Title
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Journal
Journal of Drug Targeting, 23(7-8)

ISSN
1061-186X

Authors
Kazemzadeh-Narbat, Mehdi
Annabi, Nasim
Tamayol, Ali
et al.

Publication Date
2015-09-14

DOI
10.3109/1061186x.2015.1058803

Peer reviewed

Journal of Drug Targeting

ISSN: 1061-186X (Print) 1029-2330 (Online) Journal homepage: http://www.tandfonline.com/loi/idrt20

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To cite this article: Mehdi Kazemzadeh-Narbat, Nasim Annabi, Ali Tamayol, Rahmi Oklu, Amyl Ghanem & Ali Khademhosseini (2015) Adenosine-associated delivery systems, Journal of Drug Targeting, 23:7-8, 580-596, DOI: 10.3109/1061186X.2015.1058803

To link to this article: http://dx.doi.org/10.3109/1061186X.2015.1058803

Published online: 09 Oct 2015.

Journal of Drug Targeting

http://www.tandfonline.com/idrt
ISSN: 1061-186X (print), 1029-2330 (electronic)

J Drug Target, 2015; 23(7–8): 580–596
 DOI: 10.3109/1061186X.2015.1058803

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REVIEW ARTICLE

Adenosine-associated delivery systems

Mehdi Kazemzadeh-Narbat[1,2,3], Nasim Annabi[1,2,4,5], Ali Tamayol[1,2], Rahmi Oklu[6], Amyl Ghanem[3], and Ali Khademhosseini[1,2,4,7]

[1]*Division of Biomedical Engineering, Department of Medicine, Biomaterials Innovation Research Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA,* [2]*Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA,* [3]*Department of Process Engineering and Applied Science, Dalhousie University, Halifax, Canada,* [4]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA,* [5]*Department of Chemical Engineering, Northeastern University, Boston, MA, USA,* [6]*Massachusetts General Hospital, Harvard Medical School, Division of Interventional Radiology, Boston, MA, USA, and* [7]*Department of Physics, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

Adenosine is a naturally occurring purine nucleoside in every cell. Many critical treatments such as modulating irregular heartbeat (arrhythmias), regulation of central nervous system (CNS) activity and inhibiting seizural episodes can be carried out using adenosine. Despite the significant potential therapeutic impact of adenosine and its derivatives, the severe side effects caused by their systemic administration have significantly limited their clinical use. In addition, due to adenosine's extremely short half-life in human blood (<10 s), there is an unmet need for sustained delivery systems to enhance efficacy and reduce side effects. In this article, various adenosine delivery techniques, including encapsulation into biodegradable polymers, cell-based delivery, implantable biomaterials and mechanical-based delivery systems, are critically reviewed and the existing challenges are highlighted.

Keywords

Adenosine, controlled drug delivery, controlled release, drug delivery, drug targeting, nanoparticles, targeted drug delivery

History

Received 17 February 2015
Revised 31 May 2015
Accepted 2 June 2015
Published online 5 October 2015

Introduction

The adenosine molecule ($C_{10}H_{13}N_5O_4$) is a nucleoside involving a molecule of adenine bound to ribose [1,2]. It is produced by adenosine triphosphate (ATP) metabolism and also takes part in ATP synthesis in mitochondria. Adenosine is combined with phosphate to form adenosine monophosphate (AMP) and ATP [3,4]. ATP consists of three phosphate groups bound to adenosine, and is the high-energy molecule that transports chemical energy for metabolism [3,5,6]. Moreover, ATP is believed to be involved in the regulation of central nervous system [7] and cardiac function. It affects vasodilatation, muscle contraction, bone metabolism, inflammation and liver glycogen metabolism.

Adenosine 5'-diphosphate (ADP) is formed by the hydrolysis of ATP and is converted back to ATP by the glycolysis, metabolic processes oxidative phosphorylation and the tricarboxylic acid cycle. ADP involves in platelet activation process. AMP takes part in energy metabolism and nucleotide synthesis and is used as a monomer in RNA [3,8–10].

Adenosine also functions as a ubiquitous endogenous cell signaling [11] and a modulator agent. Adenosine's important role in regulating many physiologic cell-signaling pathways (particularly in the brain and heart) is well recognized [5,12,13]. Adenosine is involved in almost every aspect of cell function by activating four G-proteins or alternately P1 receptors (AR_S: A_1, A_{2A}, A_{2B} and A_3) and the eight subtypes of $P2YR_S$ receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}R_S$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}R_S$) located on the cell surface. Many studies are developing ligands for these receptors for pharmaceuticals [5,12,13]. For example, ligand of AR antagonists are capable to treat sleep apnea, cancer pain and asthma [14,15]. A_1 agonists have been shown to be effective for Glaucoma, atrial fibrillation and paroxysmal supraventricular tachycardia [12,16]. A_{2A} agonists can inhibit the release of pro-inflammatory cytokines and have anti-inflammatory and anti-ischemic effects [17]. A_{2A} agonists have been developed for the treatment of sickle cell disease, chronic and neuropathic pain, wound healing and other disorders of the central nervous system including addiction. A_{2A} antagonists have been studied for Parkinson's disease, and its PET imaging [18]. A_3 agonists may be used for the treatment of rheumatoid arthritis, psoriasis, dry eye, glaucoma, hepatocellular carcinoma and chronic hepatitis C (genotype 1). $P2Y_{12}$ agonist and $P2Y_{12}$ antagonist can treat dry eye disease, and acute coronary syndrome, respectively [19,20].

Address for correspondence: Ali Khademhosseini, 65 Landsdowne Street, Room 265, Cambridge, MA 02139, USA. Tel: +1 617 768-8395. Fax: +1 617 768-8477. E-mail: akhademhosseini@rics.bwh.harvard.edu

Amyl Ghanem, Department of Process Engineering and Applied Science, Dalhousie University, PO Box 15000, Halifax, NS, Canada B3H 4R2. Tel: +1 (902) 494-3225. Fax: +1 (902) 420-7639. E-mail: amyl.ghanem@dal.ca

Adenosine white crystalline powder is water-soluble and insoluble in alcohols, with p*K*a values of 3.5 and 12.5, which is stable in solution with pH between 6.8 and 7.4. Lowering the pH and increasing the temperature enhance the adenosine solubility [21,22].

More than 80 years ago, Drury and Szent-Györgyi conducted some of the early research on adenosine identifying the link between adenosine and cardiac function and energy metabolism [23]. It was shown that under conditions of energy deficiency, the release of adenosine results in an increase in blood flow and respiration and a reduction in cellular work [23,24]. Since then, the effect of adenosine on these functions has been elaborated considerably [25–28].

Two brands of adenosine (Adenocard® and Adenoscan®) have been approved by the US Food and Drug Administration (FDA) and are currently available on the market [29–31] Adenocard® and Adenoscan® formulations contain 3 mg adenosine and 9 mg sodium chloride per mL of water, and have been indicated for treating ''Wolff–Parkinson–White Syndrome''. They have also been approved for use during cardiac stress tests in patients who cannot exercise adequately. The algorithm for Advanced Cardiovascular Life Support (ACLS) protocol suggests administration of the adenosine through the atrioventricular (AV) node as a primary drug for restoring sinus rhythm for treating supraventricular tachycardia (SVT) and regular monomorphic wide-complex tachycardia [32].

In addition, research suggests that administration of adenosine derivative AMP by intramuscular injection may be used for treating neuropathy [33], multiple sclerosis (MS) [34], bursitis [35], pain and tendonitis [36,37], varicose veins [38,39], pruritus [40], fever blisters [41], herpes zoster [42,43], genital herpes [42,44] and poor blood circulation [45]. Oral administration of AMP can be used for herpes zoster infection [43], and treating a blood disorder called porphyria cutanea tarda [46]. ATP can be administrated intravenously to treat pulmonary hypertension [10,47], blood pressure during anesthesia and surgery [48], lung cancer [49], multiple organ failure [50], weight loss associated with cancer [51], acute kidney failure [52], cystic fibrosis [53], ischemia [54], and cardiac stress tests [4]. ATP can also be used sublingually for quick absorption to increase physical energy [55]. ATP deficiency has been reported to be one major cause of non-healing chronic diabetic wounds which is due to low energy availability created by wound hypoxia, resulting in blood and oxygen deficiency in wound cells [56]. It has also been reported that ATP level is an indication for the extent of hepatic disease; therefore, delivery of ATP to the targeted hepatopathy tissue might be used to diagnose early hepatic disease [57]. ADP has been indicated as an initial treatment for the termination of paroxysmal supraventricular tachycardia (PVST) associated with accessory bypass tracts. It has also been applied in patients who are unable to exercise adequately as an adjunct to thallous chloride TI 201 myocardial perfusion scintigraphy. ADP is one of the favorite drugs for terminating stable, narrow-complex SVT and diagnosis of undefined, stable, narrow-complex SVT by being used as an adjunct to vagal maneuvers [58].

Under conditions of cellular distress such as seizures, the adenosine levels in cells and tissue fluids rapidly rise, and this is responsible for the termination of the seizure [59,60]. Epilepsy is a chronic disorder of the brain that is characterized by spontaneous recurrent seizures and affects approximately 70 million people worldwide [61–63]. Despite the advent of new anti-epileptic drugs, about 35% patients have experienced continuous seizures with complex partial epilepsy, which is believed to be due to intolerable systemic side effects of these drugs [63,64]. Adenosine deaminase (ADA) and especially adenosine kinase (ADK), which largely exists in astrocytes in the brain catabolize the adenosine, and are responsible for maintaining the adenosine level [60]. It has been shown that adenosine has antiseizure activities in several models of epilepsy. Nevertheless, due to cardiovascular side effects, and liver toxicity of ADK disruption the systemic administration of adenosine is not practical [65–67]. Another interesting application for adenosine is antiplatelet therapy, which has not yet been clinically used due to adenosine short half-life [68].

Despite the abundance of evidence suggesting the significant therapeutic impact of adenosine/adenosine derivatives, systemic administration of adenosine may induce severe side effects, such as suppression of cardiac function, decreased blood pressure and body temperature, and sedative side effects [26–28]. In a clinical trial reported by the FDA, among 1421 patients who received intravenous adenosine injection, 44, 40, 28, 18 and 15% experienced flushing, chest discomfort, dyspnea (urge to breath deeply), headache and throat/neck or jaw discomfort, respectively. Adenosine has low blood–brain barrier permeability and an extremely short half-life (<10 s). Adenosine is cleared rapidly from the circulation via cellular uptake primarily by erythrocytes and vascular endothelial cells. In this process, adenosine is quickly deaminated by adenosine deaminase to inosine, and subsequently is broken down to uric acid, xanthine and hypoxanthine, which eventually is excreted by the kidneys [69,70]. Therefore, sustained adenosine delivery systems are required to enhance the efficacy and consequently reduce the side effects by slow release at a concentration within the therapeutic window. Normal cells produce about 300 nM extracellular adenosine concentrations; however, in some cases such as inflammation, the concentrations may reach to 600–1200 nM. The adenosine concentration needed to activate A_1 (0.3–3 nM), A_{2A} (1–20 nM) and A_3 (about 1 μM) receptors range between 0.01 and 1 μM, and physiological adenosine concentrations are usually lower than 1 μM [71]. The adenosine level to activate A_{2B} receptor generally exceeds 10 μM in response to metabolic stress in pathophysiological conditions [72,73].

Drug delivery systems (DDSs) improve the pharmacological and therapeutic properties of drug products. These systems can maintain a steady release of drug level in a therapeutic range and reduce undesirable side effects. They also decrease the amount of drug and number of dosages needed, and provide an efficient administration of pharmaceuticals with short *in vivo* half-lives [74–77]. An ideal DDS is expressed as a system that can pass physiological barriers, shield the drug from the attacks by the immune system and selectively deliver the drug to the targeted tissue [74,78–86]. Such system can adjust both quantity and duration of drug presence in whole body or a specific tissue.

Currently, no approved system exists for controllable, sustained and long-term adenosine-associated delivery. This article aims to highlight DDSs that have been proposed for adenosine-associated delivery. This review provides an overview of various biomaterials used for adenosine-associated delivery including polymer/lipid-based and ceramic-based delivery. It also explores different techniques such as layer-by-layer assembly, pump-based and cell-based approaches with their practical application for tunable adenosine-associated delivery. Finally, the review outlines future opportunities and directions that the field may expand.

Particle-based adenosine carriers

Drug carriers generally deliver the drugs through various mechanisms including diffusion from or through the biomaterials, chemical or enzymatic degradation, solvent activation due to osmosis or swelling or a combination of these mechanisms [76]. Several attempts have been made to deliver adenosine using polymer/lipid materials. These drug carriers can be made from lipids (i.e. liposomes), polymers [i.e. poly(lactic acid), ethylene vinyl acetate and silk] and hydrogels (i.e. chitosan and polyethylene glycol). In this section, we will critically review the pertinent literature.

Liposomally entrapped adenosine

Liposomes (self-assembled enclosed lipid bilayers) are usually biodegradable, non-toxic vehicles that have been employed for encapsulating both hydrophilic and hydrophobic therapeutics in drug delivery systems [87]. Guo-Xing et al. [88] evaluated four methods for encapsulating ATP in liposomes generated from egg lecithin/cholesterol/stearylamine, including thin film-formed vesicles, reverse-phase evaporation vesicles, double emulsification vesicles and improved emulsification vesicles. The highest ATP entrapment of 38.9% was observed for positively charged liposomes made by the ''improved emulsification vesicles'' method. They reported that, after 54 days of storage at room temperature, about 11% of ATP was released from liposomes. The positively charged ATP liposomes were then injected intravenously in dogs with experimentally induced myocardial infarction. The results showed the accumulation of ATP in myocardial infarct tissue [88].

Based on liposomal entrapment techniques, Gomes et al. [89] reported the encapsulation of adenosine-3′-5′-cyclic monophosphate (cAMP) into vesicles (35–55 nm in diameter) composed of egg-phosphatidylcholine (PC), cholesterol and sulfatides. They showed that the presence of the protein kinase A in the liposome formulation not only increased the entrapment efficiency of cAMP by 5-fold, but also reduced its leakage by more than 60% in the mouse brain [89].

ATP could be used to aid in treating chronic diabetic wounds if a solution for its short half-life can be devised. It has been shown that the clearance of ATP can be prolonged by encapsulation of magnesium-ATP (Mg-ATP) into small unilamellar lipid vesicles [56,90,91]. Chiang et al. [90] developed a technique to create Mg-ATP vesicles by encapsulating Mg-ATP with highly fusogenic lipid [1,2-dioleoyl-sn-glycero-3-phosphocholine, (DOPC)] vesicles. They observed that vascular endothelial growth factor (VEGF) expressions in the wounds of mice treated with Mg-ATP-vesicles (100–200 nm) were significantly higher. This system could accelerate healing by supplying enough blood to deliver oxygen, nutrients, minerals, enzymes and by circulating hormones into the wound cells [90].

Similarly, Wang et al. [56] used Mg-ATP-vesicles (with diameters of 120–160 nm) in ischemic and non-ischemic wounds in diabetic rabbits. They concluded that intracellular ATP delivery enhances the healing process of diabetic skin wounds on ischemic (mean closure time about 15.3 days) and non-ischemic (mean closure time about 13.7 days) rabbit ears [56]. Similarly, Chien [91] investigated the intracellular delivery of fusogenic, unilamellar lipid vesicles containing Mg-ATP (with diameters of 100–200 nm) into normal or ischemic cells. The *in vivo* study on the rabbit ischemic ear wound model indicated a significant reduction in healing times in the wounds treated with ATP-vesicles (mean 18.0 ± 1.9 days) in comparison to the controls (mean 22.8 ± 4.1 days). Moreover, enhanced re-epithelialization was observed for the wounds treated with ATP-vesicles. According to their *in vitro* study, when ATP-vesicles were in contact with the endothelial cell membrane, they fused together and delivered their contents into the cytosol [91].

ADP is a platelet agonist that plays a role in stabilizing the platelet aggregation after their activation [92]. Okamura et al. [93] developed a platelet substitute with hemostatic activity by conjugating the phospholipid vesicles (PC) with a dodecapeptide (HHLGGAKQAGDV, H12) to encapsulate ADP. They prepared H12-(ADP)-vesicles with various membrane flexibilities by freeze-drying, hydration with ADP and then extrusion using membrane filters with different pore sizes. Lamellarities of vesicles were controlled by gel-to-liquid crystalline phase transition temperature of the lipid during extrusion. They showed that, by controlling vesicle membrane deformability (membrane flexibility and lamellarity), the ATP release and subsequently the hemostatic property of H12-(ADP)-vesicles can be tuned (Figure 1) [93].

Adenosine delivery using PEG

Adenosine deaminase (ADA) deficiency is a metabolic disorder that causes severe combined immunodeficiency (SCID). Bone marrow transplantation and intramuscular polyethylene glycol-modified adenosine deaminase (PEG-ADA) are the only available therapies for ADA deficiency [95]. PEG is non-toxic, non-immunogenic, non-antigenic and can be eliminated by urination. These characteristics along with low level of protein/cellular absorption have made PEG one of the most common synthetic material in polymer-based DDS. PEG has been approved by the FDA for intravenous (IV), oral and dermal applications [96,97]. In addition to utilization of PEG-based drug carriers, adenosine can be directly grafted to ADA to improve its half-life *in vivo* [95]. In 1981, Davis et al. developed a covalent coupling between PEG and ADA using cyanuric chloride agent. The circulating half-life of the modified ADA increased to 28 h [98]. In PEGylation procedure (first introduced in 1970), numerous strands of PEG are attached covalently to another molecule, such as a drug or therapeutic protein [96,97,99]. PEGylated bovine ADA (Adagen®, trade name for PEG-ADA or

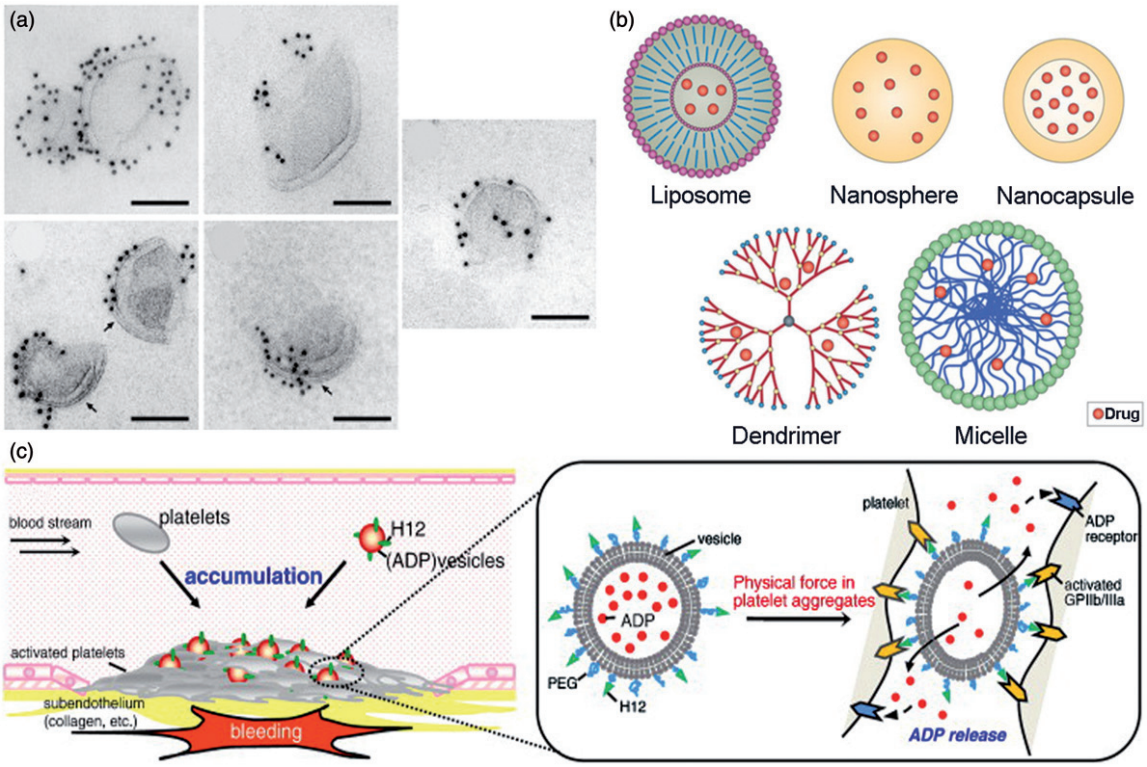


Figure 1. Schematic of ADP release from H12-(ADP)-vesicles with distinctive membrane characteristics designed by Okamura et al. (a) Electron microscopic images of the H12-(ADP)-vesicles with different membrane flexibilities showing that the H12 molecules were bound to the vesicles, scale bars show 100 nm [93]. (b) Schematic structure of nanocarriers used for drug delivery [94], (c) ADP encapsulated vesicles [H12-(ADP)-vesicles] controlled their hemostatic abilities by tuning ADP release dependent on membrane properties. It was reported that ADP release increased when either membrane flexibility increased or lamellarity decreased [93].

pegademase bovine) was approved by the FDA to enter the market in 1990 for intramuscular injection (Figure 2) [101–104]. Adagen® has been used for the treatment of SCID in patients for whom bone marrow transplantation is not possible [100]. PEG-ADA optimizes the therapeutic effect of ADA molecules by slowing its clearance and maintaining a high level of ADA activity by increasing its circulation half-life in plasma. PEG-ADA also reduces immunogenicity, decreases the enzymatic degradation of ADA molecules by protease, and limits its binding with host antibodies [100]. Long-term improvement of the immune status has been reported with Adagen® treatment [102]. Moreover, the side effects from ADA administration such as sensitization to erythrocyte antigens and virus transmission were not observed using PEG-ADA therapy in the patients with ADA deficiency [103].

So far, PEG-ADA has been successfully used in a few hundred patients worldwide and a couple of clinical studies have been reported on PEG-ADA treatment [101]. Hershfield et al. [105] could significantly improve the cellular immune function, and increase circulating T lymphocyte count by injecting PEG-ADA in two children with adenosine deaminase deficiency and SCID [105]. A clinical study on an ADA-deficit child performed by Bory et al. also showed that PEG-ADA therapy could increase the total number of lymphocytes and their response to non-specific mitogens significantly [106]. Bax et al. [107] also investigated the entrapment of native ADA and PEG-ADA within human erythrocytes via hypo-osmotic dialysis procedure to improve the *in vivo* half-life of the enzyme. It was discovered that the entrapment efficiency for PEG-ADA was only 9% due to PEG side chains, while the entrapment efficiency for unmodified ADA was as high as 50%. However, the half-life of erythrocyte-entrapped PEG-ADA (20 days) was higher than those with native ADA (12.5 days). These values were significantly more prolonged than *in vivo* plasma PEG-ADA circulating half-life (3–6 days) [107]. A similar clinical study performed by Lainka et al. [95] resulted in an impressive immune reconstitution on a 14-month-old girl with ADA deficiency [95].

Adenosine is currently used for the diagnosis of ischemic heart disease (adenosine echocardiography [108]) and a high dose is required to exert cardio-protective effects. In clinical trials, however, adenosine causes hypotension and bradycardia [109]. To reduce this associated hemodynamic consequences while administrating a high dose of adenosine, Takahama et al. [28] encapsulated adenosine in PEGylated liposomes for the delivery of adenosine to ischemic hearts. The PEGylated liposomal adenosine was prepared by the hydration method with a mean diameter of 134 ± 21 nm. To study whether liposomal adenosine has stronger cardio-protective effects compared to the conventional method, the PEGylated liposomal adenosine were evaluated for myocardial accumulation and myocardial infarct during 3 h after 30 min of ischemia followed by reperfusion in rats. The results showed that the liposomes were extensively taken up by the ischemic myocardium, but not by non-ischemic myocardium. PEG coating on liposomes could prolong their residence time in the blood [110]. No significant effects on either mean blood pressure or heart rate even at high dose of 450 μg/kg/min were observed, indicating that intravenously

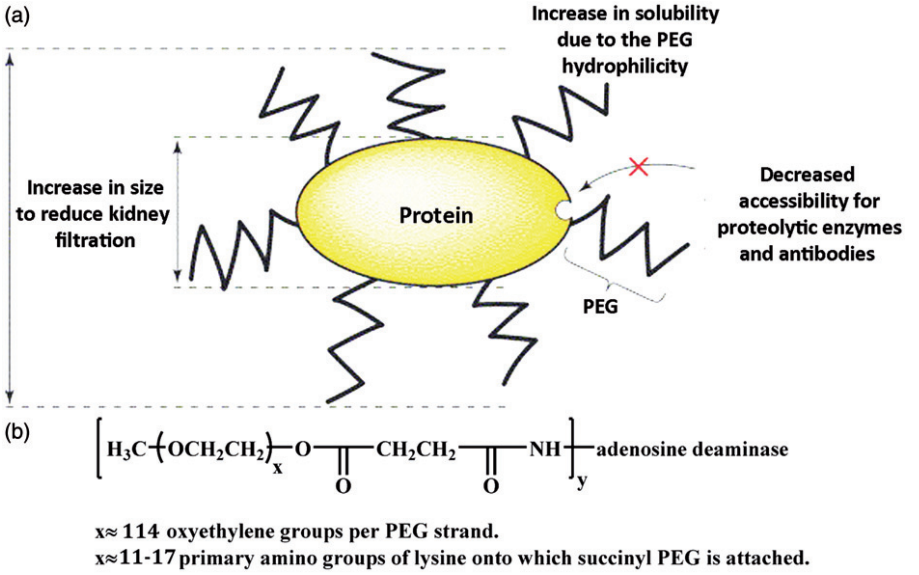


Figure 2. (a) PEGylation shields the protein surface from degrading agents by steric hindrance, and increases the size of the conjugate to decrease kidney clearance [99]. (b) In the structural formula of ADAGEN® bovine ADA covalently attached to numerous strands of monomethoxy PEG with molecular weight 5000 [100].

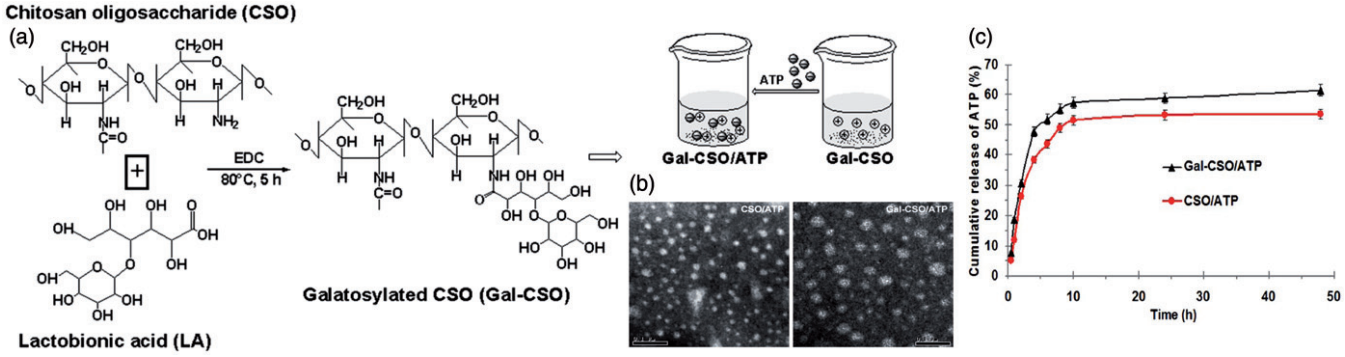


Figure 3. (a) Schematic of Gal-CSO/adenosine triphosphate (ATP) formation. (b) TEM images of nanoparticles (left) CSO/ATP and (right) Gal-CSO/ATP showing the size range of 51.03 ± 3.26 nm (the bar is 0.1 μm). (c) *In vitro* cumulative release rate of ATP from nanoparticles in PBS exhibited the initial burst release attributed to the drug adsorbed on the surface of the nanoparticles [114].

administration of high doses of liposomal adenosine to the ischemic heart was safe [28,111].

Although PEG can protect the drugs from immune cells and has been extensively used in drug delivery, its inert characteristics result in low cellular uptake. In addition, PEG-based carriers should be decorated with other molecules to pass physiological barriers such as blood–brain barrier, which add to the complexity of fabricating targeted drug carriers. In addition, PEG is also non-biodegradable and if the carrier size is big (>10 μm), its clearance from the blood might be challenging.

Chitosan

Chitosan-based nanocarriers are promising materials for drug encapsulation due to their high biocompatibility and biodegradability [112], high mucoadhesiveness [57] and immunostimulating properties [113]. As chitosan is only soluble in acidic solutions (pH < 6.5), low molecular water soluble chitosan oligosaccharide (CSO) was used by Zhu et al. [114] for ATP delivery. In their technique, lactobionic acid with a galactose group was conjugated to CSO using the freeze-drying technique [115]. Galactosylated chitosan oligosaccharide (Gal-CSO) was then loaded with ATP in a drop-wise manner as the specific adhesive ligand to the asialoglycoprotein receptor of hepatocellular carcinoma cells [116]. The ATP-loaded nanoparticles exhibited low cytotoxicity when cultured with human hepatocellular carcinoma cells. ATP loaded nanoparticles had mean diameter of 51.0 ± 3.3 nm with drug loading and encapsulation efficiency of about 26 and 89%, respectively. An initial burst release (~30%) of ATP occurred within the first 2 h in PBS while ~60% of ATP released after 48 h from Gal-CSO/ATP (Figure 3). This method has been suggested as an intracellular drug delivery system for targeting carcinoma cell in hepatopathy, however additional *in vivo* study is required to confirm the efficacy [114].

Kazemzadeh-Narbat et al. [117] encapsualted adenosine into the chitosan spherical nanoparticles (average size of 260.6 ± 20.1 nm, with zeta potential value of +29.2 ± 0.5 mV) using ionotropic gelation (5:1 chitosan:sodium tripolyphosphate mass ratio) for IV delivery. The nanoparticles had low encapsulation efficiency (3%), and loading capacity (20%) with almost 350% swelling in 6 h. The release mechanism showed a burst release, a plateau phase, followed by a steady release up to 7 days with an excellent physical stability at room temperature and at 4 °C (Figure 4) [117].

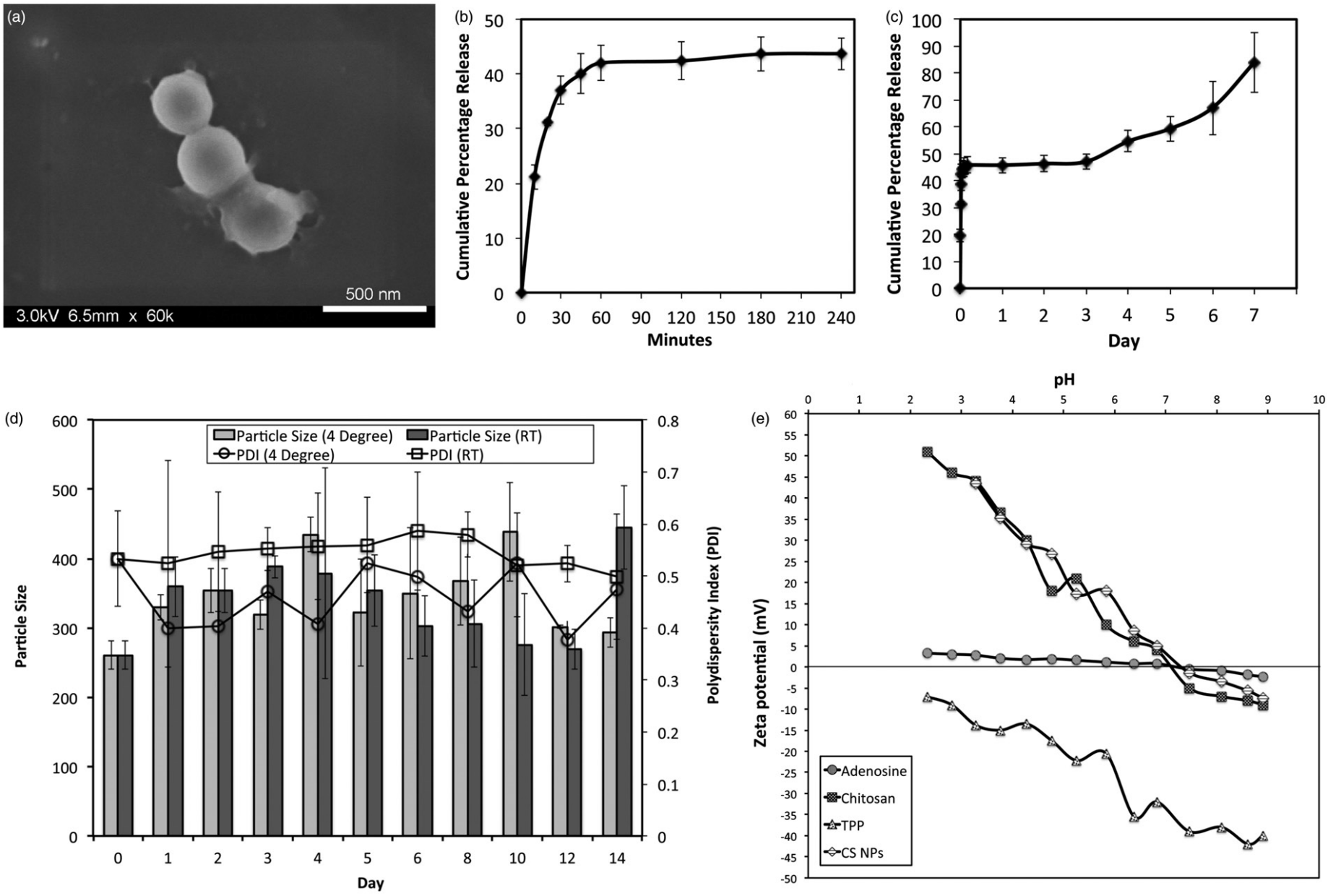


Figure 4. (a) The SEM micrograph of chitosan nanoparticles. (b) Short-term adenosine release in PBS (pH 7.4), (c) long term. (d) Particle size and polydispersity index (PDI) of adenosine loaded chitosan nanoparticles. (e) Isoelectric point and zeta potential changes with pH for the adenosine, chitosan, sodium tripolyphosphate and nanoparticles [117].

In spite of all interesting characteristics of chitosan, it can trigger inflammatory response. In addition, small size carriers might be absorbed through phagocytosis, which can result in uncontrolled release profile.

Poly(lactic acid) and poly(lactic-co-glycolic acid)

Poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymer poly(lactic-*co*-glycolic acid) (PLGA) have been extensively studied as micro- and nano-carriers for several classes of drugs due to their high biocompatibility and tunable biodegradability [118,119]. These polymers can also protect unstable compounds for a longer period of time. Similarly, these carriers have been also used for adenosine delivery.

The N^6-cyclopentyladenosine (CPA) is known as a drug with anti-ischemic properties for CNS, however due to its low stability in body CPA is unable to reach the brain [120,121]. Dalpiaz et al. [122] investigated the sustained delivery of CPA by encapsulating it into biodegradable PLA microspheres. The encapsulation method was based on emulsion–solvent evaporation. They reported the encapsulation efficiency of 1.1% for CPA encapsulated microspheres (0.11 mg/100 mg). The microspheres (21 ± 9 μm) released almost 92% of the encapsulated CPA using a column-type apparatus in 72 h in PBS (pH 7.4). This approach will maintain therapeutic doses of the CPA in the blood stream after intravenous administration [115].

In a follow-up study, Dalpiaz et al. [123] reported biodegradable PLA nanospheres as delivery systems for CPA and its pro-drug 5′-octanoyl-CPA (Oct-CPA). The PLA nanospheres were fabricated by double emulsion solvent evaporation method or nanoprecipitation, and the nanoparticles were collected by ultracentrifugagtion, gel filtration or through dialysis. The CPA loaded spherical nanoparticles prepared using the nanoprecipitation technique had an average diameter of 210 ± 50 nm and the nanoparticles prepared using double emulsion solvent evaporation method had an average diameter of 310 ± 95 nm. Although no encapsulation of CPA was obtained in either technique, Oct-CPA content in nanospheres were 0.1–1.1% w/w, with the encapsulation efficiency of 6–56%. All the Oct-CPA nanospheres had mean diameter of 220–270 nm except the nanoparticles recovered by ultracentrifugation, which exhibited mean diameter of 390 ± 90 nm. Almost 90% of the Oct-CPA was released within 4 h (Figure 5) [123].

Poly(lactic-*co*-glycolic acid) (PLGA) has also been investigated for encapsulating adenosine derivatives. As an example, a PEG scaffold loaded with PLGA has been used to encapsulate adenosine derivative for the treatment of spinal cord damage [124]. The scaffold could support and guide nerve fibers, while local delivery of therapeutic accelerates the regeneration process [124]. Rooney et al. [125] also used PLGA microsphere-loaded oligo (OPF) hydrogel disc scaffolds to deliver dibutyryl cyclic adenosine monophosphate (dbcAMP) to a transected spinal cord. DbcAMP was encapsulated into PLGA microspheres using water-in-oil-in-water (W/O/W) double emulsion solvent evaporation [126]. The mixture of microspheres and OPF solution were then poured into glass molds and cross-linked by UV light into disc shape scaffolds. It was observed that by prolonged dbcAMP release over 42-day, axonal regeneration was inhibited and rescued in the presence of Schwann cells and MSCs respectively [125].

Synthetic PLA- and PLGA-based nanocarriers are promising carriers for adenosine delivery; however, they are susceptible to phagocytosis, which can reduce their half-life. These nanocarriers can be functionalized with molecules that can make them specific to a target tissue. The release profile can also be tuned by size modification. One challenge associated with these carriers is their burst release that can prevent from maintaining a sustainable drug level over a long period of time.

Ethylene vinyl acetate

Boison et al. used a synthetic ethyl vinyl acetate (EVA) copolymer to deliver adenosine to the brain of kindled rats as a representation of human temporal lobe [127,128]. For this purpose, into the lateral brain ventricles of previously kindled rats were implanted by 0.4 mm diameter polymer rods with height of 1 mm. It was demonstrated that EVA copolymer loaded with 20 % adenosine (w/w) could release 20–50 ng/day of adenosine inducing a sustained reduction of epileptic seizures for up to 2 weeks compared with control implants. The results indicated that focal delivery of adenosine might suppress epileptic seizures without causing cardiovascular or

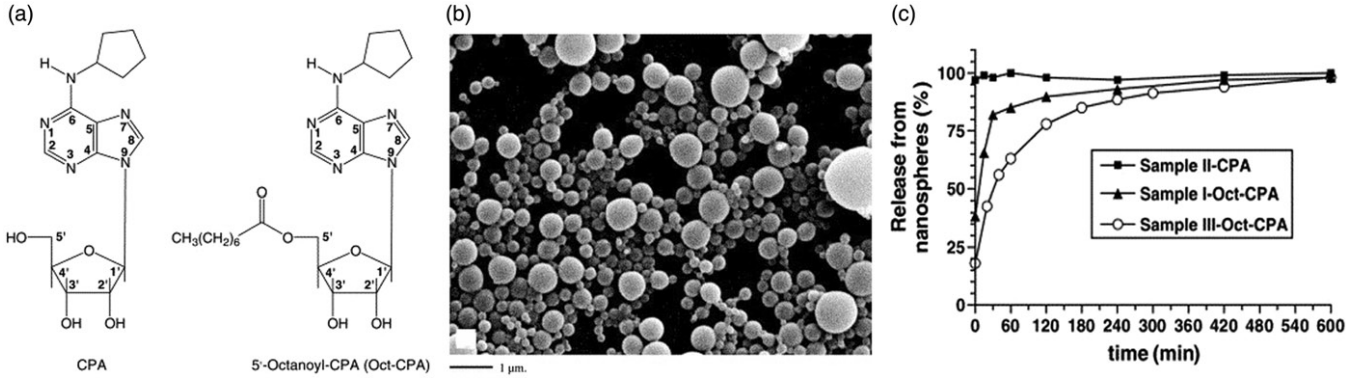


Figure 5. (a) Illustration of CPA and Oct-CPA chemical formulas, (b) SEM of Oct-CPA loaded nanospheres prepared by double emulsion solvent evaporation method, and (c) *in vitro* release of CPA and Oct-CPA from PLA nanospheres in phosphate buffer at 37 °C developed by Dalpiaz et al. [123].

sedative side effects. The implants, however, were not degradable and therefore had to be removed after experiments [127].

Silk

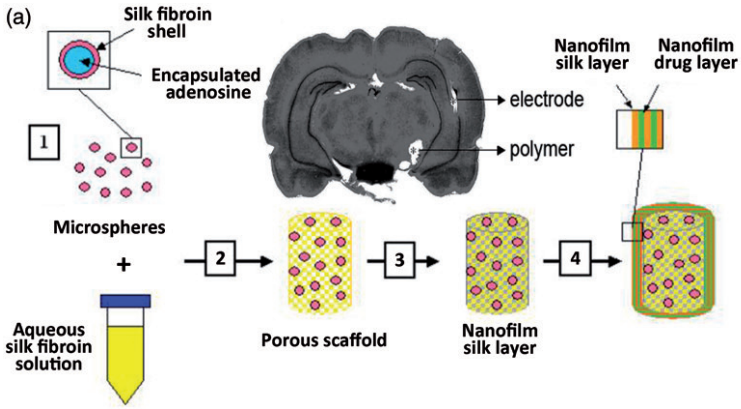
Purified silk fibroin protein is an interesting candidate for adenosine delivery as silk is a FDA-approved, biocompatible and mechanically strong polymer that degrades to non-toxic products. Furthermore, degradation of silk is tunable from weeks to years [129,130]. Although the focal adenosine delivery from silk implants precludes long-term clinical applications, it might be an alternative for short-term clinical trials prior to surgical resection. A silk-based adenosine delivery system can also be applied for preventative use in patients prone to developing epilepsy, such as those with traumatic brain injury [131,132].

Wilz et al. [132] developed a hierarchically structured controllable silk-based delivery system for adenosine. They designed brain implants with adenosine delivery capability consisting of three systems, microspheres, macroscale films and nanofilms with the nominal delivery rates of 0, 40, 200 and 1000 ng/day (Figure 6). The rate and dose of adenosine release of their implant were tuned by changing the microspheres concentration, the concentration of adenosine in the macroscale films and the quantity of nanofilm layers coating the porous scaffold system. Microspheres loaded adenosines were created in accordance with the methanol-based lipid template protocol [133]. To shape the final porous scaffold, the combination of silk solution and microspheres were embedded in a plastic container and incubated for 24 h at room temperature [134]. Macroscale and nanofilm adenosine-loaded silk coatings were then applied on the scaffolds, alternatively [135,136]. These three systems were integrated into a single implantable silk rod (0.6–0.7 mm in diameter and 3–4 mm in height; Figure 6). The *in vitro* release experiments showed that all implants released adenosine with an initial burst followed by prolonged release close to the target rates for at least 14 days. The rate of adenosine release was defined by the numbers of capping layers (implant dip into silk solution), the thickness of nanofilm, and the silk crystallinity. The *in vivo* study was conducted on a rat kindling model in which seizures were provoked by repetitive short electrical stimulation of the hippocampus. It was observed that, silk-based polymer implants could effectively retard the epileptogenesis corresponding to the released dose of adenosine. Thus, in comparison to the control, the implant with 1000 ng/day adenosine release did not exhibit any seizures during the experiment time. However, seizures gradually resumed with progressive intensity as soon as drug release from the implants began to diminish [132].

Szybala et al. [131] combined this silk-based 3D porous scaffold with engineered human mesenchymal stem cells (hMSCs) to release adenosine [137]. Polymeric scaffolds designed to release 1000 ng/day adenosine were then implanted into the rat brains. It was observed that rats were fully protected from seizures up to 10 days with sustained adenosine release. Silk-based adenosine implants exert potent anti-ictogenesis, with at least partial anti-epileptogenic effects [131].

Pritchard et al. [138] coated the solid press-fit adenosine powder reservoirs (70 ± 5 mg) with silk fibroin by dipping technique to achieve sustained, zero-order release. By controlling the silk coating, they could achieve a linear, sustained adenosine release for up to 17 days from encapsulated coated reservoirs with eight layers of silk (8% w/v) [139]. A release study on dissolvable silk films loaded with 0.5, 0.25 or 0.125 mg of adenosine per 0.2 mm^2 film has shown that almost 80% of the adenosine load was released within 15 min in PBS at 37 °C [140].

In another study, silk microspheres loaded with adenosine were fabricated in accordance with the MeOH-based lipid [1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)] protocol [136]. The resulting cross-linked microspheres were less than 2 µm in diameter, and could be loaded with 85 mg of adenosine per mg of silk. Starting with a burst release, about 75% of the total loaded adenosine released within the first 24 h, with no significant release after 3 days [136]. They showed also that creating silk hydrogel [1% (w/v) or 3% (w/v)] by suspending microspheres in a sonication did not improve release profile in PBS [140,141]. However, by suspending microspheres in aqueous-derived porous silk sponges, the release profile was improved from 3 to 7 days



(b)

Release rates of adenosine from implants (nanograms per day)

Time (in days)	Target: 40 ng	Target: 200 ng	Target: 1,000 ng
1	134.4	679	3137.0
2	89.0	513.1	2865.2
3	33.9	202.3	984.6
4	66.3	330.9	2042.7
6	21.4	104.2	426.9
10	15.6	70.3	228.7
14	9.7	43.1	166.8
Average	33.4	170.5	819.0

Figure 6. (a) Schematic of four-step fabrication of silk-based adenosine releasing implants: (1) Microsphere loaded with adenosine. (2) Mixture of microspheres with silk solution, and embedding them in the form of porous scaffolds. (3) Scaffolds are soaked in the solution of silk and adenosine, and coated with macroscale drug-loaded film coating. (4) Alternating nanofilm deposition loaded with adenosine is coated. The polymer site at infra-hippocampal and the implantation channel of the electrodes are visible in the Nissl stained coronal brain section, 20 days after transplantation. (b) The release profile of adenosine from implants, no seizure was observed at 1000 ng/day adenosine release during the experiment time [132].

with a constant, zero-order delivery for the first 3 days [140]. Also, the addition of silk coatings delayed the release of adenosine-loaded silk microspheres [136,140]. Adenosine-releasing silk microspheres can be applied by minimally invasive injection.

There is a growing interest towards the use of silk in biomedical applications. However, the challenges toward its use in drug delivery include potential inflammation and the burst release of the drug, which prevent the accurate estimation of the drug level.

Layer-by-layer assembly

Layer-by layer (LbL) assembly technique is a novel approach to fabricate multilayered coatings using sequential electrostatic adsorption of polyelectrolytes [142]. Using LbL, an ultrathin PMMA/polysaccharides nanofilm was developed by Riva et al. [143] to encapsulate adenosine deaminase inhibitor. Inhibition of adenosine deaminase results in elevation of local adenosine concentration, thereby decreasing the inflammatory effect [144]. To fabricate the polymeric nanofilm, chitosan/sodium alginate (Chi-Alg) was deposited alternatively using spin-assisted LbL method onto a PMMA structural layer. The multilayer nanofilm was subsequently loaded with an adenosine deaminase inhibitor and its fluorescent dansyl derivate by a casting deposition technique (Figure 7). In aqueous medium the nanofilm released 91.6% of drug within 6 h through diffusion, degradation, and drug–polymer liberating, following the Korsmayer−Peppas model in which PMMA acts as a release barrier. This ultrathin platform had a very low surface roughness, with thickness of about 200 nm. Combination of anti-inflammatory activity, high biocompatibility, and adhesion of nanofilm to wet tissue/mucosal surface make this a promising nanopatch for the treatment of diseases involves chronic inflammation [143].

Silica nanosphere-based adenosine delivery

The mesoporous silica nanospheres (MSN) have well-defined surface properties with very large surface area, and stable mesoporous structures that allow tuning pore sizes and volumes. These attractive features are ideal for encapsulation of therapeutic molecules [145,146]. The encapsulation of drug molecules in polymer-based delivery systems is based on adsorption or entrapment. However, MSNs are capable of encapsulating the drugs inside the porous framework by covalently capping the mesopores with caps which are chemically removable, such as size-defined cadmium sulfide (CdS) nanocrystals, or via a disulfide linkage of poly(amidoamine) dendrimers (PAMAM) to physically seal the drugs from leaching out [147,148]. Lai et al. [148] reported a mesoporous silica-based delivery system that would control the release by using trigger agents (various disulfide bond-reducing agents) as ‘‘uncapping triggers’’, such as mercaptoethanol (ME) and dithiothreitol (DTT). MSNs with mean pore diameter of 2.3 nm and average particle size of 200 nm were employed as reservoirs to soak up ATP solutions. To cap the openings of the mesopores, amidation reaction was applied using CdS [149], and the release rate was regulated by the concentration of the trigger agents (DTT or ME).

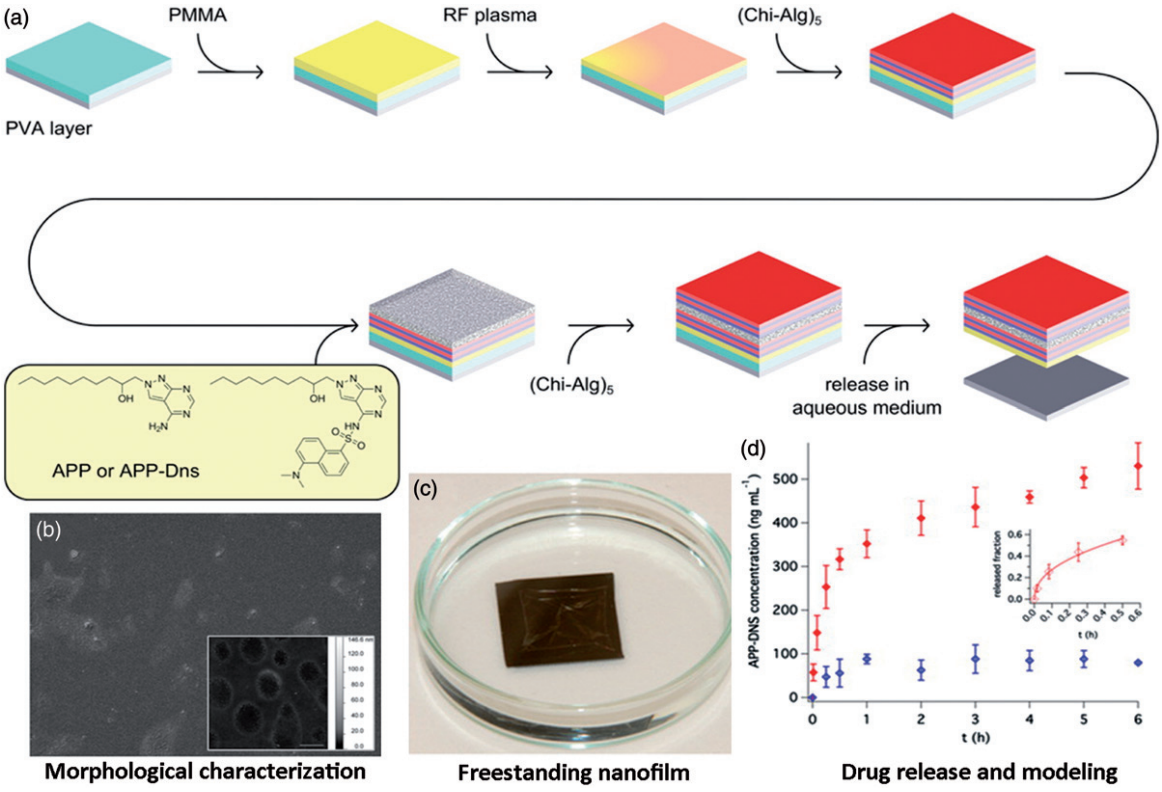


Figure 7. (a) Fabrication procedure of a free-standing layer-by-layer polymeric nanofilms (thickness < 200 nm) made of PMMA (as a barrier) and a polysaccharides assembly incorporated with an adenosine deaminase inhibitor. A thin film of PMMA first was treated with plasma then chitosan and sodium alginate was deposited on the film using spin-assisted LbL assembly. (b,c) The PMMA/LbL nanofilms characterization indicates low surface roughness, which were between 1 and 2 nm for drug loaded nanofilms and less than 1 nm for blank nanofilm. (d) The release was based on diffusion similar to the Korsmayer–Peppas model [143].

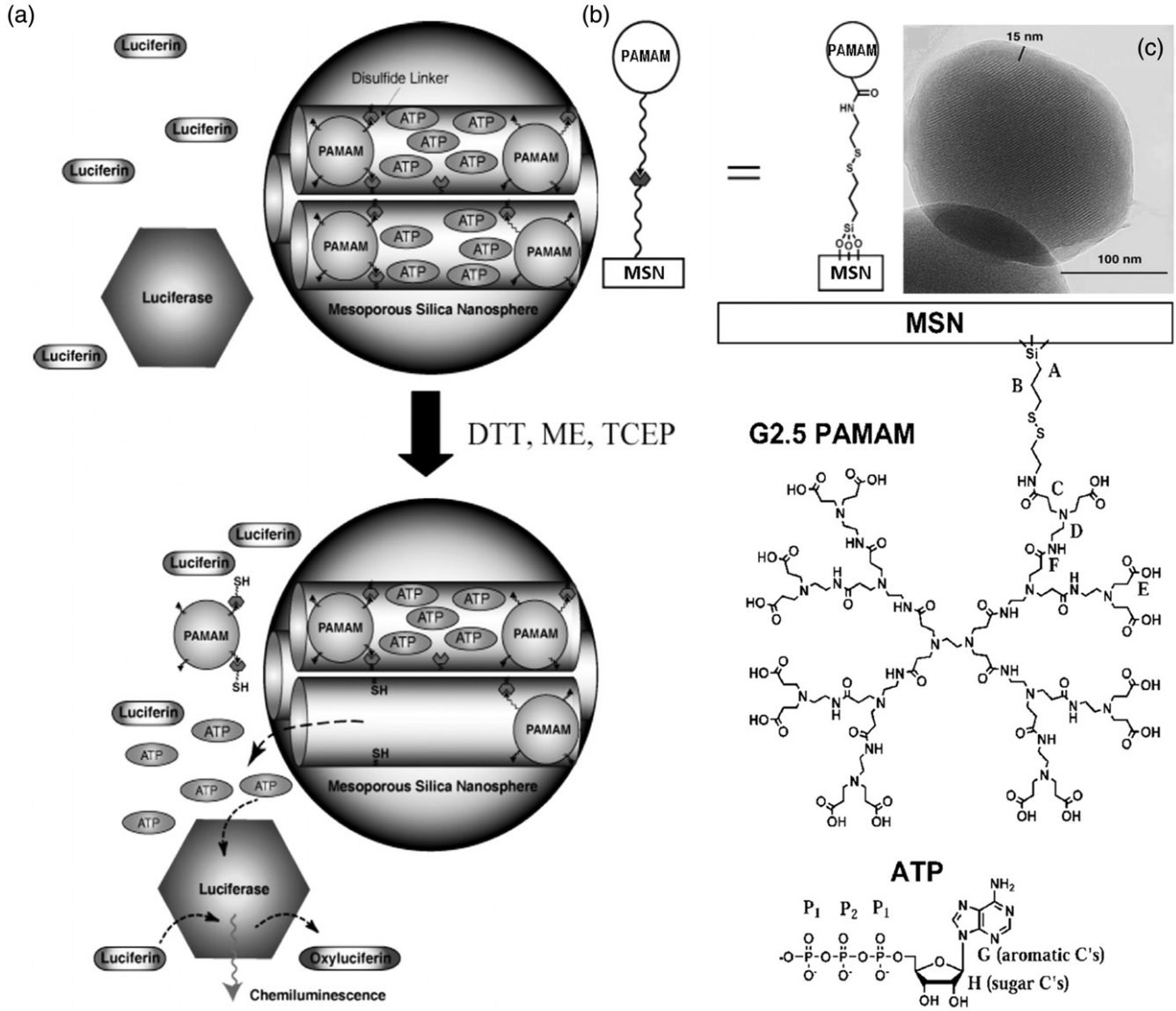


Figure 8. (a) Schematic of mesoporous silica nanosphere encapsulating ATP molecules and illustration of real-time imaging of ATP chemiluminescence developed by Gruenhagen et al. (b) The ATP release was tuned by controlling uncapping triggers and the emitted chemiluminescence signal was collected for *in vitro* release study. (c) TEM images of PAMAM dendrimer-capped MSN shows the visible PAMAM dendrimer coating encapsulating the particle [147].

The CdS-capped MSN delivery system released negligible ATP (<1.0%) in PBS over a period of 12 h. However, after 3 days of the DTT triggering, 28.2% (1.3 µmol) of the ATP molecules diffused away [148].

Gruenhagen et al. [147] conducted a similar study using ATP-loaded MSN material capped with PAMAM and CdS. Using real-time chemiluminescence imaging, chemiluminescence signals from ATP release were collected and pulse-type release kinetics was observed due to uncapping of CdS-capped particles, while the ATP release from PAMAM-capped MSN was more gradual and plateau-like profile (Figure 8) [147].

Other adenosine delivery systems

Cell-based adenosine delivery

Cell-based delivery is an alternative to micro- and nanoparticles in which, various cells are engineered to act as biological drug delivery systems [150]. Cell and gene therapies have been explored for epilepsy treatment on a local level [62,151–155]. Elevation of adenosine kinase and reduction of adenosine have been proven to increase seizure susceptibility in established epilepsy. This phenomenon can be controlled by increasing the adenosine level [61,151,156–158]. Several studies have demonstrated that kindled seizures in rats could be inhibited by focal paracrine delivery of adenosine from encapsulated cells without overt side effects. These studies encapsulated engineered fibroblasts, myoblasts or stem cells using genetic disruption of the *Adk* gene to release adenosine [61,151,156,157,159–161].

Hughes et al. [162] attempted to encapsulate cell lines within immunoprotective microcapsules before implantation by fusing a signal sequence to ADA to prevent immunosuppression in non-autologous hosts. They engineered the transfected mouse fibroblasts/myoblasts by enclosing them in microcapsules fabricated from hydrogel and alginate-poly-

L-lysine. It was observed that the cells successfully secreted adenosine deaminase (ADA) from the microcapsules and had viability of over 5 months [162].

Huber et al. [156] engineered the baby hamster kidney (BHK) fibroblasts to release adenosine. In this technique, the cells were rendered ADK deficient (shown as $Adk^{-/-}$) by inactivating the adenosine kinase and adenosine deaminase (adenosine-metabolizing enzymes) before being used for adenosine delivery. Adenosine-releasing cells were encapsulated into semipermeable polyethersulfone (PES) hollow fibers with 7 mm length, 0.5 mm inner diameter and wall thickness of 50 μm [156]. A partial epilepsy model was used for the *in vivo* study, in which the cells were implanted into the lateral brain ventricles of electrically kindled rats. *In vivo* release rate from the implanted fibers was comparable to its biological production rate, in the range of 20–50 ng/day. It was observed that electrically induced seizures were suppressed for up to 2 weeks by the local paracrine adenosine (concentrations < 25 nM) without any overt side effects such as sedation or ataxia [127]. About 90% of the transplanted cells were still in the host brain 3 days after transplantation, which resulted in 100% seizure suppression. The accumulation of released adenosine was considered to be unlikely, as the adenosine was taken up into cells by equilibratory transporters [156].

Using the same ADK-deficiency technique [156,163] Güttinger et al. [161] could induce the release of adenosine from mouse C2C12 myoblasts with the aim to achieve long-term seizure suppression. In their work, semipermeable polyethersulfone polymer hollow-fiber membranes with size of 5 mm length, 0.5 mm inner diameter and wall thickness of 50 Am containing a polyvinyl alcohol (PVA) matrix were loaded with genetically modified myoblasts [156]. One week after grafting the implants into the brain ventricles of epileptic kindled rats, all rats showed 100% protection from convulsive seizures for up to 8 weeks [161]. It was also observed that disruption of adenosine kinase after implantation did not inhibit cellular differentiation or functional activity [160].

Although localized delivery of adenosine from cells appears promising for treatment of epilepsy, long-term adenosine delivery is of great importance toward their clinical application. One limitation regarding long-term delivery of adenosine from the encapsulated cells is the reduced life expectancy of cells [156]. Local release of adenosine by engineered stem cell implants might be a solution for epilepsy therapy [62,151–154,163]. Anticonvulsant properties of adenosine, long-term survival potential and capability of stem cells in repairing the injured brain make the stem cell-derived brain implants a promising therapeutic tool to achieve focal long-term delivery of adenosine [156,160,164]. Unlike encapsulated cell grafts that release adenosine based on only paracrine action, it is believed that the release mechanism of stem cell-derived implants is a combination of paracrine effects with network interactions [156,161,165,166].

In another work by Fedele et al. [163] on mouse embryonic stem (ES) cells, both alleles of ADK were disrupted ($Adk^{-/-}$ ES) using homologous recombination. By differentiating $Adk^{-/-}$ ES cells into mature astrocytes and oligodendrocytes [167], sufficient quantity of adenosine release (up to 40.1 ± 6.0 ng per 10^5 cells/h) was obtained which was enough for seizure suppression [156]. A similar study with astrocytes derived from fetal neural progenitor cells (NPs) isolated from $Adk^{-/-}$ mouse could release 47 ± 1 ng per 10^5 cells per 24 h [168].

Li et al. [157,158] conducted a study on the following adenosine – cellular implants; (i) wild-type ($Adk^{+/+}$) and (ii) genetically altered ($Adk^{-/-}$) embryonic stem cells. The cells were then differentiated into neural precursor cells, and $Adk^{+/+}$ BHK (with normal ADK expression), and adenosine releasing $Adk^{-/-}$ BHK-AK2 (totally lacking ADK expression) cell implants. They grafted the implants into the hippocampus of rats and compared their antiepileptogenic effects. The following order of therapeutic efficacy retard kindling development was observed: $Adk^{-/-}$ NP $>$ $Adk^{-/-}$ BHK-AK2 $>$ $Adk^{+/+}$ NP $>$ $Adk^{+/+}$ BHK, indicating the superiority of embryonic stem cell-derived brain implants in terms of adenosine releasing, and sustained protection from seizures. In conclusion, the epileptogenesis and the occurrence of generalized seizures were reduced by adenosine releasing from stem cell-derived nanoparticles during kindling development. Considering the superior anticonvulsant effect of stem cell-mediated delivery over paracrine adenosine release from fibroblasts, it is speculated that the stem-cell grafts might be a potential treatment for long-term seizure suppression [157,158,169].

Van Dycke et al. isolated $Adk^{-/-}$ neural stem cells from fetuses of ADK knockout mice before they die due to hepatic steatosis within 14 day [65,168] and measured the quantity of secreted adenosine in culture medium and evaluated their differentiation potential [168,170]. It was observed that the amount of adenosine released from both non-differentiated and differentiated fetal $Adk^{-/-}$ cells was enough for suppressing refractory epilepsy.

To improve the duration of adenosine release from $Adk^{-/-}$ ES cells, Uebersax et al. [171] studied the adenosine release potential of ES cells cultured on three different substrates (1) poly(L-ornithine], (2) silk-fibroin and (3) type I collagen coated tissue culture plastic. Two different types of culture media were used for the study: (1) proliferation medium with growth factors and (2) differentiation medium without growth factors. Higher cell proliferation and lower metabolic activity were observed on collagen and poly(L-ornithine) substrates compared to SF. Compared to wild-type control cells, $Adk^{-/-}$ ES cultured on polymeric substrates, released higher concentration of adenosine (>20 ng/ml). The results showed that the differentiation of $Adk^{-/-}$ ES cells into astrocytes and subsequently release of adenosine was efficient on SF. Thus, SF might be a suitable candidate for $Adk^{-/-}$ ES encapsulation [171].

The second generation of adenosine-releasing cells is engineered human stem cells [137,172]. This method includes knockdown of ADK by gene expression with lentiviral micro-RNA vector in hMSCs and human embryonic stem cells (hESCs). This technique resulted in up to 80% ADK-knockdown for hMSCs [137]. hMSCs have advantages over cell grafts as they allow the transplantation of autologous cells, and they can be induced to neuronal differentiation. Lentiviral transduction of hMSCs with anti-ADK miRNA resulted in 8.5 ng/mL adenosine release in medium during 10^5 cells incubation for 8 h [131]. Boison

Table 1. Summary of adenosine-associated delivery systems.

	System	Advantages	Limitations
Particle-Based techniques	Liposomes	Prolonged release (up to 2 months).	Low entrapment efficiency
	PEG/PEGylated liposomal	Decreasing the enzymatic degradation, improving the cellular immune function	Very low entrapment efficiency, low cellular uptake, non-biodegradable
	Chitosan	Biocompatible and biodegradable, high mucoadhesiveness, immunostimulating properties	Potential inflammation
	PLA/PLGA	Highly biocompatible, Biodegradable	Burst release and non-sustainable short-term release
	EVA	Up to 2 weeks sustained release	Non-degradable
	Silk	Biocompatible, mechanically strong, tunable degradation to non-toxic products	Burst release, potential inflammation
	Silica Nanosphere	High encapsulation efficiency, porous structure	Non-degradable
	Layer-by-Layer assembly	Tunable release kinetics, biodegradable	Complex fabrication process
Other	Cell-based	Sustained long-term release, biocompatible	Potential inflammation, lack of established efficacy, distribution of the drug throughout the whole brain and cerebrospinal fluid, cell viability
	Pump/Inhaler-Based	Steady continued local release	Need for cyclic refilling/replacement, mechanical failure, obstruction and infection

et al. [137,172] showed that engineered hMSCs grafted into the mouse hippocampus were potent anticonvulsant and could reduce acute injury (reduction in neuronal cell loss up to 65%), chronic seizures, and seizure duration up to 35%. Knockdown of ADK was observed after applying similar lentiviral micro-RNA vector in hESCs [172]. It was reported that release of adenosine from encapsulated cell grafts is generally effective in seizure control with limited duration of action and is independent of seizure frequency [159]. Li et al. [173] demonstrated that chronic seizures reduced in a post-status epilepticus model as a proof of-feasibility study in the development of therapeutic hMSC/silk-scaffolds [171]. After grafting the engineered hMSCs into infrahippocampal fissures of models of focal spontaneous seizures, it was observed that the intensity of seizure significantly reduced [173].

Cell-based adenosine delivery has shown promising results for treatment of a range of CNS disorders and can enter clinical trials if autologous cells are used. However, the uses of genetically modified cells or non-autologous cells carry the risk of inflammation or long-term side effects which can cause other complications. Thus, future animal trials on larger animals are required to characterize the long-term effect of such treatments.

Pump/inhaler-based delivery

Cell-based adenosine-releasing systems have extensively been researched for treatment of epilepsy [159]. This approach, however, suffers from several limitations such as lack of established efficacy, distribution of the drug throughout the whole brain and cerebrospinal fluid rather than local seizure focus and the need for immunosuppression.

One approach to avoid major side effects is local delivery using pumps. Micropumps in drug delivery are electronically activated devices with usually a refillable drug reservoir that meter continued release [174]. Van Dycke et al. exhibited the antiseizure effect of sustained adenosine delivery (0.23 µl/h) via osmotic minipumps in the hippocampi of rats with spontaneous seizures [175]. They demonstrated that sustained delivery of high concentration of adenosine (33 mg per day) could lead to a significant reduction of convulsive and non-convulsive seizures without side effects, and a continuous decrease in seizure frequency [175].

Another suggested administration method for adenosine is dry powder inhalation. Administration of dry powder adenosine by inhaler was found to be very effective due to shorter administration time and more consistent delivery over the entire dose range. Lexmond et al. suggested this method as an alternative to nebulisation of adenosine 5′-monophosphate (AMP) in bronchial challenge testing. They formulated 100% pure adenosine powder and adenosine and lactose diluent using spray drying method. Several improvements were observed in their technique in compared to AMP method performed on asthmatic subjects [176].

Pump-based systems can facilitate continuous local and intravenous administration of adenosine. However, they cannot help with its short half life. Thus, in case that adenosine is employed for treatment of internal organs such as brain or heart, they should release encapsulated adenosine with longer half life. An overview of adenosine-associated delivery systems has been summarized in Table 1.

Conclusions and future directions

Local delivery of adenosine and its derivatives offers enhanced efficacy, cost-efficiency and reduction or elimination of unwanted side-effects for treatment of chronic wounds, severe immunodeficiency diseases, chronic inflammatory diseases, epilepsy, diagnosis of ischemic heart disease, early hepatic disease and spinal cord damages [177–179]. Several methods have recently been utilized for local delivery of adenosine, including synthetic adenosine-releasing polymers/liposomes, encapsulated adenosine-

releasing cells, pump-based delivery and other approaches. However, no system currently exists to offer controllable, sustained, long-term adenosine delivery through fully degradable implants. Moreover no research has been done on smart delivery to release adenosine in a targeted area (e.g. brain) without interfering with other organ's function (e.g. heart).

Polymer-based adenosine delivery systems are relatively safe and can prolong the release time and half-life of the drug. These carriers can also be used for target delivery if properly designed. On the other hand, the needs for invasive surgical procedure, local effects, and issues with their long-term effectiveness have limited their clinical use. Although long delivery durations are more difficult to achieve with polymer implants as compared to stem-cell based delivery, it seems that the efficacy of silk drug delivery implants for local delivery of neurological drugs have potential to be rapidly translated from animal studies to clinical trials [128,180].

Stem cell-based adenosine delivery systems are capable of repairing the damaged network beside the paracrine release advantage. Moreover, the cells can be injected, bringing widespread delivery potential focal delivery of adenosine via stem or progenitor cells might be an alternative for treatment of refractory patients with epilepsy, however in this technique cell survival after transplantation is challenging. The benefits of stem cell-based delivery might evolve into an exciting generation for future research, for instance cellular delivery systems using neural stem cells or myotubes to achieve improved long-term viability [157,159,181–183]. However, the long-term effectiveness/viability of cell-based systems, survival of stem cells, network interaction and immunosuppression issues for non-autologous cell sources will challenge adenosine-based stem cell therapy translation into clinical use [137,161,165,166]. In addition, it should be taken into consideration that the rat-kindling model used in most epilepsy treatment experiments is just a basic model for epileptogenesis and by no means reflects the entire histopathological changes in human epilepsy, which is a chronic disorder with a need for lifelong treatment [128,180].

Although the ATP delivery systems are in early evaluation stage, they have shown very promising results for skin wound healing especially for chronic wounds, such as diabetic wounds and pressure ulcers [56,91]. However, the significant difference between mechanisms of wound healing between humans and rodents should be considered [184]. In addition, the validity of some evaluations using immunodeficient mice should be questioned [90].

Focal delivery of adenosine via infusion from non-degradable implantable mini pumps is promising. This approach may be limited by complications including the need for cyclic refilling or replacement during the lifetime of an epilepsy patient, mechanical failure, obstruction, and infection [185,186]. A future option for clinical use of pump systems may be the steady local release of low concentrations of adenosine [159,187]. An interesting concept for future adenosine delivery can be the emerging lab-on-a-chip technology. This approach employs advanced micro- and nanotechnologies that can fit into a microchip creating a compact integration of microdevices and microfluidics for precise and controlled drug delivery [188–195].

Declaration of interest

The authors acknowledge funding from the National Science Foundation (EFRI-1240443), IMMODGEL (602694), and the National Institutes of Health (EB012597, AR057837, DE021468, HL099073, AI105024, AR063745).

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