Healthy Ageing and Longevity 13
Series Editor: Suresh I. S. Rattan

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Systemic Delivery Technologies in Anti-Aging Medicine: Methods and Applications

Springer

Healthy Ageing and Longevity

Volume 13

Series Editor

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Rapidly changing demographics worldwide towards increased proportion of the elderly in the population and increased life-expectancy have brought the issues, such as "why we grow old", "how we grow old", "how long can we live", "how to maintain health", "how to prevent and treat diseases in old age", "what are the future perspectives for healthy ageing and longevity" and so on, in the centre stage of scientific, social, political, and economic arena. Although the descriptive aspects of ageing are now well established at the level of species, populations, individuals, and within an individual at the tissue, cell and molecular levels, the implications of such detailed understanding with respect to the aim of achieving healthy ageing and longevity are ever-changing and challenging issues. This continuing success of gerontology, and especially of biogerontology, is attracting the attention of both the well established academicians and the younger generation of students and researchers in biology, medicine, bioinformatics, bioeconomy, sports science, and nutritional sciences, along with sociologists, psychologists, politicians, public health experts, and health-care industry including cosmeceutical-, food-, and lifestyle-industry. Books in this series will cover the topics related to the issues of healthy ageing and longevity. This series will provide not only the exhaustive reviews of the established body of knowledge, but also will give a critical evaluation of the ongoing research and development with respect to theoretical and evidence-based practical and ethical aspects of interventions towards maintaining, recovering and enhancing health and longevity.

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ISSN 2199-9007 ISSN 2199-9015 (electronic)
Healthy Ageing and Longevity
ISBN 978-3-030-54489-8 ISBN 978-3-030-54490-4 (eBook)
https://doi.org/10.1007/978-3-030-54490-4

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Aging is a process associated with progressive accumulation of deleterious changes (ranging from cell senescence to a decline in immune functioning and hormonal secretion) over time, leading to a loss of function in multiple tissues and an increase in the probability of death. Over the years, extensive efforts have been paid by researchers to deciphering the mechanisms of aging and treating different types of age-associated diseases. The possible link between aging and diverse physiological processes (including telomere shortening, accumulation of free-radical mediated tissue damage, and random cross-linking among biomolecules) has also begun to be recognized. Such an understanding of aging has been further enhanced recently by rapid advances in computational technologies, which make the attainment of genome-wide expression profiles of diverse tissues from individuals of different ages possible. This greatly enhances the efficiency of the identification of age-related genes, and has led to the discovery of copious potential targets that may subsequently be used for manipulation of the aging network via both genetic and non-genetic means.

The gap between basic aging research and intervention development is a major obstacle that has to be overcome before biogerontological interventions can be put into practice. Regarding the fact that aging is a systemic degenerative process, the availability of technologies that enable cells and tissues in a fully developed adult body to be manipulated systemically are in dire need. As far as cell and tissue manipulation is concerned, delivery technologies find great importance because they are the ones that enable the initiators of biological effects to get to the proper site of action. Since the turn of the last century, significant advances have been achieved in the design of delivery technologies. Advances in the development of delivery systems, along with the possibility of achieving spatialtemporal confinement of intervention execution via proper carrier design, have opened up new possibilities for the attainment of interventions to tackle a range of diseases, ranging from cancer and cardiovascular diseases to neurodegeneration and diabetes

mellitus. Despite this, the delivery efficiency of most of the existing technologies varies from tissue to tissue. This impedes the successful implementation of interventions that require cells or tissues to be manipulated bodywide.

Regarding the importance of systemic delivery technologies in the development and execution of anti-aging interventions, and the lack of books and serious discussions currently available in the field on this important topic, this edited book intends to fill this gap by comprehensively revisiting the latest advances in the chemistry and engineering of technologies for systemic therapeutics delivery, with the strengths and limitations of those technologies being explored in the context of anti-aging medicine. The content of this book is separated into six part. Part I offers an overview of the need of systemic delivery technologies to the development of anti-aging therapies, and also provides an introduction to representative experimental approaches that will be required when a technology is designed and characterized to meet the need of systemic therapeutics delivery. In Part II, III, and IV, recent advances in different strategies that may enable systemic delivery to tackle aging and related diseases will be presented. Representative practical strategies to engineer and optimize the performance of delivery technologies for applications in systemic delivery, along with their working principles, will be discussed in Part V; whereas in the last part, major technical and biological barriers that have to be overcome will be presented for the transition of delivery technologies from the laboratory to reality for applications in systemic delivery to tackle aging and age-associated diseases.

Contrary to other edited books which are presented simply as a collection of reviews that target advanced researchers, this edited book contains several special features, making it suitable not only to those familiar with the field but also to readers who are relatively new to this research area. One feature is the “Glossary” section provided in each chapter. It intends to make the content of each chapter more accessible to readers who may not be that familiar with the terminology and abbreviations used in the field. Another feature is the “Important Notes”, which concisely convey to readers the take-home-messages and recent advances in the area to be covered by the chapter. Finally, at the end of each chapter, this is a “Questions for Future Research” section, which delineates some of the important yet unsolved questions to be addressed for future research. Because of the multidisciplinary nature of the topic covered by this edited book, our book is anticipated to be an appeal to advanced undergraduate- and graduate-level students training in pharmaceutical sciences and geriatric medicine, and those with an interest in the design and development of delivery technologies.

Here I would like to thank all of the authors who have contributed chapters to this publication. We are grateful to them not only because of their support and efforts, but also because of their responsiveness and patience to our editing. A number of people have reviewed chapters of this book. We want to acknowledge

all of them for their generous participation. Thanks are extended to Mr. Eric M. Huang from the Hong Kong Polytechnic University for his administrative assistance during the editing of this book. Copious figures presented in this book have been adapted from published articles. The authors and publishers, which have granted the permission for reprinting these materials, are acknowledged.

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Part I
Fundamentals and Experimental Techniques in Systemic Delivery

Chapter 1
Systemic Delivery in Anti-aging Medicine: An Overview

Yi Wang and Wing-Fu Lai

Abstract Extension of longevity is no longer a science fiction and has been in progress of becoming scientifically achievable. The focus of current anti-aging strategies has shifted from geriatrics that is cost-effective but palliative to biogerontology that is fundamentally at the molecular level. Basic gerontological research has suggested that biological aging is closely associated with genetic/genomic factors, which has led to the development of gene therapies such as RNA-interference technology. This resulted in a subsequent need for developing reliable drug delivery systems. Numerous advanced systemic drug delivery systems have hitherto been developed but a number of challenges need to be conquered in order to make the systems practical, such as safety and effectiveness issues. For this reason, a considerable number of biogerontological intervention technologies have been taken to clinical trials but with limited success. As the first chapter of this book, this chapter will illustrate the role and limitations of technologies for systemic delivery in anti-aging medicine.

Keywords Anti-aging · Biogerontology · Drug delivery · Nanoparticles · RNA interference

1.1 Introduction

Prolongation of longevity has been a history-long desire of mankind since ancient time. Ancient Chinese emperors and European alchemists had been chasing after

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W.-F. Lai (ed.), *Systemic Delivery Technologies in Anti-Aging Medicine: Methods and Applications*, Healthy Ageing and Longevity 13,
https://doi.org/10.1007/978-3-030-54490-4_1

extension of longevity and even immortality (Lai 2013). In general cognition, however, lifespan is an impregnable natural trait and any attempts on intervention or reversion are doomed to fail. While scientists have shown enormous dedication and enthusiasm in the intervention of this natural process, and numerous encouraging progresses have been achieved (Lai 2011, 2013 ; Pazoki Toroudi et al. 2016), a practical approach to "stop the clock" is beyond reach, thus far. On the other hand, attributed to better living conditions and effective geriatric care, human lifespan was considerably extended over the last two centuries. The average life expectancy of babies bone in the twenty-first century was projected to be over a hundred years in countries such as Japan, Canada, France, etc. reported by Christensen et al. (2009).

Biological aging is often accompanied by an increasing range of age-related diseases (Guo et al. 2020). It is a time-dependent degenerative process of cells, tissues and organs, resulting in impairment of their structures and functions and eventually deterioration of health (Pazoki Toroudi et al. 2016; Kume et al. 2010). The aging process was found to be associated with extrinsic factors (such as environmental factors and oxidative stress) as well as intrinsic factors (such as metabolic pathways and genetic elements) (Herskind et al. 1996; Skytthe et al. 2003; Hjelmborg et al. 2006; Slagboom et al. 2011). Examples of these factors include, but not limited to, **mammalian target of rapamycin** (mTOR) which is a serine/threonine protein kinase that plays a role in controlling cell growth and proliferation (Lian et al. 2008; Sheaffer et al. 2008); insulin/insulin-like growth factor-1 signalling pathway which regulates the resistance to oxidative stress (Holzenberger et al. 2003); systemic chronic low-grade inflammation which contributes to age-related morbidities (Marengoni et al. 2009; Custodero et al. 2018). In addition, current aging research focuses extensively on improving health by developing novel therapeutic strategies against cellular senescence at molecular and cellular levels (Pazoki Toroudi et al. 2016).

1.1.1 A Three-Level Strategy for Anti-Aging Approaches

In order to achieve longevity extension, a three-level strategy for anti-aging has been proposed (as shown in Fig. 1.1) (Lai 2013). The first level is the pathological level which focuses on geriatric approaches. It aims to alleviate or eliminate geriatric symptoms after an age-related disease is diagnosed (Weinstein 1990). It is, therefore, a passive and palliative approach. In contrast, gerontological and engineering approaches that are preventive and proactive are more cost-effective and humane (Weinstein 1990). Therefore, they are comparatively desirable and draw more research interests. Nevertheless, the basic **biogerontology** had been progressing rather slowly in the last two decades due to an extensive focus on the development of geriatric over the last two centuries (Binstock 2003).

The second level is the molecular level, in which gerontological approaches are developed. The fundamental concept of the gerontological approach is established on genetical manipulation of metabolic pathways in order to retard aging and enhance longevity at a molecular level (Lai 2013). Techniques have been developed based on the establishment of a library of candidate genes associated with the aging

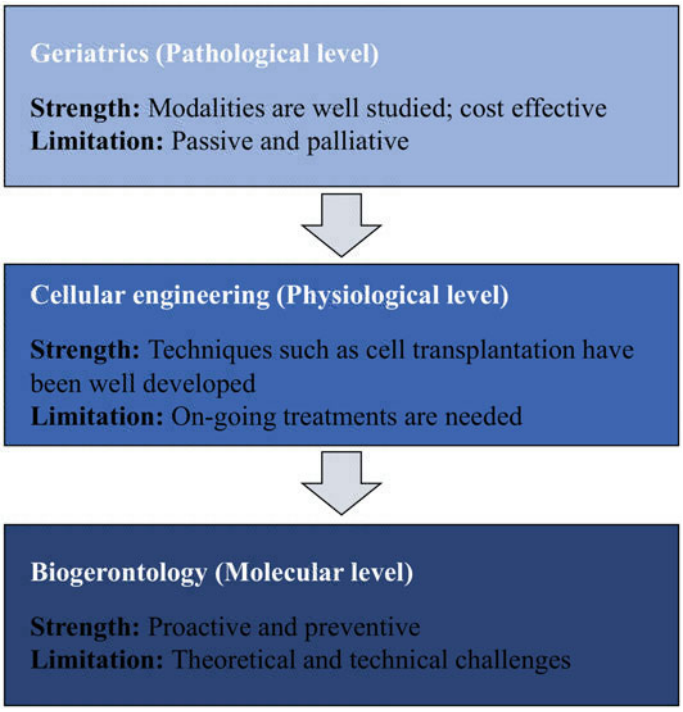

Fig. 1.1 A three-levelled anti-aging strategy

process (Swindell 2007; Kim et al. 2012). Animal models such as mice and fruit flies are often used in studies identifying candidate genes and developing intervention approaches due to their higher birth rate and shorter lifespan (as compared to humans). This makes the experiments comparatively affordable in terms of time, funds, and potential ethical concerns (Kaeberlein 2007). Moreover, eukaryote models (such as *Caenorhabditis elegans*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, etc.) are also popular experiment subjects due to their simplicity in biological structures (Kaeberlein 2007). For instance, the lifespan of fruit flies was successfully extended (while maintaining their fecundity and locomotor activity) by overexpression of the *D-GADD45* gene in their nervous system (which improves the efficiency of recognition and repairs damaged DNA) (Plyusnina et al. 2011). Recently, gene sirtuin 6 was found to be associated with an enhanced lifespan of mice due to its function in efficient DNA repairment (Tian et al. 2019). In these models, the feasibility of lifespan extension by transgenic manipulation has been corroborated, which has taken anti-aging research to the next level. However, due to the complexity of gerontological interventions, clinical applications are still yet to be feasible. Also, despite the practical plausibility of genetic manipulation, the physiological costs in genetic modulation in long term have not been completely revealed (Lai 2013).

The third level is the physiological level which involves cellular engineering approaches. In contrast to the gerontological approach that focuses on genetic manipulation, the cellular engineering approach is considerably straightforward and focuses on the physiological level. It aims at reversing diagnosed age-related damages, such as extracellular cross-links, mitochondrial mutations, nuclear mutations, cell senescence, lysosomal aggregates, etc. at a cellular level (de Grey et al. 2002). The ultimate goal of this approach is to ameliorate the damage to a threshold where pathological events can be controlled or eliminated (de Grey et al. 2002).

One of the cellular engineering approaches that has made significant progress clinically over the years is the cell transplantation technique (Fratino et al. 2013). For instance, replacement of disease-causing stem cells with normal ones by hematopoietic cell transplantation technology has been a successful clinical practice over the last four decades (Czechowicz and Weissman 2011). Cell transplantation has also been used to treat Parkinson's disease (a nervous system disease involving a progressive loss of dopamine neurons in the substantia nigra pars compacta) by inducing pluripotent cells derived from somatic cells (Chen et al. 2011). These examples illustrated the feasibility of the application of cellular engineering approaches in treating cell loss and tissue atrophy. Moreover, it was reported by Wang et al. that the phenotype of senescence (such as lowered basal cyclin-dependent kinase activity and ^{3}H-thymidine incorporation, and increased senescence-associated β-galactosidase activity) of IDH4 human fibroblasts could be rejuvenated by overexpression of *HuR* (an ubiquitously expressed *Elav*-like RNA-binding protein) (Wang et al. 2001). This study evidenced that reversion of aging and senescent phenotypes are potentially achievable at the physiological level.

Despite the possibility of manipulating the aging process at different levels, debates and secular challenges have been raised for various concerns. The primary argument concerns the necessity of developing biogerontology and engineering technologies when geriatric approaches are deemed to be more feasible and practical (Lai 2013). This resulted in the hesitancy of funding agencies to invest in anti-aging research projects (de Grey 2003). Secondly, political and society concerns would further impact funding opportunities due to society issues such as overpopulation and population aging (de Grey 2003). In aspects of biomedicine, for some genetic diseases/disorders such as Hutchinson–Gilford progeria syndrome and Huntington's disease, there is no actual curative therapy other than gene editing for embryos, which, however, is unacceptable ethically and legislatively (Li et al. 2019). For these reasons, some argued that anti-aging research is considered unjustified.

1.2 Translation of Basic Research: From Bench to Clinic

In addition to the difficulties in basic research for aging intervention, the translation of basic research into clinical practice has been another major challenge. Multiple steps are involved in the translation process (Lai 2013), including (1) basic

genetic/genomic research for identification of aging-associated genes and their functions; (2) mechanistic research on genetic/genomic manipulation of aging-associated genes; (3) development of delivery systems for therapeutic agents; and (4) clinical verifications of the previous steps. The entire process requires enormous efforts and time and involves numerous challenges which will be discussed in detail.

1.2.1 Basic Genetic/Genomic Research

The association of genetic elements and longevity was first revealed in 1988 by Friedman and Johnson (Friedman and Johnson 1988) who discovered the first gene that alters the lifespan of *Caenorhabditis elegans*. The study demonstrated a life extension of up to 65% due to the mutation of this gene. The gene was named *age-1* in the expectation that more genes affecting longevity would be found. Other examples of lifespan-associated genes include *AGTR1*, *sir-2.1*, *hcf-1*, *smk-1*, *daf-16*, etc.(Wolff et al. 2006; Rizki et al. 2011; Benigni et al. 2013), and some of which have been extensively studied for their functions and manipulation strategies (Dali-Youcef et al. 2007; Landis and Murphy 2010; Satoh et al. 2011).

In the last two decades, the computational approach has been used extensively to screen longevity-associated genes. Genome-wide transcriptional and expressional profiles of different tissues such as brain (Hong et al. 2008), skeletal muscles (Zahn et al. 2006), kidney (Rodwell et al. 2004), etc. examined by gene expression profiling and DNA microarray have been reported on individuals at various ages (Magic et al. 2007; Subramanian et al. 2005). The development of genome-wide analysis has taken the basic research on aging-associated genes to the next level. Seong et al. examined the lifespan of fruit flies undergone radiation-induced alterations in genomic expression and analyzed the genes underlying longevity extension (Seong et al. 2011). A group of genes were recognized to be responsible for the prolongation of lifespan, such as cytochrome-related genes (*Cyp1*, *Cyp4d21*, *Cyp4p3*, *Cyp6a9*, *Cyp6g1* and *Cyp318a1*), genes relating to protein turnover and ubiquitination pathways (*CG2924*, *CG7220*, *crl*, *neur*, *Roc1b*, *Ubc84D* and *Ubc-E2H*), and genes responding to oxidative stress (*GstS1*, *Jon65Ai*, *Jon65Aiv*, *Jon66Ci*, *Jon99Ci*, *Jon99Cii*, *mmd*, *Trxr-1* and *Trxr-2*).These candidate genes are worth further investigation for their roles in metabolic pathways and potential intervention approaches.

Another advanced technology, **genome-wide association studies** (GWAS), have also been widely used for genetic mapping. With the advances of single nucleotide polymorphism (SNP) genotyping technologies, GWAS are an effective approach genotyping hundreds of thousands of SNPs across the entire genome in one go and identifying new susceptibility genes with the phenotype of interest (Kronenberg 2008). This superiority makes GWAS a powerful tool to identify genetic contributors of aging-associated phenotypes. Deelen et al. applied GWAS on analyzing the genomic data of a large group of genetically independent longevous individuals and discovered that a gene *POT1* plays an important role in the telomere maintenance pathway and in turn the longevity (Deelen et al. 2013). They also found that the

influence of the insulin/insulin-like growth factor-1 signalling pathway on lifespan is associated with a group of genes including *AKT3*, *AKT1*, *FOXO4*, *IGF2*, *INS*, *PIK3CA*, *SGK*, *SGK2*, *YWHAG* and *POT1*. As compared to candidate gene studies, the application of GWAS could be limited by its current drawbacks such as poor reproducibility and false positive results. An unneglectable advantage of GWAS, on the other hand, is that it requires no prior assumption in respect to gene functions in contrast to the hypothesis-driven approaches (Kronenberg 2008). Despite the fact that GWAS have currently been used more often in studies of age-associated diseases (such as cancer, diabetes, atherosclerosis, etc.) rather than the process of aging itself (Kronenberg 2008), it is believed that GWAS have hitherto been one of the most effective tools for anti-aging research.

1.2.2 Mechanistic Research on Genetic/Genomic Manipulation

As the fact that biological aging is associated with genetic elements has been corroborated, mechanistic research on gerontological interventions should therefore not passively rely on palliative disease-oriented studies but on active and systemic approaches such as genetic/genomic manipulation. For example, the traditional treatments for lysosomal storage diseases (which are genetically determined metabolic disorders characterized by defective lysosomal enzymes causing an age-associated systemic accumulation of lysosomal aggregate) include enzyme replacement therapy (Lachmann 2011) and substrate reduction therapy (Cox 2005), which are essentially palliative. A novel gene therapy that regulates the expression of lysosomal enzymes by administrating encoding nucleic acids (Eto and Ohashi 2000; Sands and Davidson 2006), on the other hand, offers a rather curative approach.

Present genetic/genomic manipulation research for anti-aging strategies focus extensively on the **RNA-interference (RNAi) technology** (Xue et al. 2015). RNAi is a pivotal biomolecular means to regulate target gene expression (Xue et al. 2015). RNAi agents such as small-interfering RNA (siRNA), micro-RNA (miRNA) and PIWI interacting RNA (piRNA) are potential therapeutic agents for the treatment of a broad range of aging-related diseases that are associated with undesired gene expression, such as cancer, neurological diseases, autoimmune diseases, etc. (Fire et al. 1998). Small non-coding RNA molecules have been shown to mediate target gene expression in various approaches, most notably via the silencing of undesired genes by inducing mRNA degradation in cytoplasm, which subsequently supresses the expression of the corresponding proteins (Kumar and Clarke 2007). The mechanism of RNAi was first discovered by Fire et al. (1998) who reported a systemic suppression of *unc22* gene in *Caenorhabditis elegans* induced by an exogenous double-strand RNA molecule. This technology has later been used in longevity research. Bernardes

de Jesus et al. (2012) illuminated that the induction of mouse telomerase reverse transcriptase cDNA which regulates the telomerase gene has successfully prolonged the lifespan of mice by up to 24% without an increased risk of cancer.

Accounting the superior customizability of RNAi agents and the possibility of silencing almost any gene in a convenient and moderately specific manner (Xue et al. 2015), the biomedical application of RNAi has been extensively studied and reviewed by researchers and clinicians. Nowadays, RNAi technology has already become a standard experimental tool for validating gene functions (Mansoori et al. 2014). Currently, taking the advantage of numerous in vitro and in vivo data supporting the therapeutic potential of RNAi (Musacchio and Torchilin 2013; Gavrilov and Saltzman 2012), the focus of research has shifted to clinical applications. In recent years, a number of clinical trials of RNAi therapeutics especially siRNA have been conducted, with, however, limited success so far (Xue et al. 2015).

Another promising gene therapy technology is site-specific genomic integration. An example of this technology is the ΦC31 integrase system, which is a phage-derived system mediating the integration of plasmids bearing an *attB* site into pseudo-*attP* sites of the genomes of humans and mice (Thyagarajan et al. 2001; Karow and Calos 2011). This system, however, possesses risks for DNA damage and chromosomal rearrangements in mammalian cells (Ehrhardt et al. 2006; Liu et al. 2009). Site-specific genomic integration was used by Howden et al. (2008) to co-transfect a plasmid containing the P5 integration efficiency element derived from adeno-associated viruses, and mRNAs coding for Rep68/78 proteins. Gersbach et al. (Gersbach et al. 2011) inserted recombinase target site throughout the genome using *piggyBac* transposase, and then integrated plasmids into those sites and multiple transposons with an engineered zinc-finger recombinase. The site-specific genomic integration technology, together with inducible gene expression systems (that precisely control the expression of transgenes by bioactive compounds (Centlivre et al. 2010; Weyler and Morschhauser 2012)), radiation (Ito et al. 2001) or heat (Tang et al. 2008), has taken the development of gene therapy to the next level.

1.3 Importance of Systemic Delivery to Anti-Aging Medicine

With the advances in genetic manipulation technologies, silencing or overexpressing specific genes is no longer a technical difficulty at the cellular level. A significant challenge, however, lies in the delivery of therapeutic agents to somatic cells body-wide. For instance, in order to perform genetic manipulation, delivery of therapeutic nucleic acids is often required. Naked nucleic acid molecules are unstable and can be degraded shortly after administration (Musacchio and Torchilin 2013; Gavrilov and Saltzman 2012; Seth et al. 2012). Also, nucleic acid molecules may increase the risk of triggering immunogenic response (Ma et al. 2005; Draz et al. 2014). Taking the aforementioned lysosomal storage diseases for example (Eto and Ohashi 2000; Sands and Davidson 2006). Although gene therapy can be applied in a localized

(ex vivo) or a systemic (in vivo) manner, the localized treatment is carried out using macrophages obtained from cultured cells and can only be applied to tissues in which cell transplantation is viable. To genuinely tackle the disease, therapeutic nucleic acids which generate a persistent endogenous reservoir for lysosomal enzymes are needed. Also, the nucleic acids need to be delivered body-wide so as to offer a one-off solution to the disease. With advances in genetic engineering, synthesis of plasmid encoding the enzyme is no longer technically challenging, and the development of systemic delivery approaches has become the key to success.

As genetic manipulation is designed to be a treatment across the whole body, systemic delivery systems have to be well developed before the technology can be moved on to clinical trials. In fact, delivery systems have been developed rapidly in recent years mainly by investigating their therapeutic potential in various in vivo models. For instance, Zamora-Avila et al. (2009) reported that with aerosol delivery of poly(ethylenimine) loaded with RNAi agents that silence Wilms' tumour gene 1 in mice with B16F10 lung metastasis, the number and size of the tumour foci were reduced and the mean survival time of the mice was extended. Dar et al. (2015) used lipid-based nanoparticles as a carrier to deliver siRNAs to silence *ErbB2* and *AURKB* genes in mice with tumours. An improved cellular uptake by the tumour tissues and the subsequent tumour suppression were observed upon the application of the carrier. A safe and effective delivery approach plays an important role in interventive biogerontology and treatments of pathologies (Odom et al. 2007; Yannaki et al. 2010). Using delivery systems in interventive biogerontology has a number of advantages, including (1) low immunogenicity due to inertness and a small particle size; (2) easy access to the blood stream and crossing other cellular barriers due to a small particle size; (3) an enhanced circulation time that allows them to penetrate and accumulate in cells more efficiently; (4) readiness for tracking and imaging in some cases; (5) stimulation of interferon production and enhancement of natural killer cells resulting in an activation of anti-tumour activity, in cases of anti-cancer therapies (Tatiparti et al. 2017). The most frequently studied drug delivery systems are listed in Table 1.1. Although further optimization and manipulation are still required, many of these systems have already shown great potential to enhance the efficiency and bioavailability of therapeutic agents in tackling diseases.

1.3.1 Viral Nanoparticles

Viral nanoparticles (VNPs) are composed primarily of proteins and are thereby known for their innate biocompatibility, biodegradability, the ability to cross cellular barriers and effective delivery of cargo in a systemic manner (Huang et al. 2011). Viruses have evolved naturally to deliver nucleic acids and can therefore be subverted for the delivery of other molecules (Koudelka et al. 2015). VNPs can function as prefabricated nano-scaffolds with unique properties and can easily be modified (Huang et al. 2011). The interiors of VNPs can encapsulate sensitive compounds while the exteriors can be chemically modified to covalently carry drug molecules in

Table 1.1 Summary of the strengths and potential limitations of current drug delivery technologies

Types		Strengths	Limitations	References
Viral nanoparticles		• Natural stability • Innate biocompatibility • Biodegradability • Easy crossing of biological barriers • Modifiability with atomic precision • Prodigious replication • Target selection after surface functionalization	• Pathogenicity • Immunogenicity • Dose concerns	(Huang et al. 2011; Koudelka et al. 2015)
Inorganic carriers		• Bio-consistency • Extreme small size (<10 nm) • Theragnosis	• Non-biodegradability • Non-biocompatibility • immunogenicity	(Tomalia 2009; Kim and Hyeon 2014)
Organic carriers	Polymeric	• Easy surface modifications • Target selection • Theragnosis • Delivery of imaging agent	• High production costs • Potential toxicity • Undesirable entrance of blood–brain barrier	(Mallapragada et al. 2015)
	Albumins	• Biocompatibility • Versatility • Outstanding half-life	• Low loading capacity • Inconsistency in loading capacity, drug release rete	(Cortes and Saura 2010; Lamichhane and Lee 2020)
	Exosomes	• High biocompatibility • Low immunogenicity • Low cytotoxicity • High targeting accuracy • Low required dosage • Minimum side effects • Effective penetration of cell membranes	• Ineffective isolation and purification methods • Difficulties in characterization • A lack of specific biomarker • A lack of high-resolution visualization techniqe	(Li et al. 2019; Yuan et al. 2017)

(continued)

Table 1.1 (continued)

Types		Strengths	Limitations	References
	Lipid-based	• Biocompatibility • Biodegradability • Easy modifiability • Penetration of cell membranes • Low immunogenicity	• Toxicity of cationic lipids • Instability in blood • Non-specific distribution • Low transfection efficiency • Unstable for long-term storage	(Xue et al. 2015; Tatiparti et al. 2017)
	Micro/nanobubbles with ultrasound	• Exclusive cellular penetration • Well controllable release rate • Ability to carry gaseous contents	• Challenging in preparation of smaller sizes	(Duan et al. 2020; Husseini et al. 2000)
Hybrid carriers		• Combined benefits of both components • Fabricability to overcome various limitations	• Time consuming and costly to produce	(Taylor-Pashow et al. 2010; Li et al. 2014)
Xenobot		• Reconfigurability for shape, behaviour and function • Biocompatibility • Nontoxicity • Self-limiting lifespan • Self-renewing	• Potentially costly to produce • Technically challenging for development for a broad range of applications	(Kriegman et al. 2020; Kriegman et al. 2019)

precisely defined arrays (Koudelka et al. 2015). This makes VNPs a natural versatile platform for the delivery of RNAi agents (Galaway and Stockley 2013; Choi et al. 2013), conventional small-molecule drugs (Pokorski et al. 2011), imaging reagents (Koudelka et al. 2015), photosensitizers (Rhee et al. 2012) and even heterologous viral genomes for gene therapies (Azizgolshani et al. 2013). Some suggested that VNPs are almost the only efficient means for systemic delivery of nucleic acid hitherto (Lai 2013). For example, studies showed that Adeno-associated virus can safely and effectively deliver therapeutic genes for the treatments of a number of genetic diseases such as haemophilia, lipoprotein lipase deficiency, inherited retinal disease and spinal muscular atrophy with positive results preclinically and clinically (Gaudet et al. 2013; Mingozzi and High 2011; Nathwani et al. 2011). In addition, viruses were naturally designed to deliver nucleic acid and hijack the intracellular machinery of

the host to prodigiously replicate the components of progeny viruses, which allows a massive production of mammalian tissue-derived VNPs for gene therapies and inexpensive manufacture at an industrial scale (Koudelka et al. 2015).

A particular advantage of VNPs over other synthetic nanomaterials is the modifiability with functional surface appendages attributed to their monodisperse structures, which allows surface tailoring for targeting specific cells including cancer cells and immune cells (Brown et al. 2002; Lewis et al. 2006; Ren et al. 2007). In addition, the encapsidation of nucleic acids serves as a protection mechanism making the particles extremely stable naturally, which therefore allows a range of chemical modifications to be applied and facilitates structural integrity in plasma and even gastric conditions (Rae et al. 2008). For this reason, VNPs modified for targeting specific cell types allow for the application of toxic payloads (loaded into the cavity of VNPs rather than the exterior) which selectively eliminate diseased cells without off-targeting healthy ones (Brown et al. 2002; Yildiz et al. 2013; Cao et al. 2014). For example, Hibiscus chlorotic ringspot virus was developed as a carrier for a chemotherapeutic drug doxorubicin which displays a high level of cytotoxicity (Ren et al. 2007). With the conjugation of folic acid onto the capsids, the VNPs were found to selectively target ovarian cancer cells. Huang et al. (Huang et al. 2011) engineered HK97 VNPs with transferrin for tumour cell-specific targeting, and demonstrated an effective targeting via transferrin receptor in vitro.

In addition to standard chemotherapy, VNPs are often used to carry photosensitizers for photodynamic therapies. For instance, a metalloporphyrin derivative was loaded in bacteriophage Qβ VNPs with a glycan surface ligand targeting cells bearing the CD22 receptor (Rhee et al. 2012). A multifunctional MRI contrast media and a photodynamic therapy agent (named chelated Gd^{3+} and Zn^{2+} phthalocyanine) were encapsulated in an engineered cowpea chlorotic mottle virus, which was the first demonstration of the use of VNPs in **theranostics** (Millan et al. 2014). Further, retargeted adenoviral vector carrying gold nanoparticles was used for photothermal therapy for tumour cells (Everts et al. 2006). Due to the unique properties of VNPs, viruses can be employed not only for the development of drug delivery systems and novel therapeutic approaches, but also to serve as a model tool for exploring the key mechanisms behind the interactions between nanoparticles and cells (Vanova et al. 2019).

Despite multiple superiorities of VNPs as a drug carrier, limitations such as pathogenicity and immunogenicity are not to be neglected. The pathogenicity of mammalian viruses often triggers natural virus–host interactions resulting in a compromised treatment effect or even lethal immunogenic responses (Guenther et al. 2014; Wirth et al. 2013; Yla Herttuala 2012). For this reason, bacteriophages and plant viruses are regarded to be safer candidates for the development of therapeutic VNPs due to their inability to infect humans (Koudelka et al. 2015). Otherwise non-viral **nanocarriers** would become an option for drug delivery. The pathogenicity problem, however, may not be highly significant if the VNPs are functionalized to target specific cells instead of the whole body or when the required dose is minimal. Nevertheless, biological aging is a systemic degenerative process where interventions require body-wide transfection (de Grey 2003). This essentially means that a

considerably high dose would be required, and consequently a higher risk is to be confronted. Koudelka et al. (Koudelka et al. 2015) suggested that the dosage issue could be mitigated by loading the drugs using encapsulation method instead of covalently attaching the cargo molecules to internally exposed side chains, in which case the loading capacity could be increased by up to three folds (Ren et al. 2007; Aljabali et al. 2013). However, this solution would still be far from adequate to confront the dosage issue.

1.3.2 Inorganic Carriers

Inorganic carriers are consisted of hard and insoluble nanoparticles that are biopersistent and non-biodegradable, such as metal, metal oxides, carbon nanotubes, carbon fibres, magnetic particles, etc. (Tomalia 2009; Kim and Hyeon 2014). Gold nanoparticles are versatile as they can be applied as a delivery system or as a therapeutic agent (Everts et al. 2006; Bhattacharyya et al. 2011) as they exhibit anti-angiogenic property and anti-tumour activity that interfere certain cellular processes (Bhattacharyya et al. 2011). Nano-diamonds are diamond particles in an extremely small size (from 4 to 5 nm). They are suitable for surface modification, stable in photoluminescence which allows analysis for intracellular localization (Alhaddad et al. 2011) and often used to deliver therapeutic siRNA (Tatiparti et al. 2017). Alhaddad et al. (2011) employed nano-diamonds as a vector to deliver siRNA to Ewing sarcoma cells. Quantum dots are colloidal semiconductor nanocrystals produced by quantum confinement effects. They possess outstanding optical and electronic characteristics (Young et al. 2016). They are self-tracking vehicles for siRNA for cancer treatments (Tatiparti et al. 2017). Tan et al. used Quantum dots to deliver siRNA molecules that silence human epidermal growth factor receptor 2 to breast cancer tissue (Tan et al. 2007). Carbon nanotubes are a theragnostic agent that simultaneously serve imaging and therapeutic purposes (Lee et al. 2013; Zhang et al. 2014). Due to the nanoneedle structure, carbon nanotubes are capable of independently translocating into cytoplasm without causing cell death (Bhattacharyya et al. 2011). Zhang et al. (2006) used functionalized carbon nanotubes to carry an siRNA that was released from the side wall of the nanotubes and silenced the expression of telomerase reverse transcriptase in cancer cells. This activity prevented cancer cells from acquiring replicative immortality and thereby suppressed tumour growth. Another theragnostic agent is super-paramagnetic iron oxide nanoparticles. Their large surface area allows for conjugation of targeting ligands and encapsulation of both drugs and imaging agents. This dual-action probe performs non-invasive imaging task and siRNA delivery and is often used for tumour treatments. A unique advantage of this nanocarrier is that the delivery is highly target-oriented with the aid of an external magnetic field (Bhattacharyya et al. 2011; Lee et al. 2013). Despite the various advantages that inorganic nanocarriers exert, their applications in clinics have been limited by their immunogenicity, non-biodegradability and, in some cases, non-biocompatibility.

1.3.3 Organic Carriers

Organic carriers are usually based on "soft" nanomaterials that can be natural or synthetic. Organic nanoparticles are typically made of polymeric organic substances or surfactant molecules that can be assembled into large aggregates. These aggregates could be biodegradable or non-degradable. Examples are proteins, liposomes, exosomes, polyesters, chitosan, etc. (Tomalia 2009; Mallapragada et al. 2015).

1.3.3.1 Polymeric Nanoparticles

Polymeric nanoparticles are a group of (in most cases) synthetic polymeric products that are often used for both diagnostic and therapeutic purposes. They have been used to combat degenerative, inflammatory and genetic diseases associated with aging, such as cancer and developmental, infectious and immune disorders (Mallapragada et al. 2015). As drug carriers, a considerable number of polymeric nanoparticles have been approved by the FDA for clinical applications (Weissig et al. 2014). With their inherent chemical properties which allow them to be modified for targeting functions and an excellent versatility to carry various drugs, they have often been used in site-targeted therapies and to deliver antioxidants, anti-inflammatory agents, immunomodulatory compounds, growth factors, genes, RNAi agents, bioactive compounds and antimicrobials (Mallapragada et al. 2015). They are also commonly used for cell imaging and tracking (Shao et al. 2013; Chen et al. 2013; Vande Velde et al. 2012; Ren et al. 2013; Miyoshi et al. 2005). Some examples are listed below.

Poly(alkyl cyanoacrylates) is the most well-established polymeric nano-delivery system and has been used to deliver compounds that include hexapeptide dalargin (Kreuter et al. 1995; Kreuter 2015), doxorubicin (Gulyaev et al. 1999; Steiniger et al. 2004), loperamide (Alyautdin et al. 1997) and tubocurarine (Alyautdin et al. 1998). For example, Kreuter et al. (1995) reported the first successful attempt of delivering dalargin adsorbed to the surface of poly(butyl cyanoacrylate) nanoparticles to the central nervous system via intravenous injection. Apart from poly(alkyl cyanoacrylates), polyesters have also been adopted for delivery purposes. Polyesters are commercially available and have been approved by the FDA for human use (Weissig et al. 2014). They are recognized as a promising biodegradable delivery system. One of their important properties is their low cytotoxicity attributed to their rapid degradation into metabolites (Gunatillake and Adhikari 2003). For the same reason, all polyesters undergo bulk erosion (Tamada and Langer 1993; Burkersroda et al. 2002) and often cause premature release of drugs. Polyesters are often used to carry loperamide (Gelperina et al. 2010; Tosi et al. 2007), active peptides (Li et al. 2013), ritonavir (Rao et al. 2008) and doxorubicin (Gelperina et al. 2010; Wohlfart et al. 2011). Polyanhydrides also exhibit good biocompatibility and drug delivery potential (Rosen et al. 1983). A notable example of their application is the implantable wafer systems for central nervous system-directed delivery, which is

often used as therapeutics for Alzheimer's disease (Wu et al. 1994; Howard et al. 1989) and brain cancer (Brem et al. 1989; Jampel et al. 1991; Lesniak et al. 2005). An important advantage of these polyanhydride implants is their degradability into biocompatible metabolites that are readily eliminated (Domb et al. 1994). In addition, polyethers have recently attracted extensive attention for their potential as a drug carrier. These polymers are naturally derived polymers. A typical example is chitosan, a cationic polysaccharide, which is generally deemed to be a promising drug delivery vehicle (Ta et al. 2008; Shamji et al. 2009). Polyethers are not particularly susceptible to hydrolytic degradation since their ether bonds are considerably stable in water. Elimination of polyethers often takes place through enzymatic degradation either by oxidation or by dissociation prior to excretion (Ohta et al. 2005; Kawai 2002). Pille et al. (2006) reported a 90% growth inhibition of xenografted aggressive breast tumours in mice induced by anti-RhoA siRNA loaded on chitosan-coated polyisohexylcyanoacrylate nanoparticles via intravenous injection.

In summary, polymeric nanoparticles are promising and generally recognized drug delivery systems that serve diagnostic, imaging and therapeutic purposes. In addition, with appropriate chemistries and functionalization, their performance can be further improved for safety, effectiveness and target/site-specificity (Voigt et al. 2014). However, it was suggested that some of the polymeric chemicals were found to be cytotoxic to cells in the central nervous system (Mallapragada et al. 2015). In addition, future application research should focus on reducing the high cost in fabrication of these polymers and scaling up the production (Mallapragada et al. 2015).

1.3.3.2 Albumin-Based Carriers

Albumin is a highly biocompatible, non-immunogenic and negatively charged protein that is abundant in blood plasma. It has been developed into a versatile drug delivery system often used for the transportation of hydrophobic molecules such as fatty acid, hormones, bilirubin, fat-soluble vitamins, exogenous drugs (e.g. warfarin and ibuprofen) (Cortes and Saura 2010) and positively charged compounds (Lamichhane and Lee 2020). An outstanding advantage of albumin as a drug carrier is its excellent half-life that draws particular research interest. This advantage makes albumin a preferable delivery system for anti-cancer agents (Choi and Han 2018). The first attempt of using albumin as a drug carrier was performed by Stehle et al. (1997) who chemically coupled methotrexate with albumin using carbodiimide as a cross-linking agent.

Albumin molecules can be modified in various ways to serve different purposes. Due to the nature that albumin molecules carry negative charges, which limits their applications in delivering co-charged compounds, cationization has been performed to modify albumin by adding ethylenediamine to a buffered albumin solution (Byeon et al. 2016). Cationized albumin exhibited favourable pharmacokinetic properties with a longer serum half-life and an enhanced selectivity to brain tissues as compared to other organs (e.g. liver, heart, lung, etc.) (Bickel et al. 2001). Vaidya et al. (2020) demonstrated that using bovine serum albumin cationic nanoparticles as an inhalable