Chapter

Applications and Future Trends of Extracellular Vesicles in Biomaterials Science and Engineering

Esra Cansever Mutlu, Georgios V. Gkoutos, Besim Ben-Nissan and Artemis Stamboulis

Abstract

Extracellular vesicles (EVs) derived from natural resources and human cells are innovative biomaterials with vast potential for a wide range of applications. The applications of EVs are expanding rapidly, particularly in emerging fields such as biomaterialomics, information transfer, data storage, and 3D bioprinting, where principles of synthetic biology also come into play. These versatile structures exhibit diverse morphologies and compositions, depending on their cellular origin. As a result, they have been incorporated as key components in both medical and engineering fields. Their integration into these materials has facilitated research in various areas, including DNA and RNA storage, 3D printing, and mitochondrial transfer. Whilst the sustainable production of EVs using validated and standardized methods remains a significant challenge, it is crucial to acknowledge their tremendous potential and prepare for future scientific breakthroughs facilitated by EVs.

Keywords: biocomputer, DNA storage, exosome, data science, mitochondria

1. Introduction

Extracellular vesicles (EVs) play a crucial role in facilitating communication between different cellular compartments within the body by serving as carriers for the transfer of lipids, proteins, and nucleic acids [1, 2]. These vesicles are released by various types of cells and can be found in bodily fluids. Despite their sophisticated functions, only a limited number of these functions have been explored thus far.

The term "platelet-dust" was coined by Wolf in 1967 during his research at the University of Birmingham, UK, building upon previous studies that investigated the effects of platelet extraction protocols on coagulation [3–6]. Wolf's report focused on the indistinguishable properties observed amongst different platelet fractions obtained through the ultracentrifugation of plasma and serum, with regard to their coagulation activity [6].

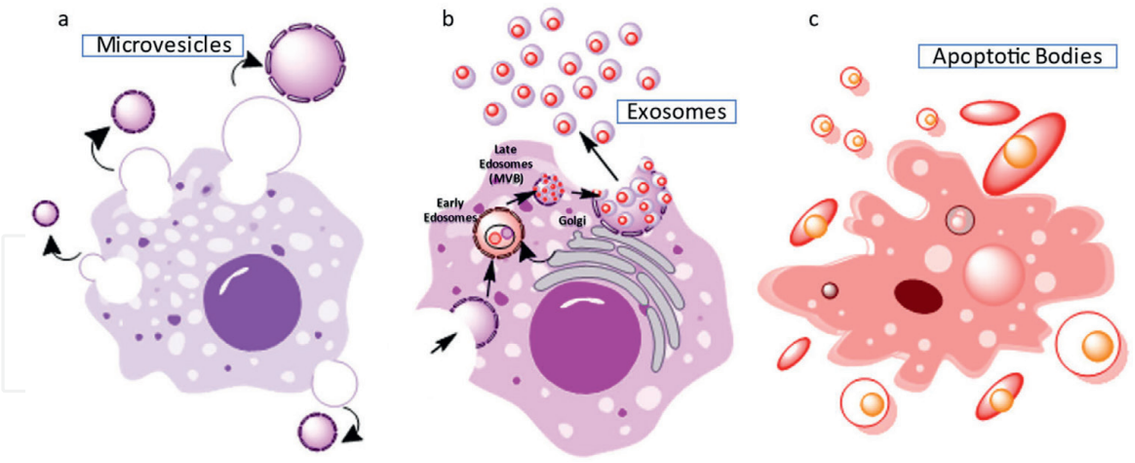

Figure 1.
Schematic representation of EVs subtypes: (a) microvesicles; (b) exosomes; (c) apoptotic bodies.

Researchers often categorize EVs (**Figure 1**) based on size, and exosomes (20–200 nm) (**Figure 2**) are particularly noteworthy due to their unique DNA and RNA content, which can be modified through cellular uptake. This ability enables them to regulate cells and tissues. Additionally, smaller vesicles than exosomes may intersect or co-release with microvesicles during the multivesicular endosomal pathway (0.1–1 μm). However, despite efforts to explore the diversity of EV subtypes, there is a lack of substantial biomaterialomics data regarding real-time release profiles [7]. Furthermore, no clear correlations have been established between the functions of EV contents. For example, recent research revealed that healthy-shaped mitochondria were found encapsulated in mitochondria-rich EVs derived from autologous stem cells [8], whereas other EV subtypes can carry mitochondria components such as mitochondrial DNA [9], or can transport mitochondrial proteins [10]. These subgroups, depending on the isolation techniques and protocols employed, can be purified as a combination of two or three subtypes. Although the relationship between motion tendency and cargo size has yet to be explored, utilizing specific biological vesicle types may provide the most effective means of targeted delivery.

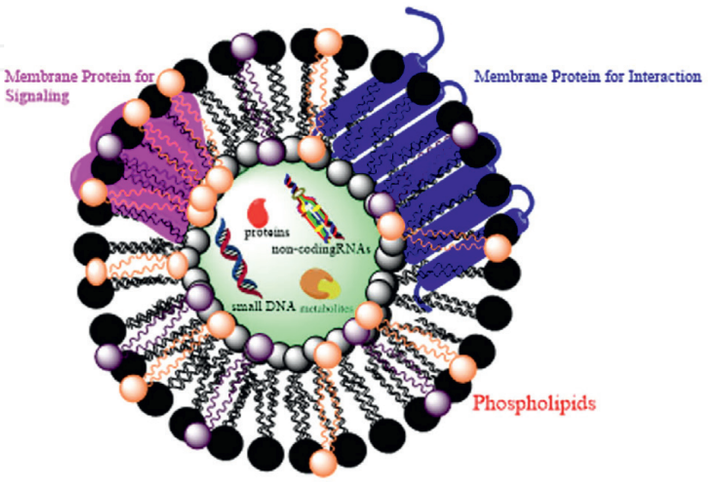

Figure 2.
Schematic representation of an exosome structure.

2. EVs in biocomputers: from synthetic biology to materials science

Biocomputers are emerging computing devices that utilize biological macromolecules, such as DNA, RNA, and proteins, to perform computational tasks [11–14]. The field of biocomputing originated with a ground-breaking study by Adleman in 1994, which demonstrated that computational tasks could be carried out using DNA [12]. Since then, research has expanded to explore various approaches and applications of biomaterial science and synthetic biology, including DNA computing, RNA computing, and protein-based computing [15]. Notably, the CELLO algorithm has led to the development of genetic Boolean gates (**Figure 3**) and three-input Boolean circuits [16], highlighting the natural biochemical properties of these biological molecules for data storage, processing, and output.

Biocomputers rely on four main components: information storage [11, 17], information processing [18], protein-based computation [19, 20], and output-and-readout [21]. DNA consists of four nucleotide bases: adenine (A), cytosine (C), guanine (G), and thymine (T), whilst RNA includes uracil (U) instead of thymine. By rearranging these bases in different-length fragments, information can be encoded and stored. Manipulating the conformation of DNA or RNA strands allows for data storage in biocomputing [22, 23]. Furthermore, these strands can be designed to selectively bind to specific target molecules or sequences, enabling logical operations such as AND, OR, and NOT gates. By combining multiple DNA or RNA strands, complex computations can be performed [24, 25].

Proteins play a crucial role in biocomputing by acting as switches or logic gates (**Figure 3**), facilitating signal processing and decision-making within a biocomputer system. Specifically designed proteins can perform computational operations, and enzymes can catalyze specific reactions [26]. Output in a biocomputer system can be achieved by detecting changes in DNA, RNA, or proteins. Alternatively, output signals can be analyzed through enzymatic reactions and translated into readable data allowing for effective communication of computational results [27].

Recently, the research on the use of exosomes in biocomputing has gained momentum, initially focusing on cell membranes and their potential as gates and

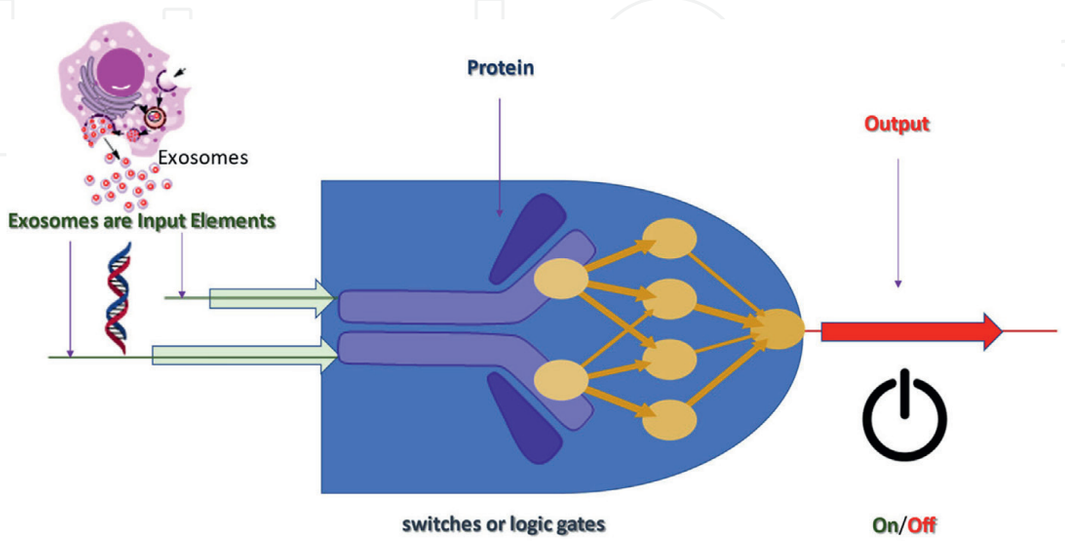

Figure 3.
A typical example of an AND gate in digital circuits, where the two A and B inputs are exosomes. Proteins can act as switches or logic gates.

signal processors. Fan et al. reported on the cost-effective and efficient utilization of exosomes for "Boolean response" in biocomputing. They proposed that the cell surface, with its complex array of surface molecules, could be harnessed for DNA computing, allowing for the design of intricate logic gates beyond traditional approaches [28, 29]. This concept involves using DNA aptamers to target biomarkers present on exosomes, triggering subsequent output signals through Boolean computation [30].

Huang et al. explored the use of nanovesicle surfaces as DNA-based logic gates, enhancing the targeting efficiency of encapsulated graphene carbon dots. They demonstrated the programmable elementary functions of nanovesicles derived from HCT116, a human colon cancer cell line, opening up possibilities for advanced biomedical applications [31]. Furthermore, exosome content derived from blood or cells can be detected from a biocomputing perspective. Oishi and Saito conducted research on hybridized gold nanoparticles (GNPs) that functioned as intra-particle DNA circuits, referred to as "DNA-walkers." Their study focused on the detection of miR-21 within or without fetal bovine serum (FBS)-derived exosomes, demonstrating the potential of this biosensor application for profiling endogenous miRNA in clinical samples from serum or cell lysates [32]. Recent studies have also shown that surface proteins of exosomes can be detected, or DNA/RNA computing devices can be employed for exosome detection [26]. Yu et al. designed a novel logic gate utilizing two types of hairpin DNAs that target specific surface proteins on different cancer cell lines. They successfully detected the presence of tyrosine kinase-like 7 (PTK7) and prostate-specific membrane antigen (PSMA) on CCRF-CEMsEV membranes, highlighting the potential of this approach [33].

More recent studies have revealed that natural vesicles, including EVs and exosomes, function as nano-machines on cell surfaces, capable of participating in biocomputation through active targeting strategies, in addition to passive accumulation [26]. In an earlier study by Yoshina et al., egg phosphatidylcholine vesicles ranging from 30 to 200 nm were investigated. The researchers utilized oligonucleotide-tethered arrays of mobile vesicles, allowing for the separation of vesicle mixtures based on their sequence-specific binding to head-labeled antisense oligonucleotides. This approach enabled the sorting of vesicles based on their specific surface binding properties [34].

Building upon this concept, a technique was developed to create nano-bio-computing lipid nanotablets (LNTs), where DNA was used as surface ligands on small unilamellar vesicles. These DNA ligands served as input "molecular barcodes" that triggered biotin-streptavidin interactions through a supported lipid bilayer (SLB). The nano-bio-computing LNTs demonstrated sophisticated performance in capturing vesicle interactions, allowing for high-resolution spatiotemporal imaging and computational analysis [35]. Furthermore, Hao et al. provided a comprehensive review of promising studies utilizing DNA technology to amplify signals upon binding to tumor-derived exosomes, highlighting its potential for various applications [30].

In a study by Meng et al., a ratiometric electrochemical OR gate assay was developed for non-small cell lung cancer (NSCLC)-derived exosomes. DNA tetrahedrons were designed as aptamers and immobilized onto gold nanoparticles to detect NSCLC-derived exosomes. These exosomes were obtained from clinical samples, and the study demonstrated that the signal input and DNA OR logic gate could be harnessed for precise nanomedicine applications [36].

DNA storage offers remarkable advantages for long-term data storage (**Figure 4**), such as dense-durable-enormous capacity and low power consumption. However, it does have some limitations, including slow read/write speeds and the requirement

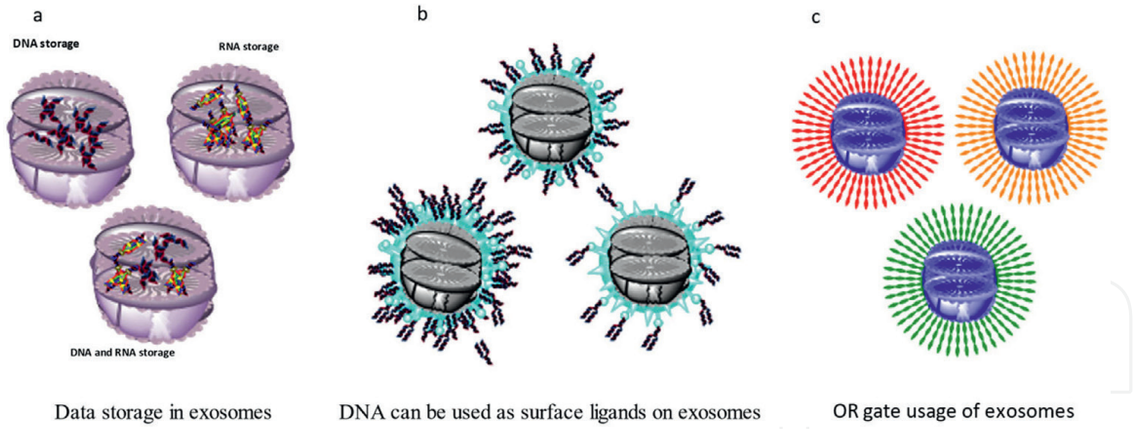

Figure 4.
Schematic representation of exosome usage in biocomputing applications.

for specialized equipment or steganography abilities [37]. Notably, EVs and exosomes have been commonly utilized as a traditional and straightforward method for DNA storage, particularly for long-term storage at temperatures between −20°C and −80°C [38]. Furthermore, modern DNA data storage approaches involve dividing and encapsulating DNA within small vesicles called Data Blocks (DBs) to mitigate error rates by decreasing the oligos size [39, 40]. Recent studies have specifically focused on utilizing EV subtypes, particularly exosomes, for DNA or RNA storage [41, 42].

A study by Madisen et al. demonstrated that dry DNA could be stored for extended periods by using cell pellets derived from plasma, with intact DNA being preserved for 7–13 years in a solution at −20° [43]. Currently, biological membranes represent a growing field of interest for DNA storage, inspiring material scientists in applications related to the "Internet of Things" to devise a so-called "DNA-of-things" (DoT) storage architecture incorporating DNA storage into 3D-printed everyday materials [18, 44, 45]. In conclusion, synthetic biology is expected to increasingly focus on utilizing EVs and exosomes as versatile tools for data storage [46–48].

3. EVs in 3D bioprinting, and mitochondrial transfer

The largest portion of the body consists of soft tissues, including the skin, organ surfaces, and the eyes. Despite the advancements in versatile reconstruction materials, there are still significant challenges to overcome for true cell-based regeneration, necessitating the development of new biomaterials [49, 50]. One of the major obstacles in 3D tissue regeneration is the weak adhesion, immunologically non-inert nature, and long-term corrosion of materials, which hinders effective tissue repair [49]. Moreover, static or semi-static nature of these materials often results in insufficient information and energy for damaged tissues during the regeneration process, leading to inadequate tissue repair and unsatisfactory clinical outcomes [51].

Whilst some personalized cell-based trials have been conducted, the survival of cells on gliding and hydrophobic materials for long-term regeneration remains a challenge [49, 51]. However, crude EVs and exosomes show promise as self-dynamic regeneration guides and self-energy centres within biomaterials [51–53]. Motile exosome structures have the potential to deliver non-coding RNAs for cell-based regeneration, whilst motile mitochondria encapsulated in EVs can serve as energy centres during tissue repair [54, 55]. Zhang et al. in their review paper stated that secretion

of EVs increases in cells under hypoxia, resulting in indirect changes of the mitochondrial function of the receptor cells via the uptake of EVs content by the receptor cells facilitating tumor progression and ischemic damage. On the other hand, EVs derived from healthy cells can have a protective effect on the mitochondria of the recipient cells. Although it has been shown that EVs can have an effect on mitochondria regulation, it is still unclear whether the EVs content enters directly into the mitochondria of the recipient cells and whether exosomes and microvesicles play a different role in mitochondria regulation [56]. In an interesting review paper, Liu et al. [57] stated that intact mitochondria could be also present within exosomes, for example, the exosomes derived from airway myeloid-derived regulatory cells. Although, the size of mitochondria is larger than exosomes and, therefore, the case of encapsulated mitochondria in exosomes seems to be an unlike event, under certain conditions, mitochondria could be present in exosomes because the morphology of mitochondria is adapted to the demands of mitochondrial fusion, fission, and transport [58].

3D bioprinting is a promising technique that can standardize the production of tissue regeneration using crude EVs. This cell-free bioink production strategy can enhance tissue regeneration by providing self-elastic and self-bioenergetic sustainable biomaterials for 3D soft tissue repair [9, 59–61].

Mesenchymal Stem Cell-derived exosomes have gained significant attention in various regeneration products, thanks to their versatility in clinical applications similar to stem cells themselves [62]. However, a systematic review by Tan et al. emphasized the need for improved validation of animal studies with MSC-derived EVs before conducting human clinical trials, highlighting the importance of generating EV subtypes that are readily accepted and compatible for regenerating damaged cells [50, 63].

Therefore, there has been a growing focus on using EV subtypes, particularly MSC-derived exosomes, in 3D printing applications. Holkar et al. demonstrated that incorporating MSC-derived EVs within 3D hydrogel scaffolds can enhance their osteochondral healing potential [64]. Similarly, Huang et al. reported that incorporating exosomes into 3D printing scaffolds can effectively promote osteogenesis, angiogenesis, and cartilage repair [65–67].

In another study, Born et al. utilized methacrylated gelatin as a bioink along with EVs derived from mesenchymal stem/stromal cells (MSCs). The researchers investigated the in vitro bioactivity and release of EVs from the photo-cross-linked gel after 3D printing. The results demonstrated that EV bioinks retained the bioactivity of the gel and facilitated sustained release of EVs [68].

Bar et al. conducted a study on 3D-printed cardiac patches for the delivery of miR-199a-3p. They activated THP-1-derived macrophages (MΦ) and isolated their EVs. The EVs were then evaluated for bioavailability and incorporated into a RGD-modified alginate solution as a bioink after electroporation of miRNA into the EVs. The researchers used a FRESH-hydrogel solution for printing and found that the EV-based patches exhibited increased bioactivity for up to 5 days, although there is a need to improve their mechanical properties [69].

4. Concluding remarks

The field of Biomaterials Science and Engineering has evolved beyond the notion that "simplest is the best way." The Chengdu Declaration has introduced new definitions and concepts such as biomaterialomics, tissue-inducing materials, and

bioink. Additionally, computational tools have enabled the synthesis of sophisticated biomaterials, necessitating the incorporation of extensive data and the utilization of machine learning and deep learning techniques by biomaterials scientists. Within this context, exosomes have emerged as an important tool in biomedical science, spanning a wide range of applications from 3D bioprinting to biocomputing [60, 70]. In the near future, biomaterialomics will pave the way for new studies, and exosomes will play a significant role within this field. Feng et al. introduced the term BioHEAs, highlighting the potential replacement of biological high-entropy alloys with high-entropy alloys incorporated with exosomes [71]. Considering these advancements, it is clear that EVs and their subtypes will serve as fundamental components in the field of Biomaterials Science and Engineering. Whilst the sustainable production of EVs using validated and standardized methods and range of engineering and biomaterials remains a significant challenge, it is crucial to acknowledge their tremendous potential and prepare for future scientific breakthroughs facilitated by EVs.

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References

[1] Wiklander OP, Brennan MÁ, Lötvall J, Breakefield XO, El Andaloussi S. Advances in therapeutic applications of extracellular vesicles. Science Translational Medicine. 2019;**11**(492):eaav8521

[2] Green DW, Watson JA, Ben-Nissan B, Watson GS, Stamboulis A. Synthetic tissue engineering with smart, cytomimetic protocells. Biomaterials. 2021;**276**:120941

[3] Ware AG, Fahey JL, Seegers WH. Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. American Journal of Physiology-Legacy Content. 1948;**154**(1):140-147

[4] Van Creveld S, Paulssen M, Vonk R. Significance of clotting factors in blood-platelets, in normal and pathologigal conditions. The Lancet. 1951;**258**(6676):242-244

[5] Van Creveld S, Paulssen M. Isolation and properties of the third clotting factor in blood-platelets. The Lancet. 1952;**259**(6697):23-25

[6] Wolf P. The nature and significance of platelet products in human plasma. British Journal of Haematology. 1967;**13**(3):269-288

[7] Jeppesen DK, Zhang Q, Franklin JL, Coffey RJ. Extracellular vesicles and nanoparticles: Emerging complexities. Trends in Cell Biology. 2023;**33**(8):667-681

[8] Ikeda G, Santoso MR, Tada Y, Li AM, Vaskova E, Jung J-H, et al. Mitochondria-rich extracellular vesicles from autologous stem cell–derived cardiomyocytes restore energetics of ischemic myocardio. Journal of the American College of Cardiology. 2021;**77**(8):1073-1088

[9] Dache ZAA, Otandault A, Tanos R, Pastor B, Meddeb R, Sanchez C, et al. Blood contains circulating cell free respiratory competent mitochondria. Faseb Journal. 2020;**34**(3):3616-3630

[10] Jang SC, Crescitelli R, Cvjetkovic A, Belgrano V, Bagge RO, Höög JL, et al. A subgroup of mitochondrial extracellular vesicles discovered in human melanoma tissues are detectable in patient blood. BioRxiv. 2017:174193

[11] Adleman LM. Computing with DNA. Scientific American. 1998;**279**(2):54-61

[12] Adleman LM. Molecular computation of solutions to combinatorial problems. Science. 1994;**266**(5187):1021-1024

[13] Adleman LM. On constructing a molecular computer. DNA Based Computers. 1995;**27**:1-21

[14] Ding T, Yang J, Pan V, Zhao N, Lu Z, Ke Y, et al. DNA nanotechnology assisted nanopore-based analysis. Nucleic Acids Research. 2020;**48**(6):2791-2806

[15] Wagner HJ, Sprenger A, Rebmann B, Weber W. Upgrading biomaterials with synthetic biological modules for advanced medical applications. Advanced Drug Delivery Reviews. 2016;**105**:77-95

[16] Dalchau N, Szép G, Hernansaiz-Ballesteros R, Barnes CP, Cardelli L, Phillips A, et al. Computing with biological switches and clocks. Natural Computing. 2018;**17**:761-779

[17] Anavy L, Vaknin I, Atar O, Amit R, Yakhini Z. Data storage in DNA with fewer synthesis cycles using composite

DNA letters. Nature Biotechnology. 2019;**37**(10):1229-1236

[18] Koch J, Gantenbein S, Masania K, Stark WJ, Erlich Y, Grass RN. A DNA-of-things storage architecture to create materials with embedded memory. Nature Biotechnology. 2020;**38**(1):39-43

[19] Nikitin MP, Shipunova VO, Deyev SM, Nikitin PI. Biocomputing based on particle disassembly. Nature Nanotechnology. 2014;**9**(9):716-722

[20] Miyamoto T, Razavi S, DeRose R, Inoue T. Synthesizing biomolecule-based Boolean logic gates. ACS Synthetic Biology. 2013;**2**(2):72-82

[21] Yazdi SHT, Kiah HM, Garcia-Ruiz E, Ma J, Zhao H, Milenkovic O. DNA-based storage: Trends and methods. IEEE Transactions on Molecular, Biological and Multi-Scale Communications. 2015;**1**(3):230-248

[22] Lim CK, Nirantar S, Yew WS, Poh CL. Novel modalities in DNA data storage. Trends in Biotechnology. 2021;**39**(10):990-1003

[23] Jiao K, Hao Y, Wang F, Wang L, Fan C, Li J. Structurally reconfigurable designer RNA structures for nanomachines. Biophysics Reports. 2021;**7**(1):21

[24] Zhang J, Qiu Z, Fan J, He F, Kang W, Yang S, et al. Scan and unlock: A programmable DNA molecular automaton for cell-selective activation of ligand-based Signaling. Angewandte Chemie. 2021;**133**(12):6807-6817

[25] Gangadharan S, Raman K. The art of molecular computing: Whence and whither. BioEssays. 2021;**43**(8):2100051

[26] Seo J, Kim S, Park HH, Nam JM. Biocomputing with nanostructures on lipid bilayers. Small. 2019;**15**(26):1900998

[27] Bose D, Roy A, Roy L, Chatterjee S. Nucleic acid sensors and logic gates. Nucleic Acid Biology and its Application in Human Diseases. Singapore: Springer; 2023. p. 271-319

[28] Fan D, Wang J, Wang E, Dong S. Propelling DNA computing with materials' power: Recent advancements in innovative DNA logic computing systems and smart bio-applications. Advancement of Science. 2020;**7**(24):2001766

[29] Song T, Shah S, Bui H, Garg S, Eshra A, Fu D, et al. Programming DNA-based biomolecular reaction networks on cancer cell membranes. Journal of the American Chemical Society. 2019;**141**(42):16539-16543

[30] Hao P, Niu L, Luo Y, Wu N, Zhao Y. Surface engineering of lipid vesicles based on DNA nanotechnology. ChemPlusChem. 2022;**87**(5):e202200074

[31] Huang H, Guo Z, Zhang C, Cui C, Fu T, Liu Q, et al. Logic-gated cell-derived nanovesicles via DNA-based smart recognition module. ACS Applied Materials & Interfaces. 2021;**13**(26):30397-30403

[32] Oishi M, Saito K. Simple single-legged DNA walkers at diffusion-limited nanointerfaces of gold nanoparticles driven by a DNA circuit mechanism. ACS Nano. 2020;**14**(3):3477-3489

[33] Yu Y, Guo Q, Jiang W, Zhang H, Cai C. Dual-aptamer-assisted AND logic gate for cyclic enzymatic signal amplification electrochemical detection of tumor-derived small extracellular vesicles. Analytical Chemistry. 2021;**93**(32):11298-11304

[34] Yoshina-Ishii C, Boxer SG. Arrays of mobile tethered vesicles on supported lipid bilayers. Journal of the American Chemical Society. 2003;**125**(13):3696-3697

[35] Seo J, Kim S, Park HH, Choi DY, Nam J-M. Nano-bio-computing lipid nanotablet. Science Advances. 2019;**5**(2):eaau2124

[36] Meng F, Yu W, Niu M, Tian X, Miao Y, Li X, et al. Ratiometric electrochemical OR gate assay for NSCLC-derived exosomes. Journal of Nanobiotechnology. 2023;**21**(1):1-15

[37] Song X, Reif J. Nucleic acid databases and molecular-scale computing. ACS Nano. 2019;**13**(6):6256-6268

[38] Doricchi A, Platnich CM, Gimpel A, Horn F, Earle M, Lanzavecchia G, et al. Emerging approaches to DNA data storage: Challenges and prospects. ACS Nano. 2022;**16**(11):17552-17571

[39] Blawat M, Gaedke K, Huetter I, Chen X-M, Turczyk B, Inverso S, et al. Forward error correction for DNA data storage. Procedia Computer Science. 2016;**80**:1011-1022

[40] Matange K, Tuck JM, Keung AJ. DNA stability: A central design consideration for DNA data storage systems. Nature Communications. 2021;**12**(1):1358

[41] Organick L, Ang SD, Chen Y-J, Lopez R, Yekhanin S, Makarychev K, et al. Random access in large-scale DNA data storage. Nature Biotechnology. 2018;**36**(3):242-248

[42] Parker MT, Knop K, Sherwood AV, Schurch NJ, Mackinnon K, Gould PD, et al. Nanopore direct RNA sequencing maps the complexity of Arabidopsis mRNA processing and m6A modification. eLife. 2020;**9**:e49658

[43] Madisen L, Hoar DI, Holroyd CD, Crisp M, Hodes ME. DNA banking: The effects of storage of blood and isolated DNA on the integrity of DNA. American Journal of Medical Genetics. 1987;**27**(2):379-390. DOI: 10.1002/ajmg.1320270216

[44] Paunescu D, Puddu M, Soellner JO, Stoessel PR, Grass RN. Reversible DNA encapsulation in silica to produce ROS-resistant and heat-resistant synthetic DNA 'fossils'. Nature Protocols. 2013;**8**(12):2440-2448

[45] Oskam CL, Haile J, McLay E, Rigby P, Allentoft ME, Olsen ME, et al. Fossil avian eggshell preserves ancient DNA. Proceedings of the Royal Society B: Biological Sciences. 2010;**277**(1690):1991-2000

[46] Ramasubramanian L, Kumar P, Wang A. Engineering extracellular vesicles as nanotherapeutics for regenerative medicine. Biomolecules. 2019;**10**(1):48

[47] Martella A, Pollard SM, Dai J, Cai Y. Mammalian synthetic biology: Time for big MACs. ACS Synthetic Biology. 2016;**5**(10):1040-1049

[48] Mateescu B, Jones JC, Alexander RP, Alsop E, An JY, Asghari M, et al. Phase 2 of extracellular RNA communication consortium charts next-generation approaches for extracellular RNA research. Iscience. 2022;**25**(8):104653

[49] Wan R, Hussain A, Behfar A, Moran SL, Zhao C. The therapeutic potential of exosomes in soft tissue repair and regeneration. International Journal of Molecular Sciences. 2022;**23**(7):3869

[50] Mutlu EC, Kaya Ö, Wood M, Mager I, Topkara KÇ, Çamsarı Ç, et al. Efficient doxorubicin loading to isolated dexosomes of immature JAWSII cells: Formulated and characterized as the bionanomaterial. Materials. 2020;**13**(15):3344

[51] Bjørge I, Kim S, Mano J, Kalionis B, Chrzanowski W. Extracellular vesicles,

exosomes and shedding vesicles in regenerative medicine–a new paradigm for tissue repair. Biomaterials Science. 2018;**6**(1):60-78

[52] Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X, et al. Large cardiac muscle patches engineered from human induced-pluripotent stem cell–derived cardiac cells improve recovery from myocardial infarction in swine. Circulation. 2018;**137**(16):1712-1730

[53] Willms E, Cabañas C, Mäger I, Wood MJ, Vader P. Extracellular vesicle heterogeneity: Subpopulations, isolation techniques, and diverse functions in cancer progression. Frontiers in Immunology. 2018;**9**:738

[54] Pant T, Juric M, Bosnjak ZJ, Dhanasekaran A. Recent insight on the non-coding RNAs in mesenchymal stem cell-derived exosomes: Regulatory and therapeutic role in regenerative medicine and tissue engineering. Frontiers in Cardiovascular Medicine. 2021;**8**:737512

[55] Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nature Medicine. 2012;**18**(5):759-765

[56] Zhang Y, Tan J, Miao Y, Zhang Q. The effect of extracellular vesicles on the regulation of mitochondria under hypoxia. Cell Death & Disease. 2021;**12**(4):358

[57] Liu D, Dong Z, Wang J, Tao Y, Sun X, Yao X. The existence and function of mitochondrial component in extracellular vesicles. Mitochondrion. 2020;**54**:122-127

[58] Ju G, Wang L, Liu J, Xie F, Su B, Wang X. Abnormalities of mitochondrial dynamics in neurodegenerative diseases. Antioxidants. 2017;**6**(2):25

[59] Gu C, Feng J, Waqas A, Deng Y, Zhang Y, Chen W, et al. Technological advances of 3D scaffold-based stem cell/exosome therapy in tissues and organs. Frontiers in Cell and Developmental Biology. 2021;**9**:709204

[60] Sun Y, Zhang B, Zhai D, Wu C. Three-dimensional printing of bioceramic-induced macrophage exosomes: Immunomodulation and osteogenesis/angiogenesis. NPG Asia Materials. 2021;**13**(1):1-16

[61] Yerneni SS, Lathwal S, Shrestha P, Shirwan H, Matyjaszewski K, Weiss L, et al. Rapid on-demand extracellular vesicle augmentation with versatile oligonucleotide tethers. ACS Nano. 2019;**13**(9):10555-10565

[62] Wei W, Ao Q, Wang X, Cao Y, Liu Y, Zheng SG, et al. Mesenchymal stem cell–derived exosomes: A promising biological tool in nanomedicine. Frontiers in Pharmacology. 2021;**11**:590470

[63] El-Andaloussi S, Lee Y, Lakhal-Littleton S, Li J, Seow Y, Gardiner C, et al. Exosome-mediated delivery of siRNA in vitro and in vivo. Nature Protocols. 2012;**7**(12):2112-2126

[64] Holkar K, Kale V, Ingavle G. Well-orchestrated physico-chemical and biological factors for enhanced secretion of osteogenic and angiogenic extracellular vesicles by mesenchymal stem cells in a 3D culture format. Biomaterials Science. 2022;**10**(16):4458-4473

[65] Huang J, Xiong J, Yang L, Zhang J, Sun S, Liang Y. Cell-free exosome-laden scaffolds for tissue repair. Nanoscale. 2021;**13**(19):8740-8750

[66] Chen P, Zheng L, Wang Y, Tao M, Xie Z, Xia C, et al. Desktop-stereolithography 3D printing of a

radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration. Theranostics. 2019;**9**(9):2439

[67] Kim DK, Lee S, Kim M, Jeong Y, Lee S. Exosome-coated silk fibroin 3D-scaffold for inducing osteogenic differentiation of bone marrow derived mesenchymal stem cells. Chemical Engineering Journal. 2021;**406**:127080

[68] Born LJ, McLoughlin ST, Dutta D, Mahadik B, Jia X, Fisher JP, et al. Sustained released of bioactive mesenchymal stromal cell-derived extracellular vesicles from 3D-printed gelatin methacrylate hydrogels. Journal of Biomedical Materials Research Part A. 2022;**110**(6):1190-1198

[69] Bar A, Kryukov O, Etzion S, Cohen S. 316Engineered extracellular vesicle-mediated delivery of miR-199a-3p increases the viability of 3D-printed cardiac patches. International Journal of Bioprinting. 2023;**9**(2):316-330

[70] Levesque-Tremblay G. News and views. Regenerative Engineering and Translational Medicine. 2019;**2019**(5):446-449

[71] Feng J, Tang Y, Liu J, Zhang P, Liu C, Wang L. Bio-high entropy alloys: Progress, challenges, and opportunities. Frontiers in Bioengineering and Biotechnology. 2022;**10**:977282