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Synthetic immunosurveillance systems: Nanodevices to monitor physiological events

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Abstract

The field of nanotechnology has recently seen vast advancements in its applications for therapeutic strategy. This technological revolution has led way to nanomedicine, which spurred the development of clever drug delivery designs and ingenious nanovehicles for the monitoring of cellular events *in vivo*. The clinical implementations of this technology are innumerable and have demonstrated utility as diagnostic tools and fortifying machineries for the mammalian immune system. Recently engineered viral vectors and multi-subunit packaging RNAs have verified stable enough for long-term existence in the physiological environment and therefore reveal unique potential as artificial immunosurveillance devices. Physiological and pathological events recorded by nanodevices could help develop “biocatalogs” of patients’ infection history, frequency of disease, and much more. In this article, we introduce a novel design concept for a multilayer synthetic immune network parallel to the natural immune system; an artificial network of continuously patrolling nanodevices incorporated in the blood and lymphatic systems, and adapted for molecular event recording, anomaly detection, drug delivery, and gene silencing. We also aim to discuss the approaches and advances recently reported in nanomedicine, especially as it pertains to promising viral and RNA-based nanovehicles and their prospective applications for the development of a synthetic immunosurveillance system (SIS). Alternative suggestions and limitations of these technologies are also discussed.

Keywords

Nanomedicine; Nanovirology; Theranostics; Drug-delivery; Viral vectors

1. Introduction

In comparison to most complex eukaryotes, the mammalian immune system is unique in that it contains, in addition to innate immunity, adaptive immunity. Innate immune surveillance is constantly active and works to neutralize localized infections and mildly virulent pathogens. This system is considered “nonspecific”, as it is continually ready to respond to infection.

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Acquired or adaptive immunity on the other hand is activated by events with high rates of heterogeneity and virulence (Vesely et al., 2011; Klimpel, 1996). As the body is constantly exposed to pathogens, it must be able to discern threats from commensal events. Characterizing these distinctions is critical for immune response, as failure in doing so can lead to autoimmune disease and potential death of the host organism. The proposed Pattern Recognition Theory by Charles Janeway revealed the complexity of innate immunity and its adaptive arm (Janeway, 1989; Gayed, 2011). The subsequent years were marked by a series of relentless investigations of immune cells, which eventually led to the identification of Th1 and Th2 cells and the discovery of type 2 immunity-derived nuocytes (Neill et al., 2010). Today, a substantial amount of understanding has been accumulated about human immune defense, yet we continue to witness elevated rates of pathogenic infections and cancer deaths worldwide, with the latter claiming over 7 million lives each year (Ferlay et al., 2010).

In the human immune system, the failed activation of lymphocyte is a common precursor of neoplasia and carcinogenesis. Once neoplastic cells escape innate immune defense, acquired immune programs are activated; the failure of the latter usually results in an immune crisis. This is further accelerated when the body is under stress and with an increase in age. Events such as gene disruption, reduction in hormone production, and thymus shrinkage (involution), aid drive this deficiency. During the past several years, immunologists have attempted to artificially ameliorate immune defense by engineering T-cells better adapted for patrolling and antigen clearing (Stone et al., 2012; Bowerman et al., 2009; Masiero et al., 2005); the refinement of appropriate cell receptor profiles and the reduction of antigen-receptor mis-pairing are examples of such attempts (Sadelain, 2009). Nevertheless, the challenges of T-cell engineering are numerous, as it has been difficult to develop stable T-cell lines capable of long-term function *in vivo*. Recently, new attitudes for the amelioration of immune response have emerged and they include the employment of nanotechnology to carry out failed immune programs (Hyejung and Miqin, 2013; Guo, 2005; Azizgolshani et al., 2013). The introduction of these nanotechnological tools presents a wide array of clever medical applications. This was validated with the advent of oligonucleotide packaging motors, which revealed tremendous therapeutic potential after the discovery of microRNAs (miRNA) in 1993 (Ambros, 2013; Bartel, 2004). Since then, the use of RNA interference (RNAi) moieties has been successfully conceptualized as gene delivery vehicles for cell and tissue specific purposes.

In recent years, there have been substantial efforts to develop stable RNA species and nanoparticles capable of sustaining the harsh heterogeneous physiological environment (Guo, 2005; Shu et al., 2011a; Germer et al., 2013). The incorporation of nucleotide derivatives to enhance RNA stability has been employed and showed great promise for gene delivery and gene silencing (Guo, 2005; Lai et al., 2009; Abdelmawla et al., 2011). Similar studies have predominantly focused on RNA or cell engineering to improve immune defenses, yet few have elaborated on the potential of an auxiliary artificial immune network to enhance or complete failed immune tasks. We present this article as an exploratory, interdisciplinary, platform to the possibilities of a synthetic immune surveillance network. It is not a matter of concern to us if every mechanism presented here has already been validated, but to discuss how recent advances in nanotechnology can be implemented for the development of synthetic immunosurveillance systems (SIS).

2. The role of commensals in immune response

The pathobiome and commensal microbiota are “steel-sharpening” components of the immune system. In the 1980s, the publication of the “hygiene hypothesis” allowed investigators to explore the beneficial relationships between bacteria and the human immune system. Today, we know that secondary metabolites produced by microbes aid in immune defense. In the intestine alone, nearly 10^{14} bacteria from 1^3 different species make-up the enteric microbiome (Patten and Collett, 2013) where they control pathogenic and probiotic thresholds and have recently been shown to influence immune cell response to vaccination (Eloe-Fadrosh et al., 2013; Fujimura et al., 2010). On the skin, they regulate keratinocyte TLR3-mediated inflammation response and provide a microscopic cushion against pathogenic entry (Lai et al., 2009). Due to this intertwined relationship involving bacteria and healthy cells, the immune system constantly has to distinguish differences between nosocomial bacteria and infectious pathogens. One of the ways by which this is accomplished is opsonization, a process by which immune cells coat pathogens with complement antibodies, marking them as targets for phagocytosis and immune attack (Male et al., 2006).

In the past decade, several strains of phagocytosis-resistant bacteria have been driving agents of pathogenic outbreaks (Pfaff-McDonough et al., 2000; Mead et al., 2006; Gaul et al., 2013). Currently, the treatment methods principally used to solve this problem have heavily relied on synthetic antibiotics. Yet, several findings, including results from our group, have revealed that antibiotic resistance is readily acquired by such pathogenic strains (Woappi et al., 2013; Oliver et al., 2000). Better antibiotic-free armamentarium against phagocytosis-resistant pathogens can be established with nanotechnology. For instance, nanovectors can help coat resistant strains with appropriate complements and can be engineered to deliver reactive oxygen species (ROS) to invading pathogens. Furthermore, the attachment of toll-like receptors (TLRs) or nod-like receptors (NLR) onto nanovectors can aid neutralize and transport pathogens to inflammasomes for destruction (Jones and Weiss, 2011; Yang et al., 2013).

3. Designing a synthetic immune surveillance network

In 2003, an attempt to halt T-cell trafficking, a major factor in tumor-induced immune suppression, was carried out in a double blind, placebo-controlled trial for multiple sclerosis (MS) patients (Polman et al., 2006). The trial used Natalizumab, a monoclonal human antibody, to accomplish trafficking hindrance. As an α_4 integrin antagonist, Natalizumab was designed to reduce brain lesion development in patients and was successful in doing so. Clinicians noted fewer inflammatory brain lesions and fewer relapses over a six-month period in the treatment group. And the successful outcome of the study exposed the vulnerability of similar therapeutic targets. For instance, the development of anti-cytokine and anti-CD25 antibodies, known as Daclizumab and Basiliximab, has been quite successful in the clinic (http://clinicaltrials.gov). Similar immunological trials have been fruitful, yet the scarcity of reliable therapeutic antibody-based trials is rooted in the difficulty of antibody specificity design, particularly to cancer cell receptor targets. The challenges lies in the fact that receptors present on diseased cells are also often found on healthy cells. In response to

this, immunologists and clinicians have attempted to identify possible alternatives for cancer therapy. This is again seen in the high number of attempts to engineer T-cells capable of producing ultra-sensitive antibodies adept for swift diseased cell and pathogen elimination (Wilen et al., 2011; Rita et al., 2010). As this approach is fairly new and its technology still developing, clinical trials have been relatively unsuccessful. As of today, the NIH reports over 100 clinical attempts to use engineered T-cells in patients – none deemed convincingly effective (http://clinicaltrials.gov). Nanotechnology, conversely, provides an exceptional supplementary platform for immune surveillance and immune defense.

In recent years, the merge of therapeutics and diagnostics – theranostic medicine – has emerged as a propitious tool for disease treatment at the molecular level. In the last 5 years alone, numerous studies have used nanovehicles to achieve gene delivery or immune activation, and proposed the implementation of intelligent nanodevices to carry theranostic functions (Guo et al., 2005; Sukhanova et al., 2012; Mok and Zhang, 2013; Bhaskar et al., 2010; Kateb et al., 2011; Lehner et al., 2013; Cohen and Shoushan, 2013; Etheridge et al., 2013). Though the ideas have been multifunctional, they have mostly focused on specific types of nanomaterials to carry individual cellular missions. The concept we are presenting here, however, is a multilayer synthetic immune network parallel to the natural immune system. An artificial network of continuously patrolling nanodevices adapted for molecular event recording, anomaly detection, drug delivery, and gene silencing. Although artificial, the nanodevices would be incorporated in the blood and lymphatic networks and would follow natural physiological flows. The system could be endogenously maintained by self-replenishing scaffolds of functional transcripts, or supported exogenously through oral and intravenous inoculations. And though nanovector trafficking routes have been successfully established in mice (Rae et al., 2005), the challenges of establishing a stable network of patrolling nanovectors in humans are colossal; to bring such a model to fruition five key obstacles would have to be overcome:

- **a.** Designing competent and non-toxic nanovectors capable of long-term stability in the physiological environment.
- **b.** Disguising nanodevices to be recognized as *self*.
- **c.** Incorporating sensitive molecular sensors and footprint reporters capable of detecting and recording abnormal molecular events.
- **d.** Monitoring nanovectors trafficking routes *in vivo*.
- **e.** Interpreting biochemical signatures.

4. Engineering competent and non-toxic nanovectors

Nanodevices assembled from nucleocapsid vectors can enclose stable miRNA species and have shown promising results in animal models (Azizgolshani et al., 2013; Sainsbury et al., 2010). Recently, Azizgolshani and colleagues successfully incorporated gene silencing transcripts within nucleocapsids (Azizgolshani et al., 2013). The capsids were then used as vehicles to neutralize specific gene expressions in mice. What make nucleocapsids particularly effective as vectors are their naturally stable, yet malleable, coating proteins

(Fig. 1). Their capsid proteins (CP) are capable of short antigenic peptide (epitope) presentation and can often serve as scaffolds for agglomeration of immobilized receptors and nanoelectrodes (Hafenstein and Fane, 2002; Steinmetz et al., 2006). Furthermore, capsid-coating arrangements can be modeled to recognize and record physiological changes, which could then be interpreted *ex vivo* (Guo, 2005; Steinmetz et al., 2006). In the model we propose, nanovehicles migratory routes would follow the principles underlying chemotaxis and aid in immune cell recruitment or the recruitment of additional drug-carrying nanovectors to a diseased site. Physiological hydrostatic pressures and RNA motors would then help guide the nanodevices to appropriate tissue reservoirs and cellular compartments (Fig. 2) (Mackay, 2001; Guo et al., 2005). An elaborate list of factors comprising immune migratory patterns and their potential as therapeutic targets is reviewed by Luster et al. (2005).

4.1. Plant viruses in nanotechnology

In living systems, capsid proteins (CP) generated from *Tobamoviruses* and *Bromoviruses* (infecting plants) have been shown to be significantly more stable than those generated from *Alphaviruses* (infecting animals) (Azizgolshani et al., 2013; Strauss and Strauss, 1994; Thornberry and Nagaich, 1962; Alonso et al., 2013). Tobacco mosaic virus (TMV), a virus belonging to the *Virgaviridae*; Cowpea Chlorotic Mottle Virus (CCMV), a virus belonging to the *Bromoviridae* family; and Cowpea Mosaic virus (CPMV), a virus of the *Comoviridae* family, represent some of the best studied plant-infecting viruses to date. A paramount progress in plant virus research was made when Lomonossoff and Shanks (1983) derived the complete nucleotide sequence of CPMV. Three years ago, Kumar et al. (2011), with the help of several African phythopathologists, characterized protein-based phylogenetic relationships between numerous plant viruses found in that region, many of them possessing protein capsid characteristics similar to CCMV, revealing legumoviruses as abundant sources of potentially stable nanovectors. If appropriately disguised to evade immune responses, CCMV and alike viruses will surely present more unique applications to nanomedicine.

More recently, chemists successfully remodeled plant viral capsids as mammalian gene vectors by assembling CCMV hybrid virus-like particles *in vitro* and reconstituting them for cytoplasmic RNA release (Azizgolshani et al., 2013). These nanovehicles were capable of packaging numerous RNA molecules of various lengths and were robust enough to protect them from nuclease degradation. The study revolutionized the field, as it affirmed the efficacy of plant viruses as therapeutic vehicles capable of long-term mammalian gene delivery in human systems. What makes plant-based vectors particularly attractive for mammalian cell targeting is the fact that, in addition to their stability *in vivo*, they possess lower risks of toxicity and virulence in mammalian cells. Their spontaneous packaging nature also encourages self-sustainability *in vivo*, making them ideal platforms for long-term patrolling devices in a synthetic immune surveillance network (Sainsbury et al., 2010; Roy et al., 2010). So far, the most widely explored biomedical applications of these vehicles have been for gene delivery, but the implementation of viral nanotechnology should be extended to chemotherapeutic targeting, molecular abnormality detection, and interference of pathogen-evasion.

In healthy tissue, stimulation of T-cell, B cell, and dendritic cell (DC) interactions is one of the primary events following identification of an antigen peptide. However, the disruption of leukocyte trafficking is often the result of integrin receptor instability or a disturbance in the multistep cell-recruiting cascade (Luster, 1998). Pathogens can then easily evade immune surveillance once these regulatory checkpoints are disrupted. The understanding of this process encouraged focusing on the repair of cell adhesion molecules as a way to inhibit disruption of immune cell migration. Since the outer coating of several viral capsids naturally carry glycoproteins (Partridge et al., 1974), CP could be harnessed to also carry mammalian glycoprotein ligands, which can then be modeled to combat immune defense evasion and promote leukocyte trafficking and migration to inflammatory sites.

5. Nanovirology

One of the chief architectural characteristics of viral vectors is their nanoscale. On average, viruses range between 5 and 300 nm in size and consequently make efficient gene delivery vehicles to the tightly structured cellular compartments (Prangishvili et al., 2006; Wu et al., 2013a). Evolutionary, viruses have adapted to infiltrate living cells and to effectively evade host antigen-detection. This efficient infiltration feature was at the origin of the exploration of viruses as synthetic gene delivery vehicles. That and the ability to evade host antigen-detection is a major advantage viruses have over organometallic nanoparticles and other nanovectors. Furthermore, viral capsid repetitive geometry presents major architectural benefits. Their structural arrangements offer vast amounts of surface area for the addition of supplementary receptors and bioactive aptamers. Inside their capsid encasing, viruses naturally withhold bioactive genomic elements and can be engineered to carry libraries of therapeutic oligonucleotides (Lindbo et al., 2001; Sainsbury and Lomonossoff, 2008; Lomonossoff, 2001; Azizgolshani et al., 2013). The recent discovery of Pandora viruses has redefined our understanding of viral genomics and reveals the immense amounts of genomic information viruses can stably cargo while inside a host (Philippe et al., 2013). These newly identified features are quite exciting, as they offer a great deal of prospective therapeutic applications. The exponential growth of virology-based nano-therapeutic research has opened a gateway to a new subset of virus biology which can be termed “nanovirology”, the study of virological applications to nanotechnological designs.

6. Vector stability

One of the things that has made viral vectors outstanding above most nanovehicles has been their stable CP protein characteristics. Cowpea (*Vigna unguiculata*) viruses, for example, can be resistant to pH levels as low as 3 and possess an average thermal inactivation threshold of 60 °C, surpassing normal physiological temperatures (Table 1) (Singh et al., 2006). In mammalian systems, CCMP virus particles can remain stable over 96 h (Rae et al., 2005; Ma et al., 2012). Such remarkable durability is a result of protein organizational patterns usually structured in arrangements of multiple subunits assembled in quaternary complexes (Sainsbury et al., 2010; Ma et al., 2012). Plant virus capsid conformations have encouraged the design of many stable viral vectors, and recent technological advances have demonstrated that CCMV CP can be engineered to possess even greater physical and thermodynamic stability (Rae et al., 2005; Sainsbury et al., 2010; Steinmetz et al., 2006).

When applied to naturally stable strains, capsid engineering can offer a wide platform of ultra-stable vector libraries competent for therapeutic applications (Mateu, 2011; Aljabali et al., 2010).

One of the main challenges of using viral vectors as vehicles lies in the designing of empty viral capsids, which can be refilled with non-toxic bioactive elements. The use of CPMV to target vascular and neovascular endothelial cells has explored this remodeling and has revealed encouraging results in human systems (Koudelka et al., 2009). Furthermore, in an attempt to study virus-traveling routes *in vivo*, Aartsen et al. (2010) successfully incorporated a green fluorescent protein (GFP) gene into CCMV sequences and were able to use it as a nanovehicle in mice. The same year, another research group further exposed CCMV potential as a gene expression vector, expanding on previous findings revealing such vectors reliable for long-term carriage of molecular cargo, including siRNAs, nanoemulsion droplets, pH-controlled chromophores, and heterologous RNA species *in vitro* and *in vivo* (Lindbo et al., 2001; Sainsbury and Lomonossoff, 2008; Lomonossoff and Montague, 2001).

7. Oligonucleotides as drug-delivery vehicles

MicroRNAs (miRNA) versatility and pico scale have served as excellent tools for both nanovector design and drug delivery. However, their simplistic and exposed structural arrangement raises stability and sustainability concerns in physiological environments. This has led to substantial efforts in the development of RNA families resistant to exonuclease inactivation, yet competent for drug delivery (Guo, 2005a, 2005; Bartel, 2004; Shu et al., 2013). In the last 10 years alone, several nucleotide derivatives have been created to help design stable RNA species (Guo, 2005a; Shu et al., 2013) (Table 2). miRNA polymer stacking and the addition of nucleotide derivatives, such as 2′-F-pyrimidine, are examples of rearrangements used to stabilize RNA species (Guo, 2005a; Shu et al., 2013). Similar RNA rearrangements have also been shown to prevent exonuclease degradation and misfolding, all while retaining bioactive function (Guo, 2005a). These modifications have rendered miRNAs capable of ligand–cell receptor binding and competent for blood circulation. In 2010, RNA-based vectors carrying anti-HIV moieties were successfully employed to establish a stable vector expression system *in vitro* (DiGiusto et al., 2010).

The main challenges of working with RNA in human cells are the immune programs designed to recognized and neutralize foreign genomic elements. Therefore, in addition to stability, it will be desirable to develop multifunctional RNA complexes capable of evading immune barriers. The successful assembly of packaging RNAs (pRNAs)-silencing RNAs (siRNA) complexes in 2011 marked a major step in that direction and demonstrated that RNA/siRNA chimeric nanovehicles could successfully infiltrate cancer cells and utilize cellular machinery (*i.e.* Dicer) to unleash gene silencing transcripts (Shu et al., 2011b). And although the biochemical instability of RNA moieties is often regarded as a disadvantage, it is important to note that their sensitivity and swift conformational folding patterns make them ideal for monitoring subtle changes in physiological activity. In fact, their role and use as aptamers have been widely explored and honed to record pathological events *in vivo* (Guo, 2005a; Guo, 2005).

7.1. Bacteriophages-derived packaging RNAs

In general, bacteriophages are not as chemically stable as bromoviruses. And though this has been inconvenient for prolonged biomedical studies, some bacteriophage proteomic and genomic components have been effective vehicles for mammalian gene targeting. This is exemplified with bacteriophages phi29 (φ29), which remodeled chimeric packaging pRNAs were used as vehicles for gene delivery and nanomaterial assembly (Guo, 2005). These RNA moieties typically exist as part of φ29 DNA-packaging motor and upon manipulation can be chimerically arranged to form dimers. As such, they have been exceptionally efficient for drug delivery in cancer cells (Guo et al., 2005; Guo, 2005). Findings have also shown that the fusing pRNA with receptor-binding aptamers, siRNA, ribozyme, or other chemical groups does disturb dimmer formation or interferes with RNA moieties (Shu et al., 2011; Guo et al., 2005; Guo, 2005). Another element that makes pRNAs efficient vectors is their condensed nanoscale which allows them to easily penetrate various cell compartments, whereas this can be difficult for capsid-enclosed vectors. A limited risk of untargeted gene disruption is seen in the high fidelity of beta like corynephages chromosomal integration and should encourage us to investigate their pRNA gene-delivery potentials (Oram et al., 2007).

7.2. Construction of therapeutic-harboring aptamers

Aptamers are a family of RNA- and DNA-based oligonucleotides that bind to selective targets, including proteins, organic compounds, and nucleic acids (Germer et al., 2013). They are typically obtained by *in vitro* screening and their usefulness is exhibited by swift ligand recognition through the formation of a series of binding pockets analogous to antigen–antibody interactions (Germer et al., 2013; Eloe-Fadrosh et al., 2013; Guo, 2005). These oligonucleotides have presented some promising applications for synthetic nanovector designs, as they can also gather information about the environment they are presented to. One of the methods by which we have been able to identify such aptamer families is through Systematic Evolution of Ligands by Exponential Enrichment (SELEX). SELEX allows for the screening and production of oligonucleotides and aptamers from randomized RNA libraries. By applying similar techniques, receptor-binding RNA aptamers have been successfully conjugated onto pRNAs motors and can even be engineered to harbor siRNA or other therapeutic molecules (Guo, 2005). Similar approaches have recently been investigated in cell culture and animal models, and they have resulted in the successful binding and co-entry of therapeutic particles into cells (Kraus et al., 1998; Guo et al., 2005; Khaled et al., 2005). This technology holds promise for long-term use in a synthetic immunosurveillance network and the treatment of chronic diseases.

7.3. DNA-based nanovectors

In recent years, the use of DNA-based nanostructures has been increasingly employed in theranostics (Kay et al., 2010; Li et al., 2011). What makes these vehicles particularly promising is their increased stability compared to RNA vehicles and their sustained transgene expression in quiescent cells and tissues (Liu et al., 2012). In a SIS, the use of DNA-based vectors should be encouraged as support scaffolds for pRNA vehicles. Drawbacks surrounding DNA nanotechnology are mostly technical, and as DNA-based structures they do retain the potential for integration into host genome and transcription into

inappropriate message. These drawbacks have however been thoroughly addressed in recent DNA-based nanovector designs which are now arranged into conformations unfit for transcription or polymerization. In-depth descriptions of DNA nanotechnology have been provided in some recent reviews (Pinheiro et al., 2011; Pei et al., 2014).

8. Oncolytic viruses

Oncolytic viruses are a group of viruses, which preferentially infect and kill tumorigenic and cancer cells. One of the best-studied oncolytic virus is OBXY-015, an adenovirus engineered for high specific infection targets. The virus is rendered relatively harmless by its inability to neutralize p53 and its incompetency in infecting non-diseased primary cells (McCormick, 2000). Upon completion of its regular life cycle, OBXY-015 lyses host cancer cells, resulting in a swift elimination of neoplastic and cancerous cells without jeopardizing the growth of healthy adjacent cells. Families of similar oncolytic viruses have been celebrated as prospective therapeutic tools and the redesign of their genomic profiles has rendered them quite specific and useful against certain cancer types (Zeyaullah et al., 2012). Some of the potential challenges hindering the use of oncolytic viruses as SIS vectors include their unstable biochemistry, which is often targeted by interferon-mediated immune responses and can render them unfit for long-term trafficking in the physiological environment (Mullen and Tanabe, 2002). Furthermore, their toxicity and possible virulence in mammalian cells present major biosafety concerns. Investigators have, however, attempted to establish non-toxic oncolytic rhabdoviruses engineered to express interferon antagonist (Chiocca, 2002). Bell and McFadden (2014) provide an excellent review of the disadvantages and benefits of contemporary oncolytic viruses.

9. Molecular sensors and footprint reporters for abnormal molecular events

Over the past 20 years we have obtained a greater understanding of antigen presentation on the cell surface. In the context of mammalian immunity, the roles of Class1a, Class 2b, and Class 3 receptors have been extensively studied. Today we know, for instance, that Class 1a receptors are primarily present in CD8+ T-cells and can recognize both cytokines and cytotoxicity, while Class 2 receptors are mainly found in CD4+ T-cells and focus centrally on cytokines. Class 3 receptors are even more complex in their recognition behavior, as they can identify super antigens, bacterial toxins, and retroviral particles (Male et al., 2006). These immune responses are quite effective at dealing with extracellular threats, but the few deficiencies they do possess have greatly disadvantageous effects on the host. For example, T-cells are able to use the major histocompatibility complex (MHC) for antigen recognition, yet they cannot recognize peptide antigen presented by multiple MHC molecules and are thus restricted to receiving message from a single MHC molecule at a time (Wang and Reinherz, 2002). This results in a slow immune response to a rapidly evolving pathobiome, and forces T-cells to operate myopically in their antigen recognition and molecular sensory patterns. Such drawbacks can be greatly ameliorated by the use of artificial sensors coated on nanovector surfaces.

The SIS design should be modeled to incorporate anomaly detection and event recording. In humans, immune response is primarily triggered by inputs from cell sensors. The detection of conserved microbial motifs, unmethylated CpG DNA, flagellin, lipopolysaccharide (LPS), and even products of tissue damage such as HMGB1, S100B, and uric acid, can stimulate T-cell activation and immune response (Male et al., 2006; Vesely et al., 2011; Banchereau and Palucka, 2005). Often however, immune defense activation only results after the detection of extremely elevated inflammatory gradients (Banchereau and Palucka, 2005). This is one of the reasons neoplastic cells can evade immune sensors and undetectably proliferate throughout the body, so long as they maintain low inflammatory signal production levels. The practicality of synthetic nanovectors is that they can be equipped with ultra-sensitive lucin rich domains (LRR) and help in chemokine detection as well as antigen peptide-presentation to T-cells. Through the permeation and retention effect (EPR), certain nanomaterials have been able to accumulate and be retained in tumorigenic locations (Schroeder et al., 2012), upon which they are internalized into various cellular compartments by translocation domains to then release gene silencing transcripts, endosome-disrupting chemicals, or apoptotic signals to diseased cells (Guo et al., 2005; Schroeder et al., 2012). The construction of virus-like particles containing siRNAs and cellular toxins was shown to be just as effective, and specific, at targeting cancer cells (Azizgolshani et al., 2013).

Nanosensors can also be harnessed to recruit additional nanovehicles to an infected tissue where they could then be internalized by cells for drug delivery or could stay on the cell surface to enhance antigen presentation and phagocytosis. Therefore, in addition to polyvalent oligonucleotides and aptamers, the applicability of other materials such as heavy metal, quantum dots, nanocrystals, and radioisotopes should be explored, as they can potentially be conjugated onto vectors as nanosensors for the detection of cancer signatures (Guo et al., 2005; Schroeder et al., 2012). Similar approaches have begun to be explored with the development of ligand-modified virus glycoproteins assembled onto viral vectors to detect temperature-sensitive intracellular trafficking (Guibinga et al., 2004).

9.1. Nanovehicle-mediated recording of molecular events

Contemporary vector designs have been arbitrarily tailored for gene delivery and have immensely negated molecular event recording. Part of this is due in large to the fact that most gene delivery vectors are usually designed to be episomally present in cells, therefore leaving them little time to exist intracellularly, creating less opportunity for event recording. In the human immune system, memory B and T-cells are natural “data recording” devices. Their “memory” is essentially a recollection of antigen-fighting events encountered during an infection. These experiences are stored and recollected by transcriptional regulations mediated through epigenetic programs. Therefore, upon reintroduction of a previously encountered antigen, these cells can initiate appropriate responses (Youngblood et al., 2013; Light et al., 2010). Similar principles are beginning to be applied to nanovector designs. As seen in the construction of chimeric pRNA vehicles carrying receptor-binding aptamers and therapeutic molecules assembled by interlocking oligopeptide loops (Abdelmawla et al., 2011; Guo et al., 2005).

Another promising technology for molecular sensors has been the monitoring of oligonucleotide modifications via chemical probing (Moazed et al., 1986; Yap and Musier-Forsyth, 1995). Some of these chemical modifications have been accomplished with carbodiimidemetho-p-toluene sulfonate (CMCT), dimethyl sulfate (DMS), and kethoxal, which work by methylation and nucleic acid modification (Moazed et al., 1986; Yap and Musier-Forsyth, 1995; Stern et al., 1988). The change in specific nucleic acid base arrangement, for instance, can confer information about the composition of a cellular milieu and reveal exposure to a particular physiological solvent (Guo, 2005a). The recorded interactions can then be deciphered and analyzed *ex vivo* by primer extension to better characterize cellular milieus (Youngblood et al., 2013; Stern et al., 1988). The internal bases of specific protein and RNA species have also been labeled with photo affinity crosslinking agents to analyze inter-and intra-molecular interactions. This could then be interpreted and used to convey information about a particular cell or tissue environment (Guo, 2005a; Chee et al., 2010). Recently, Wu and colleagues were able to construct DNA origami nanostructures capable of monitoring biochemical changes and measuring chemical binding efficiencies occurring within a cell (Wu et al., 2013b).

9.2. Viral capsids as detection devices

Nanovirology presents unique potential for molecular event recording since virus particles can stably exist in cells and are naturally suited with the ability to detect and record subtle changes in the cellular environment. This is perhaps best seen in latency reactivation where, upon integration, viruses can detect changes in cellular conditions and select to either activate replication programs or to remain latent. During a latent infection, viruses record inputs about environmental conditions and use that information to determine appropriate periods of reactivation (Sinclair and Sissons, 2006; Kepler et al., 2007). In 2011, clinical findings revealed a 12-year-long latency period observed in a patient infected with a type 2 vaccine-derived poliovirus. Even with such prolonged latency, the group of researchers was still able to detect a bioactive viral capsid protein region within the patient cells (DeVries et al., 2011). Such observations provide supporting evidence for the long-term genomic stability of viral elements *in vivo*. In addition, the repetitive geometry of viral capsids offers a great deal of constructional advantages, as it can support multiple nanostructures, many of which can serve as antigen-detection apparatuses (Soto and Ratna, 2010; Steinmetz and Evans, 2007). This advantageous geometry has enabled the engineering of immobilized receptor arrangements onto capsid surfaces (Soto and Ratna, 2010). And due to their intracapsid positioning, capsid-bound peptides are well exposed to the physiological environment and can thus readily gain information about cellular events. A recently proposed peptide-presentation apparatus has been CPMV's βB-βC loop located on the small viral coat protein. In addition to detecting pathogenic peptides, the βB-βC loop can equally behave as a vaccine and immune system booster when exposed to viral epitopes (DiGiusto et al., 2010).

It would also be equally important for nanovectors to recognize extracellular events, and to be able to detect exogenous and endogenous signals of infection and tissue damage. The inhibition of TLRs signaling by pathogens is an enormous challenge for natural immune surveillance (Takeda and Akira, 2005). This is mostly due to the antigen-sensitivity

inhibitory properties of pathogenic secretions. In the body, the nucleotide-binding oligomerization domain receptors (NLRs) are part of the main line of pattern recognition components active in innate immune response. The recognition of Flagellin proteins by NLRC4, for example, is a key characteristic by which cells distinguish differences between pathogenic strains and commensals (Latz et al., 2013; Franklin and Latz, 2012). The sensitivity of natural immune surveillance could be enhanced via viral vectors carrying nanosensors, leucine-rich domains, and TLR receptors.

10. Monitoring nanovehicles trafficking routes *in vivo*

The tracking and monitoring of viral vectors and nanomaterials would be essential to the development of a functional SIS. As of today, contemporary methods for tracking viral elements in mammalian cells are largely based on sequencing. This has been useful for mice *in situ* studies, as high amounts of viral cDNA can accurately determine viral accumulation in specific tissue regions (Rae et al., 2005). But in human studies, similar applications are limited since acquisition of tissue samples often requires invasive procedures.

The foundations for the development of noninvasive nanovector tracking were laid in the 1970s by Gunter Blobel while studying protein synthesis and peptide compartmentalization within cells. He observed that certain proteins were ubiquitously transported throughout cells while others were fairly immobile. Soon he noted that the immobile proteins served as processing stations for nascent peptides. In addition, the proteins tagged newly-synthesized peptides and sent them to various cellular compartments. The concept became known as the molecular “zip code” (Editorial, 1999). Ever since, Blobel’s discovery has been used to map specific molecular patterns and locations within cells (Jansen, 2001; Luster and Tager, 2004). The molecular “Zip code” is a concept that provides a great element of specificity for therapeutic delivery and synthetic immune surveillance trafficking.

Another great noninvasive tracking technique is Galas and Schmitz’s “footprinting” assay, which consists of using radical nucleic acid species to disclose protein–DNA interactions and establish high resolution mapping of RNA splicing patterns (Wang and Padgett, 1989). The technique has mostly been used on the bench but recent advances in chemistry and biophysics could allow these analyses to be remodeled for *in vivo* work.

The monitoring of nanodevices equally offers a lot of potential for *in vivo* imaging, as recently demonstrated by the use of CPMV-bound probes used in mammalian cell imaging (Koudelka et al., 2009; Lewis et al., 2006). Results from those studies revealed that high resolution intravital imaging can be accomplished through fluorescently-labeled viral particles to yield images of normal and tumor vasculature *in vivo* (Hafenstein and Fane, 2002). Similar approaches using RNA nanoparticles bound to numerous chimeric RNA building blocks for specific cell recognition and image detection have also been developed for molecular imaging (Guo, 2005a). This live monitoring of nanovector trafficking routes would help clarify a mix of immunological ponders. For instance, as of now, cell surface antigen distinguishing Foxp3+ Treg cells from activated effector cells have yet to be identified, and the differences between immune-suppression and immuno-modulation remains ambiguous. Nanodevices could clarify these topics by incorporating themselves in

tissue stem cells and tracking the developmental pathways of various cell lineages. Furthermore, SIS may help enhance macrophage polarization and provide answers to debates about the possibility of an extrathymically regulated Foxp3+ network, which would help in future discoveries of auto-immune disease origins.

10.1. Piezoelectric immunosensors

Noninvasive monitoring of nanovehicles in living cells has been greatly honed with Curies' piezoelectric effect. In general, piezoelectric biosensors work by measuring and recording changes in liquid viscosity, density, pressure, and ionic fluctuations (Suleiman and Guilbault, 1994; Ngeh-Ngwainbi and Suleiman, 1990). The use of piezoelectric technology in the field of enzyme chemistry has also been successful at revealing live protein interactions within mammalian systems. In a clinical setting, the coupling of nanovectors with piezoelectric probes presents unique applications for theranostics, as piezoelectric-probed vectors can help monitor physiological changes in blood pressure, arithmetic heart beats, and body temperature.

In the development of SIS, the use of piezoelectric sensors would be coupled with molecular engineering to give rise to bioactive nanovectors competent for therapeutic applications and event recording. Hypothetically, a carrier cell engineered to produce viral capsids could be injected in a patient to produce large amounts of viral-based nanovectors. Nano-scaled piezoelectrodes mounted onto antibodies would be incorporated in lipid-based vesicles and introduced in the bloodstream to be engulfed by genetically engineered carrier cells. While in the cytoplasm, the vesicles would unload the antibody-bound piezoelectrodes which will then bind to specific receptors found on viral coating proteins. This agglomeration would result in the intracellular assembly of functional piezoelectrode-equipped nanovehicles. These nanodevices would then be released from carrier cells into the bloodstream and lymphatic networks to both patrol and be localized in tissues such as in lymph and blood vessels to monitor physiological events. This self-sustaining system would provide an artificial network of continuously patrolling nanodevices adapted for molecular event recording, anomaly detection, drug delivery, and gene silencing (Fig. 3). The application of this technology is quite promising and will surely bring numerous therapeutic advantages to nanomedicine.

Several studies have also harnessed piezoelectric technology to serve as immunesensors conjugated to DNA and RNA aptamers (Ngeh-Ngwainbi and Suleiman, 1990; Tombelli et al., 2009; Konecny et al., 2006). In 2005, the immobilized piezoelectric biosensors from thiolated DNA probes were developed by Tombelli et al. (2005). More recently, a major improvement was recently made in the way we monitor viral capsids when a group of biophysicists, using dielectrophoretic impedance measurements, successfully detected norovirus capsids *in situ* (Nakano et al., 2012). The *in vivo* monitoring of nanovehicles presents huge potentials for data mining. Live tracking of nanovectors traffic routes in a living system would be a noninvasive approach towards the discovery of diseased tissue, cancer pathways, and tumorigenic signatures. Furthermore, by labeling pre-mRNA transcripts with appropriate piezoelectric tags, events occurring in the spliceosome can be more easily recognized and followed. This allows piezoelectric-probed vectors to be

localized and stationed in lymph and blood vessels to monitor molecular events. The use of iron oxide-based nanoparticles has been quite efficient for this task (Mok and Zhang, 2013). And as a consequence of their metal-based composition, they make excellent piezoelectric conductors. If used in concert with the aforementioned designs, classical molecular techniques, and tandem affinity purification assay (TAP) (Wang and Padgett, 1989), this technology could be particularly helpful in monitoring *in vivo* events.

11. Data mining and interpretation of molecular events

In the 1980s, a number of theoretical immunological concepts were discovered and their validations on the bench influenced many research approaches outside of the field. Before long, computer scientists proposed the implementation of immuneconcepts to computational programs. This resulted in the conception of artificial immune systems (AIS), softwares designed to metaphorically mimic events of the natural immune system and utilize them for computational processes (Timmis et al., 2008). Today, AIS have amassed large amounts of data about the immune system and have been practical for data mining, pattern recognition, and data analysis, therefore possessing the capacity, if appropriately remodeled, to translate biochemical signatures and predict nanovector performances in living systems.

In silico analysis tools will be paramount for the interpretation of *in vivo* molecular events. And computational search alignment techniques will be needed to anticipate potential physiological obstacles and to help map nanovehicles' traveling routes *in vivo*. Furthermore, the promising field of transcriptomics has greatly evolved in recent years and is serving to design gene-silencing transcripts with unprecedented stability and specificity to ligand sites (Azizgolshani et al., 2013; Pfaff-McDonough et al., 2000; Sukhanova et al., 2012). Kong and colleagues established the Nanominer database, which catalogs interaction outcomes of human transcriptome samples and various nanoparticles (Kong et al., 2013). Similar platforms, in concert with advanced metagenomic systems, genetic mapping, graph matching, and AIS mathematical algorithms should be employed collectively for *in vivo* mining and statistical analysis, and tailored to aid in the triage of nanovectors with potential in an artificial immune network. These technologies can also help us identify impending gaps in such artificial systems.

12. Future perspectives and five-year view

The introduction of foreign elements is systematically counterbalanced by natural immune defenses and remains one of the central obstacles of using nanodevices for *in vivo* therapeutic applications. Methods such as synthetic pseudotyping have been used to help enable nanodevices evade immune defenses but a lot of progress remains to be made in the field. Discher's "passport" system could perhaps be expanded to nanovehicles, which could in turn be coated with *self* molecules, such as CD47 and polyethylene glycol (PEG), to evade macrophage recognition and antigen-neutralizing defenses (Rodriguez et al., 2013). Biotoxicity is another major concern of nanovehicles. This is especially true for organometallic nanoparticles, which, while targeting diseased cells, can often cause disadvantageous effects to neighboring healthy cells. Despite this drawback, their rising

cost-efficiency and effectiveness in carcinogenic cells has allowed them to remain as reliable platforms for nanovehicle design.

Moreover, long-term efficacy of nanovectors *in vivo* is a central component for the development of SIS. What makes this exceptionally crucial, beyond its fundamentality for the proper performance of the system, is that the degradation of one of is functional arm can lead to the crumpling of the whole network. This concern is more pronounced in oligonucleotide-based vectors which have demonstrated great gene delivery capabilities in preclinical experiments but still possess a great deal of instability in physiological environments. Thus, development of nanomaterials capable of long-term trafficking and infiltration into cell compartments will be needed. Therefore, viral-based vectors still represent the most promising segment of nanovehicle technology, as they can be quite specific in their cell targeting. Their current uses in gene therapy continues to show great promise and viral-based vectors remain the most clinically trialed nanovehicle platforms. These nanovehicles do nonetheless continue to raise biosafety concerns due to their capacity to integrate mammalian genomes and their potential to revert back to virulence *in vivo*. The introduction of plant-infecting viruses is perhaps the best answer to these apprehensions, as they are virtually non-virulent in mammalian systems, possess fidelity of chromosomal site integration and can be readily engineered to evade immune defenses. Biosafety concerns surrounding viral-based vectors have been largely curbed in recent years and the introduction of alternative bioactive vehicles such as bacteria-based vectors, microbots, have been presented as alternative, efficient, cargo-delivering vehicles (Park et al., 2013; Jiang et al., 2013). The use of bacteria as nanovehicles, bactofection, is promising but quite nascent, thus more research will be needed to better expose the applicability of such bioactive nanovectors.

Lipid-based vesicles such as solid lipids, liposomes, vesosomes, and nanocells remain some of the most commonly used nanovehicles for *in vivo* therapeutic applications. What makes them particularly attractive is their marked enhanced uptake in target cells and their ease in infiltrating tumor cells or target tissue at large. Tocopherols, particularly vitamin E, for example, have shown exceptional promise as immune surveillance vehicles and are particularly promising as SIS vehicles due to their similarity to physiological molecules which enables them to be easily camouflage within the physiological environment (Nishina et al., 2008). Nonetheless, the majority of tocopherols can be easily detected and voided by the immune system and they are still possess great limitations due to their elevated biotoxicity in normal cells.

Polymeric-derived vectors such as dendrimers, functional monomers, and micelles are also rising as some of the most stable and target-specific nanovectors *in vivo*. Their synthesis has become quite cost-effective in recent years and their main advantage lies in their ability to enhance gene and drug transport across specific cells and tissues. Biocompatibility as seen in their marked reduced ability to be processed by physiological metabolic processes is likely to be their most paramount drawback in a SIS. This highlights the fact that in addition to finding the perfect vectors for the development of artificial immune systems, it will be equally important for us to develop ways to interpret the information these nanovehicles accumulate. In upcoming years, it will be crucial to remodel or develop technologies better

suited to interpret signals and messages delivered by nanovehicles in a SIS. The patterns of transcriptional events, the folding of proteins, RNAs and ribozymes interactions are key chemistries, which can confer a lot of molecular information to be used in this prospective clinical tool. Fig. 4 compares promising nanovehicles and their respective advantages and disadvantages in theranostic applications.

13. Conclusion

In the past several years, therapeutic nanovehicle concepts have traditionally been based on unilateral drug-delivery designs. In this review, we have highlighted several promising nanotechnologies and their potential for the development of a synthetic immune system. Oligonucleotide-based nanovehicles such as packaging RNAs and DNA-based vectors are quite promising, but their central drawbacks remain to be biostability and biosafety, as DNA-based nanotechnologies have the potential to be translated into inappropriate transcripts. We have however outlined the high instability of RNA-based vectors as an advantageous feature, which can be harnesses to confer information about subtle changes in physiological biochemistry. We emphasize that viral-based vectors perhaps present the most promising scaffold for future nanovector designs due to their innate biostability and complex nanoarchitecture which makes them ideal for transportation of therapeutic elements. The creation of synthetic immune surveillance will undoubtedly be challenging and will require sustained amalgamation of interdisciplinary technologies. Advances in chemistry, biology, medicine, physics, bioinformatics, and engineering should be constantly consolidated to give rise to these multifaceted artificial networks. It will be vital to frequently explore past discoveries and to investigate the development of future technologies to capture concepts with applicability for biocompatible nanovector designs and synthetic immunosurveillance systems.

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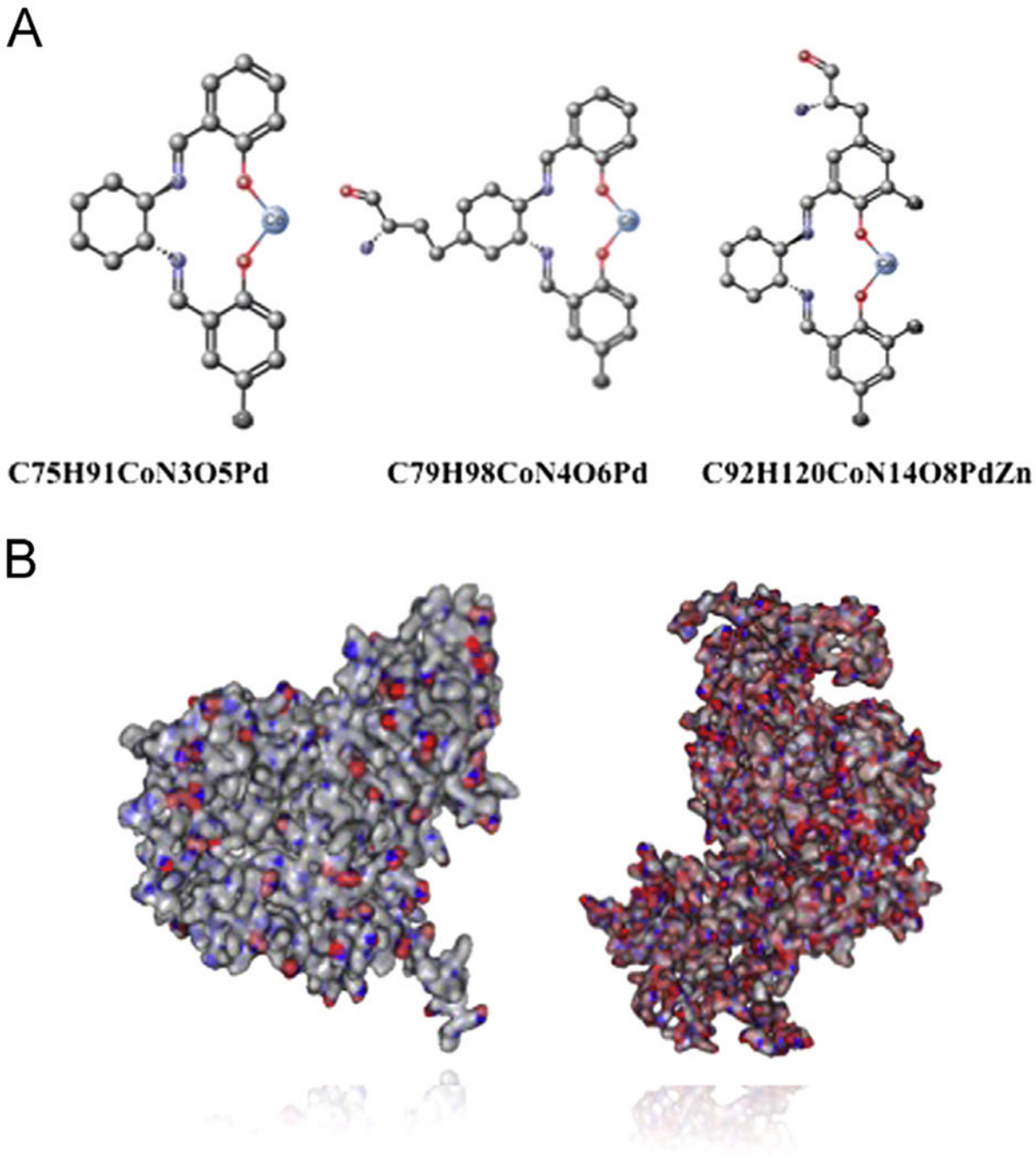

Fig. 1.

Nano-scaled elements with potential for nanovector assembly.(A) Isotype structures and formulas of three (1R,2R)-(−)-1,2-Cyclohexanediamino-N,N’-bis(3,5-di-t-butylsalicylidene)cobalt(II)-based organometallic chemicals with physiologically-favorable stability for nanovector assembly. (B) Electrostatic potential of two capsid proteins from plant-infecting and animal-infecting viruses with unique prospective as nanovehicle platforms. (Left) Cowpea Mosaic Virus (CPMV) capsid protein subunit; and (Right) Nanoporouscrystals of Chicken Embryo Lethal Orphan (CELO) Adenovirus Major Coat

Protein. Blue and red colors indicate positive and negative electrostatic potential, respectively. Protein ID obtained from Protein Data Bank (PDB).

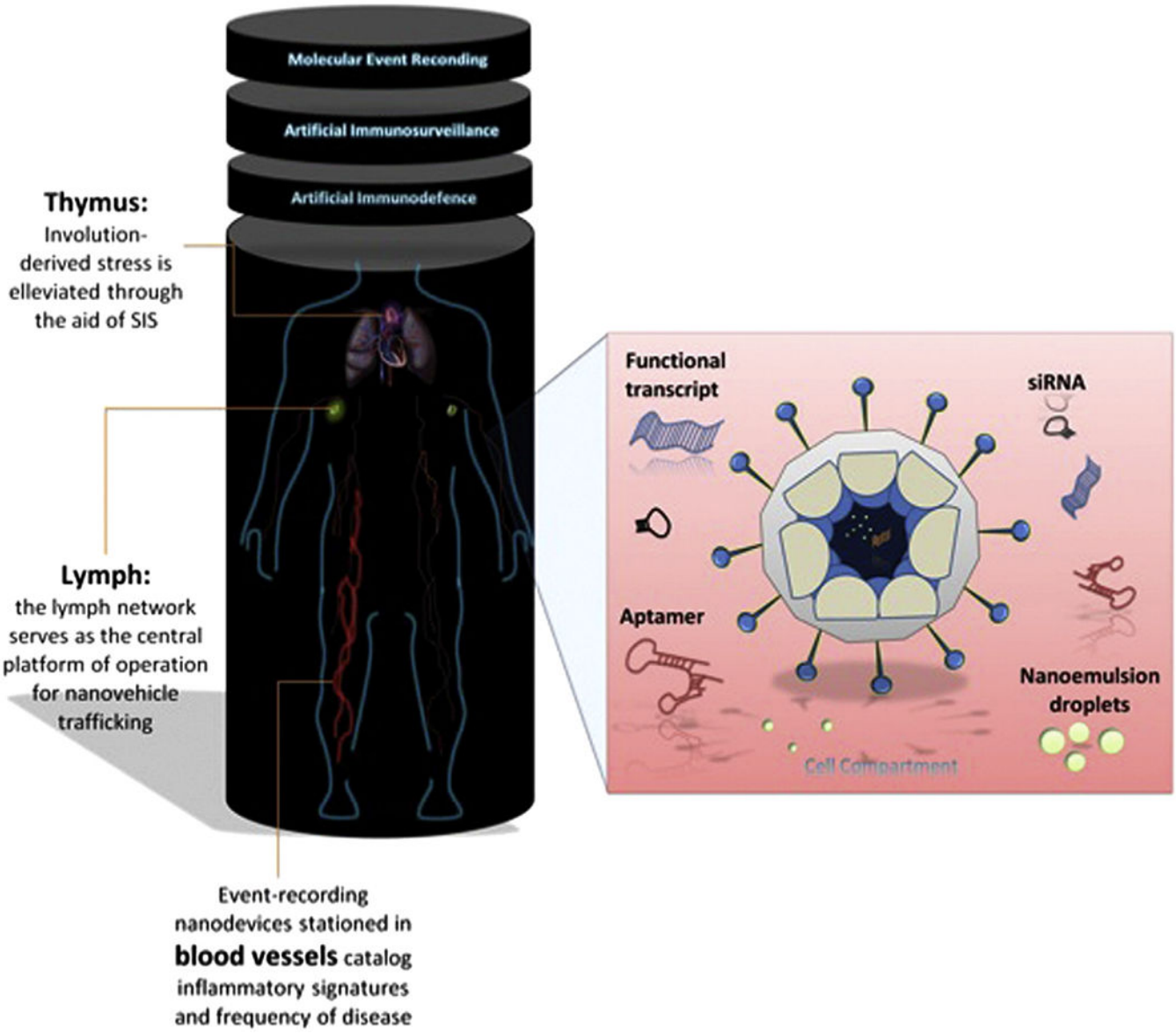

Fig. 2.

Illustration of an active synthetic immunosurveillance system (SIS) in an aging patient. (Top to bottom) Molecular event recording, artificial immune surveillance, and artificial immune defense represent the three pillars of SIS. Involution offsets T-cell production and leads to malignant growth-stimulated inflammatory response. Patrolling viral-based nanovehicles are equipped with RNA motors and pH-controlled nanochromophores to detect inflammatory signatures. The vehicles then migrate to cellular compartments via the lymphatic and blood networks to release functional transcripts, siRNAs, therapeutic aptamers, or chemotoxic nanoemulsion droplets. Probed transcripts are then released by the nanovehicles and dispersed into the cellular milieu to monitor molecular events and catalog disease frequency.

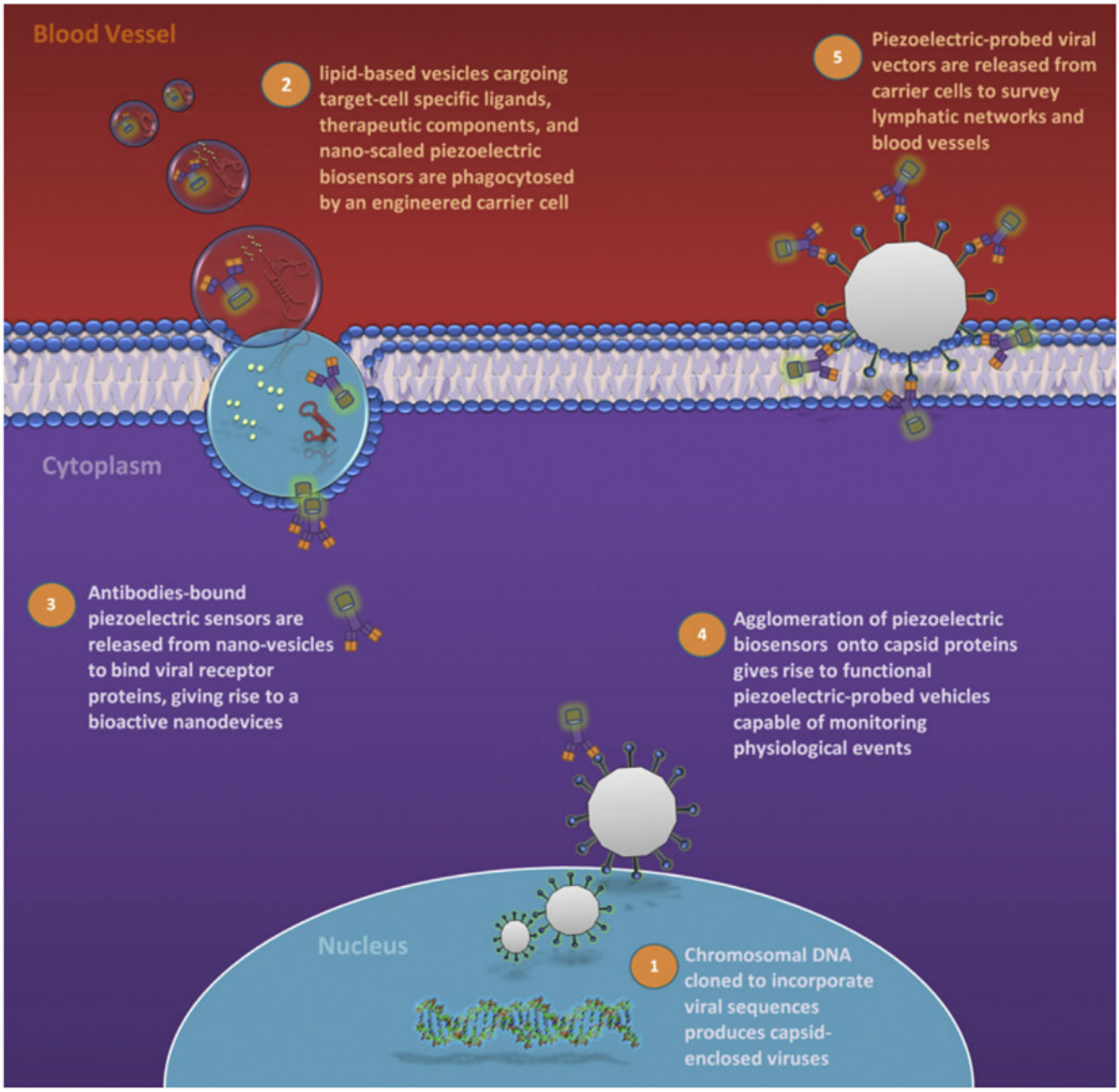

Fig. 3.

One arm of SIS will utilize lipid-based vesicles and viral vectors to carry artificial immune surveillance. A carrier cell engineered to produce viral capsids produces large amounts of viral-based vectors. Antibodies-bound nano piezoelectrodes are packaged inside lipid-based nano-vesicles and introduced into the bloodstream to be phagocytosed by a carrier cell. While in the cytoplasm, vesicles unleash nano piezoelectrodes, which bind specific viral coating receptors, resulting in the assembly of functional piezoelectrode-equipped nanovehicles inside the carrier cell. The assembled probed nanodevices are then released into the bloodstream to patrol and be incorporate inside blood and lymphatic networks.

Fig. 4.

Workflow of promising nanovehicles and their respective advantages and challenges in a SIS. (Clockwise) Viral and bacterial-based vectors perhaps represent the most promising arm of nanovehicle technology. Liposomes, solid lipids, and oligonucleotide based vectors could be readily incorporated into the immune defense arm of SIS. As well studied nanotechnologies, organometallic-based vectors provide a potent therapeutic component to nanovehicle designs. Dendrimers, functional monomers, and micelles are rising as some of the most stable and cell-specific nanovectors *in vivo*. Tocopherols, from which vitamin E is derived, have shown promise as immune surveillance vehicles.

Table 1

Cargo-delivering and clinically trialed nanovehicles with potential applicability in a synthetic immunosurveillance system (SIS).

Nanovector	Recent Use	System	Status	Stability	SIS application	Source
AMT-011	Adeno-associated viral vector encoding familial lipoprotein	Lipase deficient patients	Ongoing	N/A	Surveillance and artificial defense	Amsterdam molecular therapeutics
AntiPTEN-siRNA	Reduction of endogenous PTEN mRNA	HeLa cells	Bench	>70 °C	Artificial defense	Santel et al. (2006)
CCMV	Electrostatic interactions	*in vitro*	Bench	>59 °C/pH 5–7	Surveillance, artificial defense, and event recording	Konecny et al. (2006)
CPMV	Targeting tumor neovascular endothelium *in vivo*	*in vivo*: tumor neovascular endothelium	Bench	>60 °C	Surveillance. artificial defense, and event recording	Mateu 2011
KSHV	virus-activated cytotoxic therapy	HIV patients	Complete	pH 3–4	Surveillance, artificial defense, event recording	Uldrick et al. (2011)
M13 viron	Viron –mediated phage display	*in vitro*	Bench	>60 °C/pH 6.7–8	Surveillance, event recording	Paschke (2006)
Magnetite	Therapeutic delivery; tumor imaging	*in vivo*	Phase 3 trials	>50 °C	Artificial defense and event recording	Tareen and Krishnamurthy (1981), Douziech-Eyrolles et al. (2007)
MCH	Functional pH-responsive monomer	*in vitro* nano-assemblies	Bench	>pH 7.4, 37 °C	Surveillance, artificial defense, event recording	Mastrotto et al. (2013)
MVA	Modified vaccinia Ankara for malaria prevention	Healthy persons	Complete	<4 °C, >37 °C/pH 7.4	Surveillance and artificial defense	University of Oxford
OncoVEXGM-CSF	Oncolytic viral vector	Malignant melanoma patients	Complete	N/A	Surveillance and artificial defense	BioVex limited
ONYX-015	Antitumor adenovirus	Head and neck cancers	Ongoing	>25 °C	Surveillance and artificial defense	Rogulski et al. (2000), Makower et al. (2003)
pJL-TRBO-G	TMV vector tailored for gene delivery	Plant systems	Bench	N/A	Surveillance, artificial defense, event recording	Lindbo (2007)
pMC.ApoE.hFIX	Minicircle DNA vector	Quiescent cells and tissues	Bench	>37 °C	Surveillance and artificial defense	Kay et al. (2010)
pTRAERH H5tr	Plant vectors encoding haemagglutinin (HA) surface glycoprotein gene	Mice, chicken and plants	Bench	>37 °C	Surveillance, artificial defense, event recording	Mortimer et al. (2012)
rAAV2.REP1	Adeno-associated Vector encoding rab-escort protein 1	Choroideraemia patients	Phase 1	N/A	Artificial defense	University of Oxford
RCNMV	Therapeutic cargo	HeLa cells	Bench	>59 °C/pH 5–7	Surveillance, artificial defense, and event recording	Lockney et al. (2010)
RRE decoy	HIV-1 inhibition	*in vivo*	Complete	>37 °C	Surveillance and artificial defense	Lee et al. (1994)
SUV	Lipid nanoparticle-delivering siRNAs	*in vitro*	Bench	>50 °C	Artificial defense and event recording	Sahay et al., 2013

Nanovector	Recent Use	System	Status	Stability	SIS application	Source
TMV	Therapeutic protein expression	*in vitro*	Bench	>90 °C	Surveillance, artificial defense, and event recording	Pansri et al. (2009)
Tat/Rev shRNA	RNA therapeutic vehicle	*in vivo*	Phase 1	N/A	Artificial defense	City of hope/benitec
VCSM13 helper phage	Viral gene amplification disruption	*in vitro*	Bench	N/A	Surveillance, artificial defense, event recording	Paschke (2006), Pansri et al. (2009)
Vitamin E	siRNA delivery in liver	*in vivo*	Bench	>30 °C/pH 7.4	Artificial defense	Nishina et al. (2008)
φ29 pRNA	Gene delivery and nanomaterial assembly	*in vitro*	Bench	N/A	Surveillance, artificial defense, and event recording	Shu et al. (2011)

N/A=Not available; information about clinically trialed vectors were obtained from NIH/clinicaltrails.gov.

Table 2

Base mutagenesis of RNA species yielding to exonuclease-resistance and higher melting point.

RNA species	Modification	Synthesis	References
Aptamer – CD4	5′UCAGACAGAGCAGAAACGACAGUUCAAGCCGAA3′	SELEX-based	Douziech-Eyrolles et al. (2007)
Aptamer – VEGF	5′ CGGAAUCAGUGAAUGCUUAUACAUCCG3′		
Aptamer – WT1	5′ GAUAUGGUGACCACCCCGGC 3′		
Aptamer – β-catenin	5′ GGACGCGUGGUACCAGGCCGAUCUAUGGACGCUAUAGGCACACC 3′ 5′GGAUACUUUAACGAUUGGCUAAGCUUCCGCGGGGAUC3′		
Gap-mer – GEM231	5′ G *C *G *UGCCTCCTCACU *G *G *C *3′	Oligonucleotide mixed backbone; 3′-endo sugar conformation	Allerson et al. (2005)
Gap-mer – GEM-231	5′ G *C *G *G *GCCTCCTCACU *G *G *C *3′	Deixynucleotide substitution by 2′ o-methylribonucleotides	Allerson et al. (2005)
RNA species	3′ C *G *G *C *U *ACACAGAUAACUUC U AP 5′	Silyl ethers-mediated modifications	Wang et al. (2008)
siRNA – antiPTEN 10	5′ GGGUAAAUACAUUCUUCAU 3′ 3′ CCCAUUUAUGUAAGAAGUA5′	2′ Oligopeptide modifications	Allerson et al. (2005)
siRNA – antiPTEN 11	5′ P *GGGUAAAUACAUUCUUCAU 3′ 3′ CCCAUUUAUGUAAGAAGUA *P 5′	2′ Oligopeptide modifications	Allerson et al. (2005)
siRNA – antiPTEN 12	5′ GGGA *AAG *UAU *C *UUG *UUU *AU 3′ 3′ CCCU *UUC *AUA *G *AAC *AAA *UA 5′	2′ Oligopeptide modifications	Allerson et al. (2005)
siRNA – antiPTEN 5	5′ AAGC *AAC *GAGAAGCGAU *AA 3′ UUCG *UUG *CUC *UUCG *UA *UU 5′	2′ Oligopeptide modifications	Allerson et al. (2005)
siRNA – APOB	5′ GUCAUCACACUGAAUACCAAU GC U GG *A 3′ 3′ C *A *CAGUAGUGUGACUUAUGGUUA *CG *A *CC U-Toc 5′	α-Tocopherol phosphoramidite conjugation to siRNA antisense 5′ end	Shen et al. (2005)
siRNA – GL3	5′ CUUACGCUGAGUACUUCGATT 3′ 3′ TTGAAUGCGACUCAUGAAGCU 5′	2′-F pyrimidines-mediated modification	Layzer et al. (2004)
siRNA – SOD1	5′ C *G *A *UGUGUCUAUUGAAG *A *U *U *C 3′	Silyl ethers-mediated modifications	Wang et al. (2008)
siRNA – VEGFR-1	5′ B-CUGAGUUUAAAAGGCACCCTT-B 3′ 3′ T *TGACUCAAAUUUUCCGUGGG 5′	Complementary sense and antisense single strands annealation, desalted, and lyophilized	Shen et al. (2005)
T4 RNA hairpins	5′ A *U *A *GGAGC *UUCG *G *C *U *CCUAU 3′	Thermal denaturation	Tuerk et al. (1988)

*Nucleic acid base substitution.