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Mini Review

A Novel Nano-Structure for Central Nervous System Drug Delivery: Sustained Release of Therapeutic Agents from Core-Multi-Shell Nano-Carriers

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- Axon regeneration
- Sustained release

Abstract

The use of novel polymer nanocomposites has attracted considerable interest in the biomedical field. In this article, we review and propose an innovative nanotechnological platform for central nervous system (CNS) drug delivery, based on remotely tunable, multifunctional, and biocompatible polymeric nanostructures. Engineered magnetic nanocarriers with tailored size, volumetric transition range, and magnetic properties based on biocompatible, thermo-responsive oligo(ethylene glycol) methacrylate biopolymers can be designed for sustained and sequential release of growth factors after internalization or surface attachment to the target cells. Precise control of nano-sphere size in the range of 100-300 nm, coupled with a higher and broader volumetric transition range (32-42°C) is ideal for sustained release of growth factor to the target site. More importantly, super-paramagnetic behavior of the nanocarriers, even after polymer shell shrinkage can generate stable and easily controllable loss mechanisms inside ac magnetic field exposure.

ABBREVIATIONS

CNS: Central Nervous System; LCST: Lower Critical Solution Temperature; FDA: Food and Drug Administration; PNIPAM: Poly (N-Isopropylacrylamide); PEG: Poly-Oligo-Ethylene Glycol

INTRODUCTION

Current treatments used for central nervous system (CNS) injury include surgical, thermal, and pharmacological interventions largely targeted at decreasing neuronal loss and the inflammatory response initiated after acute injury [1-3]. However, these therapies require implementation during a limited treatment window and are not designed to encourage the axon regeneration and synaptogenesis necessary for recovery during chronic CNS injury. Although stem-cell therapy is very promising, pre-clinical studies using undifferentiated stem cells indicate that these cells do not form neurons in the CNS and may lead to the development of tumors [4-6]. In addition, potential of the traditional therapeutic approaches are limited because of difficulties in accessing the CNS due to the blood brain barrier and the presence of non-specific, off-target effects [7]. Thus, given the complexity of CNS injuries and recovery responses, it is likely that a combinatorial treatment approach targeting neuronal loss, axon regeneration, and synaptogenesis will provide a more effective treatment paradigm.

ROLE AND POTENTIALS OF NOVEL NANO-CARRIERS IN CNS INJURY TREATMENT

From the above discussion, it is evident that an innovative approach possessing novel therapeutic potential needs to be implemented in order to overcome the existing challenges to treat CNS damage related conditions. Nano-structured materials and smart surfaces that could target specific cells (e.g. corticospinal neurons) and be remotely tuned to release measured doses of therapeutic agents carry excellent treatment potential for developing novel clinical solutions [8,9], which may improve treatment efficacy, decrease therapy time, and decrease the quantities of the therapeutic agent necessary for effective treatment 10-50 fold [10]. However, several issues (e.g. biocompatibility of nanotubes or nanoparticles [11,12], external tunability of nanostructures [13,14] or difficulty in targeting the

specific cells) limit the use of conventional nanoscale systems for biomedical applications. Among various small-scale systems, nano-conjugates made from materials with magnetic properties are especially attractive possibilities for designing unique platforms [15-18] as they meet almost all criteria to achieve combinatorial therapeutics (e.g. specific targeting, triggered release, efficient clearance). Moreover, these nano-carriers can be actuated by magnetic fields, a strategy that is easy to implement remotely [19-21], providing an advantage over other types of actuation.

Improving the efficiency of remote magnetic field actuation for drug delivery could be achieved by designing core-shell magnetic nanostructures [7,22,23]. Several groups have synthesized magnetic nanoparticle systems using smart polymers like poly (N-isopropylacrylamide) (PNIPAM) [24-26], because of its perceived intelligence to external stimuli [i.e., possession of lower critical solution temperature (LCST) close to the normal physiological temperature, at around 33°C]. However, biocompatibility of the PNIPAM based systems is questionable [27]. In our laboratory, oligo(ethylene glycol)-methacrylate-based magnetic nanostructures have been designed that are thermo-responsive (LCST ~34ºC), non-toxic at relatively higher concentrations (100-250 µg/mL), and anti-immunogenic by Food and Drug Administration (FDA) standards [22]. Although these nanocarriers are promising candidates for delivering therapeutic agents, there are many limitations of the system that should be addressed before this can be used as a CNS drug delivery vehicle. Most importantly, burst release of the therapeutic agents arising from the sharp LCST behavior of the nanocomposites is a major obstacle. Thus, the designed system needs to be engineered to achieve a higher LCST with broad volumetric transition range (~30-42ºC) to allow sustained release of the growth factors, instead of the burst release that is characteristic of presently available systems after internalization or surface attachment to the target cells.

HYPOTHESIS

In this hypothesis, we would like to propose an innovative approach that exploits magnetic nanocomposites, which can be synthesized by a two-step polymerization method in order to achieve efficient drug release, targeting and accessibility to the CNS in a single platform.

Here we propose new core-multi shell magnetic nanocarriers made with poly(oligo(ethylene glycol)) (PEG) derivative thermo-responsive biopolymers. The most outer shell (i.e., the second shell) will have significantly higher volume phase transition temperature than that of the inner shell, and the shells collectively will broaden the transition temperature of the system. The polymeric shells will act as the reservoir of the drug molecules, while the magnetic core will act as nano source of heat, and thus, will initiate release of imbibed drug from the tunable excipient by causing volumetric shrinkage of the polymer network when exposed to the oscillating magnetic field. Both inner and outer shell sizes will be tuned by varying oligomers concentrations, surfactant concentrations, and by the addition of a small amount of acrylic acid (AA), especially during the formation of the outer shell.

The proposed hypothesis is innovative because, if successful, it will lead to a new class of externally tunable nano-scale systems that have the following advantages: (a) the nanospheres will possess a higher LCST with a broader transition range to allow sustained release of drugs after internalization or surface attachment to the target cells; (b) size, and magnetic properties of the nanospheres will be precisely tuned for sequential release and temperature regulation; and (3) super-paramagnetic behavior of the designed system will be preserved at the collapsed state (i.e., above LCST) for efficient heating inside an ac magnetic field. Figure (1) depicts a schematization of our hypothetical nano-tool.

DISCUSSION & CONCLUSION

Novel design techniques can be adopted to synthesize multifunctional nanocarriers with tailored size, compositions and LCST that may be suitable for various synergistic applications. Thermo-responsive behavior of the nanospheres is a result of two competitive effects: (a) the hydrogen bond between the PEG segments with water; and (b) the intra and intermolecular interactions of the hydrophobic segments. The hydrogen bonding between the PEG segments and water leads to polymer network's solubility at lower temperatures; however, at elevated temperatures, the hydrogen bonding becomes weaker while the interaction between hydrophobic segments becomes stronger, leading volumetric transition. The oligomers of PEG ethyl ether methacrylate (PEGEEMA, transition temperature ~24ºC), and PEG methyl ether methacrylate (PEGMA, transition temperature ~61ºC) will be chosen as properly mixing these two oligomers can lead to a LCST that is close to physiological temperature [28]. Three main categories of magnetic nanocarriers were synthesized in our lab to support this hypothesis [29]: (a) γ-ferric oxide (γ-Fe_2O_3) core-single shell nanospheres (type A); (b) γ-Fe_2O_3 core-double shell nanospheres (type B); and (c) magnetite (Fe_3O_4) core-double shell nanospheres (type C). For synthesis of core-single shell nanospheres (i.e., type A), highly water dispersible magnetic colloidal nanocrystal clusters in dextran (γ-Fe_2O_3 in dextran) were synthesized first that facilitate formation of the shell around them by acting as seeds during the free radical polymerization process (part 1, Figure 1a). Type B nanospheres were synthesized by first preparing the type A nanospheres and then using these as seeds for formation of the second shell (part 2, Figure 1a). Fe_3O_4 encapsulated core-double shell nanospheres (type C) cannot be prepared by this approach as the initiator used during the second shell formation completely oxidizes magnetite to γ- Fe_2O_3. To prevent this, a double shell nanosphere was synthesized first, and Fe_3O_4 nanomagnets were formed inside the outer shell (Figure 1b). Addition of a small amount of acrylic acid (AA) can increase the size of the shells significantly, which is particularly important to incorporate Fe^{3+} and Fe^{2+} ions inside the outer shell for Fe_3O_4 double shell nanospheres synthesis. Increase in shell size was observed as more AA becomes ionized to make the nanosphere swell in water [30].Polymerization was conducted at 70ºC for mainly two reasons [30]: (a) the collapsed inner shell serves as a nucleus for further polymerization of the outer shell and avoids formation of new particles; and (b) the collapsed shell hinders the outer shell polymer from interpenetrating into the inner shell area. As a result, a broader volumetric transition range was observed for multi-shell nano-carriers (Figure 2). In figure (2), the left dashed region indicates

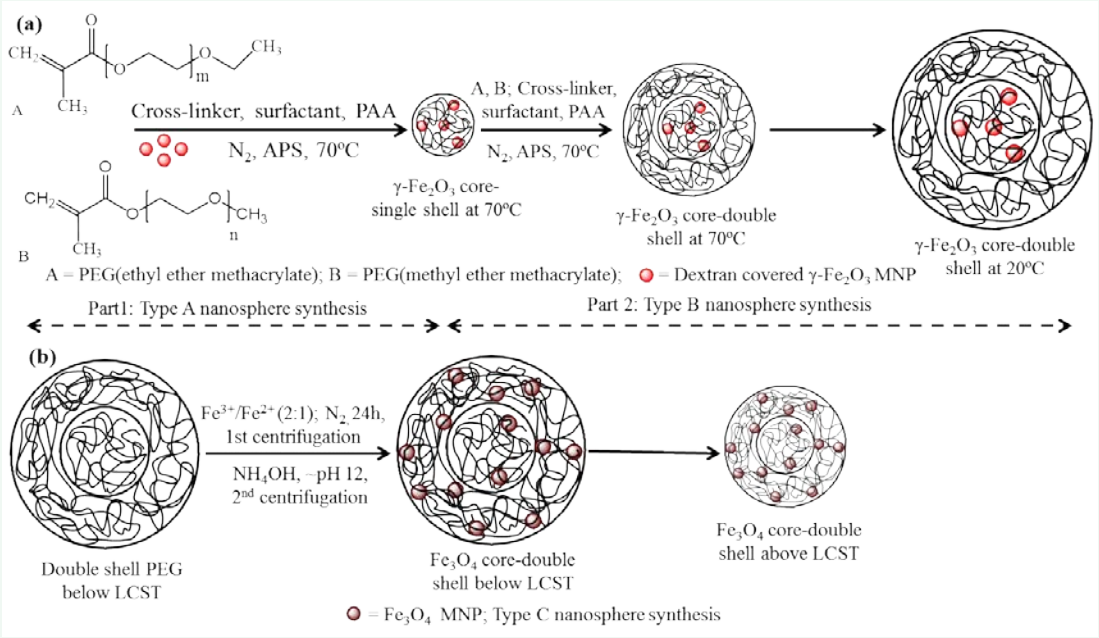

Figure 1 Schematic diagram of core-multi shell magnetic nanospheres: (a) type A (part 1) and type B (part 2) nanosphere synthesis; (b) type C nanosphere synthesis. The transition temperature of the outer shell is significantly higher than that of the inner shell to facilitate higher and broader LCST. Moreover, response in ac or dc magnetic field can be tuned by selecting nano-carriers with different magnetic nano-crystals.

the first decrease in size due to inner shell shrinkage between 28-34ºC, while the right dashed region points to the second decrease in size due to outer shell shrinkage between 34-42ºC. More importantly, super-paramagnetic behavior of the nanocarriers, even after polymer shell shrinkage indicated stable and easily controllable loss mechanisms inside ac magnetic field exposure. Thus, the nanostructures can be designed with tailored size, significantly broader LCST than the presently available systems, and with varying magnetic properties by choosing suitable magnetic nano-crystals. In these aforementioned investigations [28-30], scanning and (high resolution) transmission electron microscopy (SEM and (HR)TEM) were performed to assess the nanosphere morphology and size distribution, and to observe the encapsulated nano-magnets, respectively. Dynamic light scattering (DLS) measurements were performed to examine the volumetric transition behavior using a Malvern NanoZS system equipped with a Helium-Neon laser (632.8 nm) as the light source. Magnetic properties of the nanospheres were measured using a Lakeshore model 7300 Vibrating Sample Magnetometer (VSM) at ambient temperature and at 38ºC.

To be useful in biomedical applications, the designed nanospheres must have low toxicity and little innate bio-reactivity. We assessed the cytotoxicity of the previously mentioned multi-shell magnetic nanospheres by analyzing nuclear morphology of PC12 model neural cells and found little fragmentation and blebbing or DNA condensation in cells not exposed to magnetic nanospheres or exposed to nanospheres containing 200 μg/mL Fe_3O_4 [29], thereby indicating the nano-scale system's excellent bio-compatibility and potential to satisfy our current hypothesis. In the next phase of investigation, the effectiveness of the synthesized nano-scale system to deliver peptide trophic factors, the most potent extracellular signals needs to be evaluated. To do this, beta-nerve growth factor (β –NGF) will be loaded inside the polymer shell so that it may later be remotely released by application of an external ac magnetic field modulated heating of the medium, and thus causing the volumetric shrinkage of the thermo-sensitive polymer shells of the attached nanospheres. As amount of AA can effectively control the mesh density of the thermo-activated polymer network, increased pore sizes will be able to readily engulf the polypeptides during the drug loading process. Because NGF has been demonstrated to provide directionality to axon extension, the nanosphere surface may be further derivatized by covalently attaching a laminin binding peptide [31,32]. This strategy will allow attaching the derivatized nanospheres to an applied laminin substratum. Entrapment

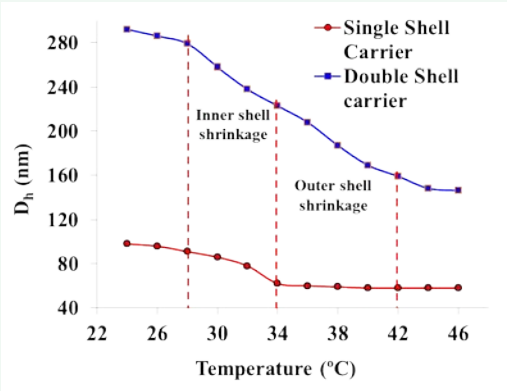

Figure 2 Proposed temperature dependence of hydrodynamic diameter (Dh) of core-multi-shell nano-carriers. Presence of the outer shell raises and broadens the LCST of the system for multi-shellnano-carriers than that of the core-single shell nanospheres.

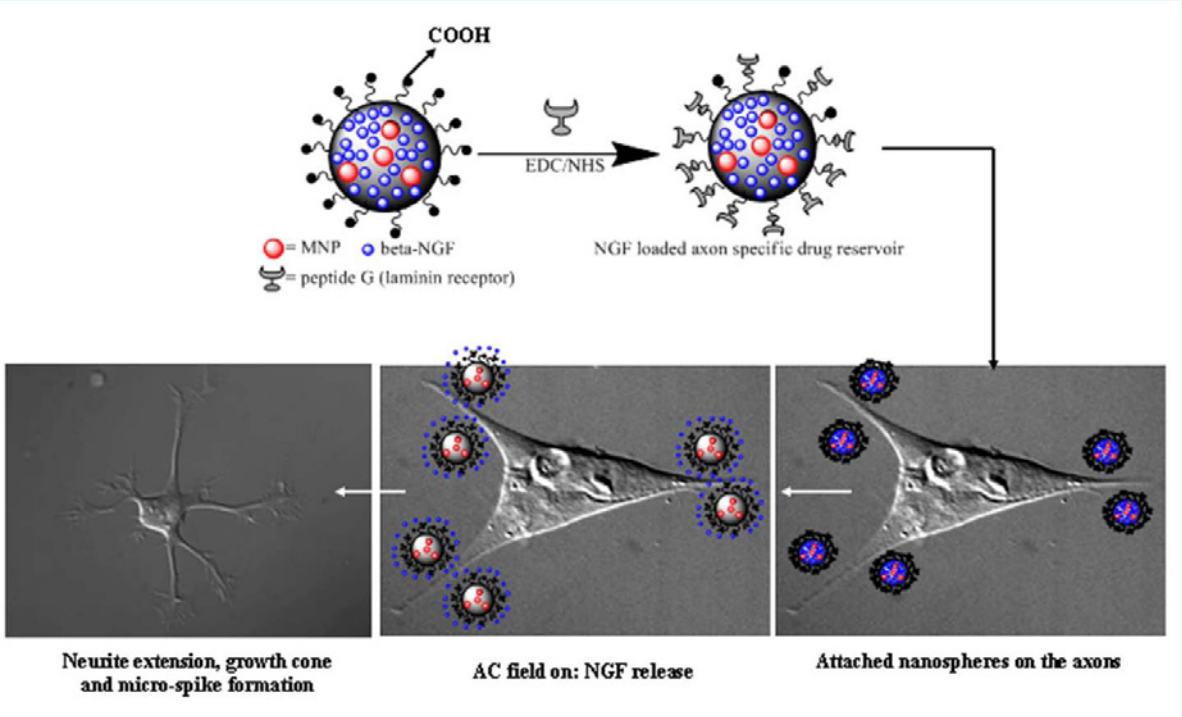

Figure 3 Proposed scheme of ac magnetic field modulated external release of β-NGF, and neurite growth.

of NGF bound nanospheres should provide for sustained TrkA activation and continuous signaling to promote neurite extension [33,34]. A schematic is demonstrated in Figure (3).

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