porous polymer graft by slowly releasing the stem cell trafficking chemokine, stromal cell-derived factor-1α (SDF-1 α) [25].

2.3 Carboxymethyl chitosan-based coacervates

Carboxymethylation increases the water solubility of chitosan and the resulting carboxymethyl chitosan (CMC) has become a commonly studied polymer with a variety of applications, including drug delivery. The polyanionic nature of CMC enables its coacervation with positively-charged molecules via electrostatic interactions. This method was employed using unmodified chitosan as the polycation to deliver doxorubicin in an oral formulation that slowly released it within the GI tract, significantly increasing its intestinal absorption and bioavailability in the blood [26]. The group went on to encapsulate the coacervate beads within sodium alginate shells for further stabilization and protection in the low pH gastric environment [27].

2.4 Mussel adhesive proteins

Another common coacervate system exploits the unique properties of charged proteins secreted by marine organisms such as tubeworms and mussels. These polyelectrolyte proteins undergo complex coacervation to produce a robust underwater adhesive which facilitates anchoring to a surface or cementing together sand and shell fragments. Recombinant mimics of these mussel adhesive proteins (MAPs) have been characterized and evaluated for numerous applications [28]. Of particular importance for drug delivery, coacervation of recombinant hybrid MAPs with hyaluronic acid (HA) creates micron-sized droplets that are stable for at least 8 days in phosphate-buffered saline [29]. Recombinant MAPs also retain their adhesive qualities after coacervation with HA, allowing their use as a device coating or easy integration into tissue engineered scaffolds [30]. Although these systems have yet to be used in a drug delivery setting, these explorations are anticipated in the near future.

3. Expert opinion

Compared with other drug delivery vehicles including micro- and nano-particles, liposomes, hydrogels and dendrimers, coacervate is a very recent development, with just over a decade of history. Despite the short history, coacervate delivery vehicle as a class has already shown that: (1) it forms rapidly in water by self-assembly, (2) it can substantially improve the bioactivity of proteins *in vitro* and *in vivo*, (3) it readily coats a biomaterial surface either alone or combined with other molecules, (4) it can increase the efficacy of cell therapy, and (5) targeting is feasible. Self-assembly in water and affinity to proteins that significantly reduce protein denaturation and burst release make coacervate a rational choice for controlled release of proteins and peptides. Ionic coacervates can be less stable compared with conventional drug delivery vehicles in biological fluids because of their ionic nature, and this needs to be addressed for a systemic delivery route when the coacervate will be carried by blood. To this end, much can be borrowed from strategies for stabilization of liposomes. Furthermore, active targeting of a specific tissue or pathology (e.g. cancer, infarction, or inflammation) will greatly expand the utility of this new class of drug delivery vehicles. Other future growth areas are “control” over release kinetics including stimulus-responsive release and external guidance of the coacervate post-injection. In all, we anticipate that coacervate will evolve into a versatile tool for controlled drug delivery in the

near future and a likely catalyst for the “big bang” of this field will be a successful clinical translation of any coacervate vehicle.

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