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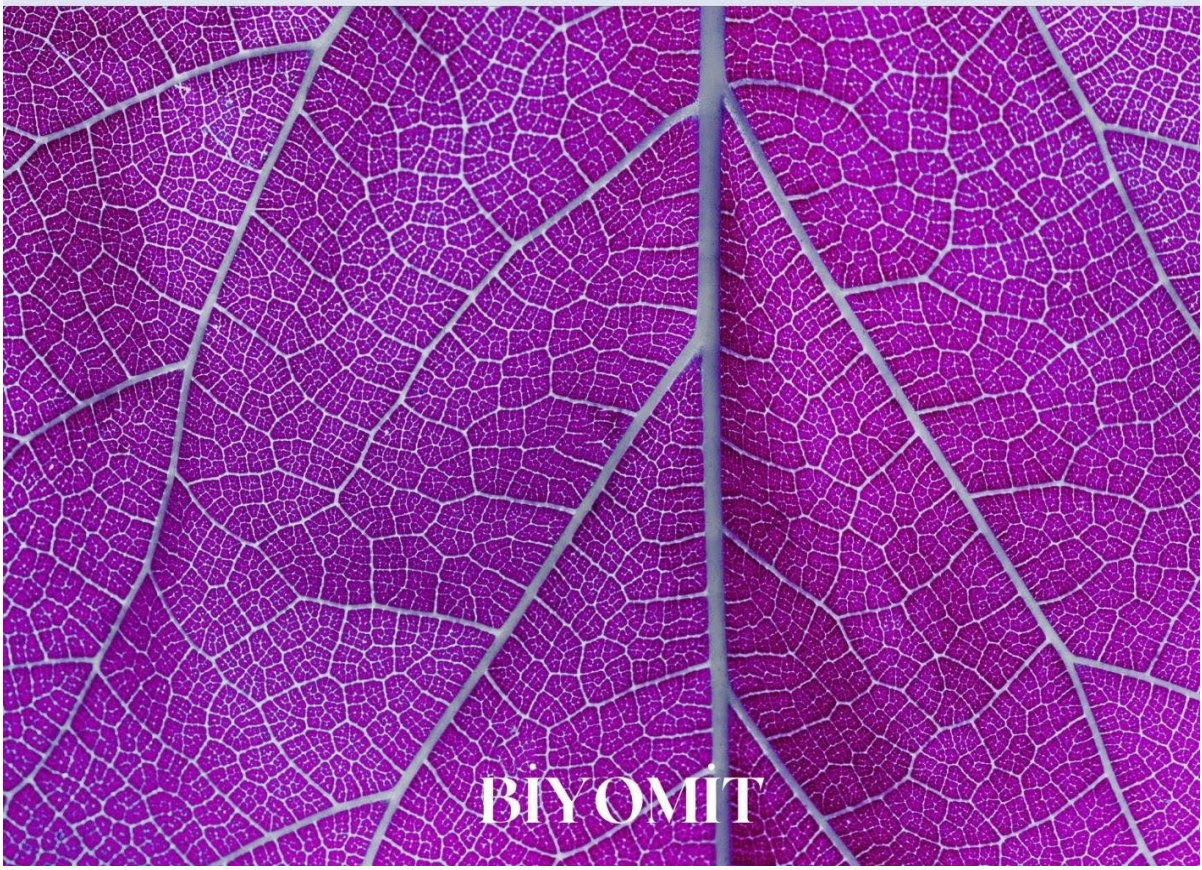
Yilmaz KAYA

Biotechnology and Application Areas



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Editor

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CHAPTER 1

Phytofabricated Nanoparticles: A Promising Alternative to Agrochemicals and Possible Effects on Phytopathogens

Aydin Atakan and Sevgi Marakli

Phytofabricated Nanoparticles: A Promising Alternative to Agrochemicals and Possible Effects on Phytopathogens

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Abstract

Pyhtopathogens pose a significant threat to sustainable agriculture, causing significant economic losses. Nanotechnology has been increasingly applied for the control of plant pathogens affecting agricultural crops, animals and even human beings. There are many ways to produce nanoparticles (NPs) including physical, chemical and biological methods. Phytofabricated NPs as a biological method meet desired criteria against plant pathogens for sustainable agriculture due to its environmentally friendly nature, low cost and high efficiency. This chapter on reviewing literature has mentioned antibacterial, antifungal and antiviral effects of green synthesized silver, zinc, copper, iron, titanium and calcium NPs on phytopathogens, emphasizing application types and concentrations of NPs. It seems that phytofabricated NPs will be commonly used in agriculture instead of agrochemicals. Therefore, it becomes essential to analyse the

efficiency of NPs against phytopathogens not only morphological and biochemical analyses but also molecular aspect.

Keywords: Antifungal effect, antibacterial effect, antiviral effect, silver (Ag), zinc (Zn), copper (Cu), iron (Fe), titanium (Ti), Calcium (Ca)

1. Introduction

Feeding the increasing World population and sustainable agriculture are one of the most important topics in the present era. According to reports, it will be estimated that World population will reach 8.5 billion by 2030. Therefore, more food will be reqiured for demand. Improving agricultural productivity is a vital to meet this request and for this reason, both farmers and scientist have been studied to enhance plant growth, development, quality and yield by using different biotechnological methods [1].

Diseases caused by fungi, bacteria and viruses are account for almost 70% of total plant diseases. In addition to natural products, synthetic products such as pesticides, fungicides, bactericide which are generally petrochemical derivatives have been commonly used to control plant diseases [2]. On the other hand, these chemicals pose strong hazardous to soil, groundwater and even plant [3-6]. Furthermore, the most important of the adverse effects is phytopathogen resistance to agrochemicals that have an

important role in these pathogens control. This situation mostly arise from excessive and unconscious usage of agrochemicals. [7]. Therefore, it is needed to improve alternative control methods with high efficiency and low pollution for agriculture and even providing plant protection.

Nanotechnology as a modern science area deals with materials at nanoscale via knowledge and experience in physics, chemistry and biology areas [8, 9]. Nanomaterials should be in the range of 1-100 nm according to the US National Science Foundation and National Nanotechnology Initiative [10]. When compared to macroscale, biological, morphological and physicochemical characteristics are incompatible at nanoscale. Nanomaterials may be categorised based on their morphology including NPs, nanobars, nanocables, nanotubes, nanobelts, nanofibers, nanospheres and quantum dots, and composition as organic or inorganic [11, 12]. NPs can be optical, electrical, magnetic, chemical and mechanical characteristics due to their small size (<100 nm) and large specific surface area [13]. Microwave irradiation, ultrasonication, laser vaporization, solid-state thermal decomposition, hydrothermal, and sol-gel methods have been commonly used for NPs synthesis [14]. Moreover, UV-Vis (Ultraviolet-visible) spectroscopy, zeta potential, FT-IR (Fourier Transform Infrared spectroscopy), TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy), AFM (Atomic Force Microscopy), DLS (Dynamic Light Scattering),

XRD (X-Ray Diffraction) etc. have been used for detection and characterisation of NPs [15].

The nanotechnology has been widely applied in different fields such as medicine, biotechnology, chemistry and food industry. In addition, nanotechnology has also been used in agricultural practises as nanofertilizers and nanopesticides over the last few decades [16]. The extraordinary advances in nanoscience have led to use plants and their various parts to synthesise nanoparticle. The obtained products were expressed as green synthesis of nanoparticles (GS-NPs) (Figure 1). They exhibit excellent antifungal, antibacterial and antiviral effects with eco-friendly, clean, safe, cost-effective, easy, and even effective sources for high productivity and purity properties [17]. GS-NPs are more effective than the others as the NPs get attached with plant amino acids, proteins, enzymes [18]. Moreover, GS-NPs could act as reducing and stabilizing agents in the NPs synthesis processes and therefore, they have gained interest to minimise the negative environmental effects of agrochemicals [19, 20].

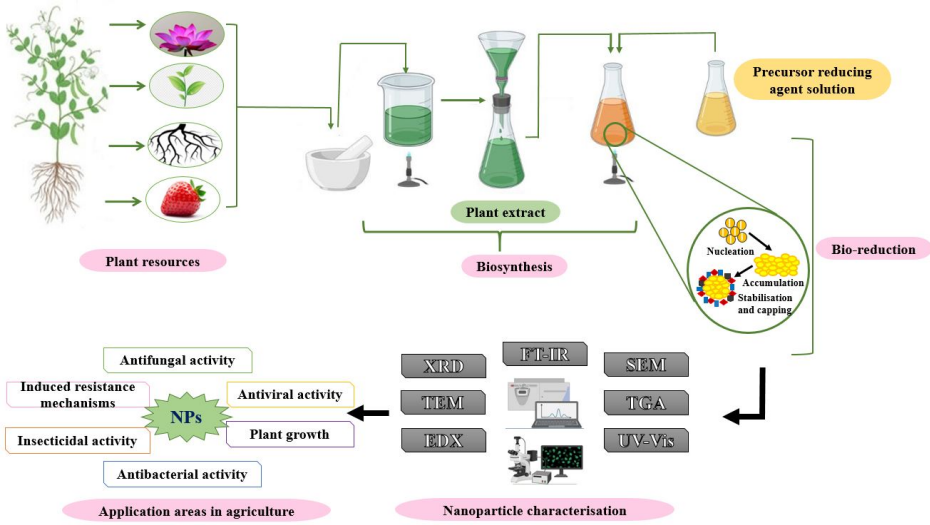

Figure 1. Schematic representation of synthesis, characterisation and application areas of NPs [21-24].

NPs are one of the newest alternatives in terms of the control of plant pathogens. The effect of NPs is based on their the small size and the high surface/volume ratio [25]. At this point, many possible mechanisms in the action of NPs against phytopathogens have been mentioned in Figure 2.

It is worth noting that the method of GS-NPs have been commonly used to synthesise NPs such as Ag, Zn, Cu, Fe, Ti and Ca. For controlling phytopathogens, plant-derived NPs are utilised for their antimicrobial, antifungal, and antiviral effects [15]. Different investigations clearly indicate the effects of GS-NPs in terms of leaf area, biomass, leaf length, root growth, synthesis of secondary metabolites, shoot length, germination

rate, lipid peroxidation, enzymatic activities, chlorophyll content, seed germination, and many more [27].

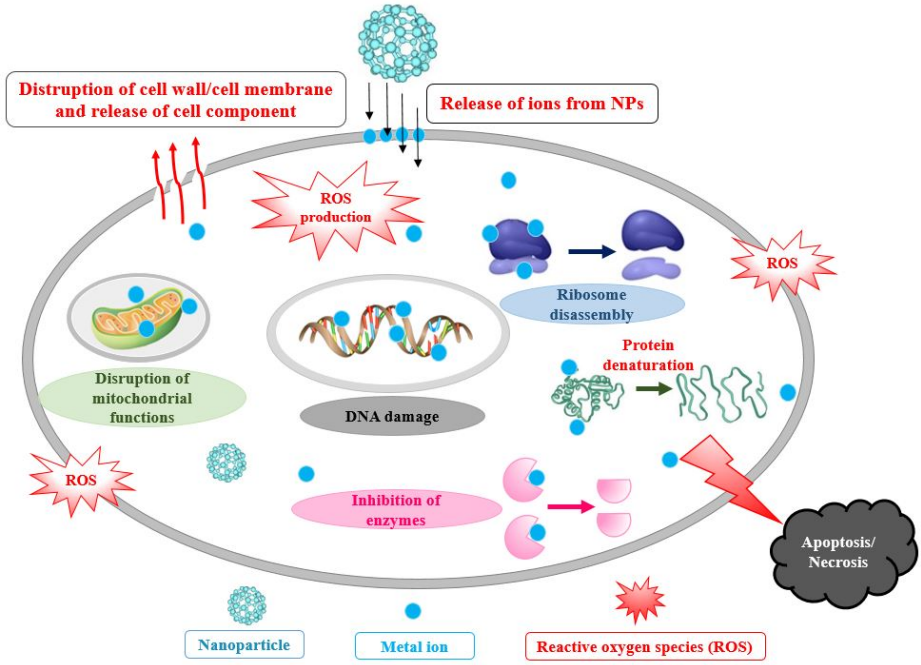

Figure 2. Mechanisms of action of GS-NPs against phytopathogens [26].

In this chapter, we summarised the recent advances in the uses of phytofabricated Ag NPs, ZnO NPs, Cu NPs, Fe_2O_3 NPs, TiO_2 NPs and Ca NPs against phytopathogens to control them and also provide the food security and sustainability.

2. Green Synthesis Ag NPs and Applications

Ag NPs have gained interest to use potential antimicrobial agents [28]. Various researchers have worked on the synthesis of Ag NPs from different plant parts to examine their antibacterial potential against phytopathogens [29]. One of them was performed by Vanti et al. [30]. They characterised and evaluated the efficacy of *Gossypium hirsutum*-derived Ag NPs against plant pathogens *Xanthomonas axonopodis* pv. *malvacearum* and *Xanthomonas campestris* pv. *campestris*. These two phytopathogens cause losses in cotton and Brassicaceae family, respectively. As a result of the disc diffusion method, they observed different zones of inhibition at 50 and 100 μg mL^{-1} concentrations. Similarly, Olfati et al. [31] examined the antibacterial effects of Ag NPs from *Calendula officinalis* on *Pectobacterium caratovorum* by using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and biofilm inhibition analyses. They reported effective concentrations of 40, 60 and 40 ppm for MIC, MBC and biofilm inhibition, respectively.

In addition to antibacterial activity, many researchers have carried out green synthesis of Ag NPs to explore their antifungal potentials [32]. Mallaiah [33] studied with ornamental flowers of *Crossandra* spp. infected by Fusarium wilt in pot culture. They reported complete mycelial inhibition at 800 ppm of Ag NPs

application whereas this ratio was 98% at 700 ppm. Moreover, *Melia azedarach*-derived Ag NPs against *Fusarium oxysporum* in tomatoes were also analysed by Ashraf et al. [34]. They concluded that 60-140 μg mL^{-1} of Ag NPs supressed fungal mycelial growth with 79-98% inhibition. Jebril et al. [35] studied the antifungal effect of Ag NPs obtained from *Melia azedarach* against *Verticillium dahliae* in eggplants. They obtained significant results in 60 ppm and 20 ppm for *in vitro* and *in vivo*, respectively. Similar *in vitro* results were reported against *Colletotrichum coccodes*, *Monilinia* sp., and *Pyricularia* by Lee et al. [36]. Moreover, synthesis of Ag NPs from strawberry indicated antifungal effects on *Ralstonia solanacearum* and *F. oxysporum*, respectively [37].

3. Green Synthesis and Applications

Phytopathogens show resistance against commercially available fungicides and bactericides even at higher concentrations, causing significant loses in crop production. Phytofabricated ZnO NPs are environmentally friendly, non-toxic, bio-safe, and biocompatible properties, and therefore provide an alternative as compared to agrochemicals. The antifungal and antibacterial effects of ZnO NPs makes them a suitable candidate for the control of plant diseases [38, 39]. Rajendran et al. [40] determined the inhibitory effect of ZnO NPs derived from *Rubus fairholmianus* root extract. For this purpose,

they revealed antibacterial activity on *Staphylococcus aureus* by using agar well diffusion, MIC and bacterial growth assay. Khan et al. [41] also analysed ZnO NPs synthesised from *Matricaria chamomilla* against *R. solanacearum* causing distruptive bacterial wilt disease in tomato and other crops. They concluded the highest growth inhibition of *R. solanacearum* at 18.0 μg mL^{-1} ZnO NP concentration. In addition, phytofabricated ZnO NP presented significant antifungal properties at 0.2 mg mL^{-1} concentration.

Ahmad et al. [42] examined *Eucalyptus globules*-derived ZnO NPs against *Alternaria mali*, *Botryosphaeria dothidea* and *Diplodia seriata* as major phytopathogens of apple orchards. Fungal growth inhibition was observed at 100 ppm concentration and inhibition rates were determined as 76.7% for *A. mali*, 65.4% for *B. dothidea* and 55.2% for *D. seriata*. Another fungus, *Alternaria alternata* caused early blight disease in *Solanum lycopersicum* was analysed by Zhu et al. [43]. They synthesised ZnO NPs from *Cinnamomum camphora* and examined the effects against this fungus. They reported strong inhibition at 20-160 mg L^{-1} for mycelial growth and 20 mg L^{-1} for spore germination and germ tube elongation, and even 20 mg L^{-1} for the best antifungal effect. Ali et al. [44] also studied with fruit rot symptoms caused by *Rhizoctonia solani* in grapefruit. They observed that ZnO NPs at 1.0 mg L^{-1} concentration synthesised from *Trachyspermum ammi* indicated very strong antifungal activity both *in vitro* and *in*

vivo experiments. Similar study was determined the effect of ZnO NPs derived from seed coat of almond on carrot plant infected with pathogenic fungus *R. solani.* In addition to antifungal affects, plant growth and photosynthetic pigments increased after spraying 50 ppm and 100 ppm ZnO NPs. Moreover, molecular docking analyses confirmed the interaction between ZnO NPs and *R. solani* by mechanical enfolding [45].

In additon to bacterial and fungal pathogens, plant viral infection is also one of the most important problems for agriculture and food security. For this reason, Abdelkhalek and Al-Askar [46] evaluated the antiviral activity of ZnO NPs (100 µg L^{-1}) against tobacco mosaic virus (TMV). They reported that the double foliar application of ZnO NPs 24 h before and also 24 h after TMV inoculation reduced by 90.21% viral accumulation level. Moreover, they oserved that *PAL*, *PR-1*, *CHS*, and *POD* genes showed varying expression results in ZnO NPs applied plants.

4. Green Synthesis Cu NPs and Applications

Copper has been used traditionally as a major component in agriculture to manage crop protection and improvement. It is a non-toxic for mammals but toxic for microorganisms, offering as an antimicrobial agent. It was shown that the antimicrobial activity of Cu NPs is higher than the Cu compounds [47].

Shende et al. [48] examined the antifungal and antibacterial effects of Cu NPs derived from *Ocimum sanctum* leaf extract on thebacterial and fungal pathogens including *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas axonopodis* pv. *punicae*, and eleven fungal pathogens including *R. solani*, *Alternaria carthami*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Colletotrichum lindemuthianum*, *Drechslera sorghicola*, *F. oxysporum* f.sp. *carthami*, *F. oxysporum* f.sp. *ciceri*, *F. oxysporum* f.sp. *udum*, *Macrophomina phaseolina*, *Rhizoctonia bataticola* and *Rhizopus stolonifer*. The highest antibacterial Cu NPs effects were observed on *X. axonopodis* pv. *punicae* (0.03 mg mL^{-1} MIC). In addition, antifungal effects showed varying results such as 0.06 mg L^{-1} MIC for *A. carthami*, *A niger*, *F. oxysporum* f.sp. *udum* and 0.03 mg mL^{-1} for other pathogens. Ahmad et al. [49] performed similar investigation on apple. They synthesised and characterised Cu NPs form Neem leaf extract (*Azadirachta indica* A. *Juss*) and determined significant antifungal activities on *A. mali* and *B. dothidea*.

Iliger et al. [50] synthesised Cu NPs using extracts of Eucalyptus (E) and Mint (M) leaves to control *Colletotrichum capsici* causing fruit rot disease in chili. They also used Carbendazim 50 WP and copper oxychloride 50 WP as standards. The highest mycelial inhibition was observed at 1000 ppm of Cu NP-M with 99.78% and then 1000 ppm of Cu NP-E with 93.75%. Cu NP-M indicated better results because of smaller size. When

compared to standards, they revealed high efficiency of Cu NPs at lower concentrations. Similar antifungal effects against *F. oxysporum* f.sp. *ciceri* was observed in chickpea by Sathiyabama et al. [51]. They synthesised curcumin derived Cu NPs and applied on chickpea plant to control wilt disease. They reported the growth inhibition for this pathogen but increase in growth parameters including root, shoot length, number of leaves and dry weight for plant.

5. Green Synthesis Fe_2O_3 NPs and Applications

Iron acting as a co-factor of enzymes is an essential for plant nutrition [52]. Iron oxide (Fe_2O_3) is a crucial micronutrient for plants and has important roles in plant metabolic processes such as respiration and photosynthesis. Initially, Fe_2O_3 NPs due to high catalytic activity and low toxicity were involved in the biological remediation of polluted soil and water [27, 53]. Smaller NPs have higher efficiency for penetrating the pathogen cell membrane. This accumulation disrupts protein and DNA structures and finally leads to the death of pathogens [54]. Therefore, the mechanism of antimicrobial activity of Fe_2O_3 NPs similar to other NPs is based on accumulation in the cytoplasm. The green synthesis of Fe_2O_3 NPs allows for the production of a large number of antimicrobial compounds with eco-friendly agents, providing sustainably energy and time [55]. Subsequently, Fe_2O_3 NPs can be widely used in agricultural practises including

biofortification, nanofertiliser, and anti-phytopathogen agents [27].

For this purpose, Fe_2O_3 NPs have been commonly studied to manage plant pathogens. One of these studies was conducted by Alam et al. [56]. Antibacterial activities of Fe_2O_3 NPs synthesised from *Skimmia laureola* leaf extracts were investigated against tomato wiltness caused by *R. solanacearum* both *in vitro* and *in vivo*. In this context, different concentrations of Fe_2O_3 NPs (1 mg mL^{-1}, 3 mg mL^{-1} and 6 mg mL^{-1}) were applied on pathogen. *In vitro* analyses showed that 6 mg mL^{-1} concentration of Fe_2O_3 NPs drastically inhibited the *R. solanacearum*. Furthermore, various concentrations (0, 1, 3 and 6%) of Fe_2O_3 NPs were applied rhizosphere region of plant in *in vivo* analyses and the disease severity was successfully decreased at the highest concentration (6%) of Fe_2O_3 NPs. They suggested that Fe_2O_3 NPs have strong antibacterial effects depending on concentration.

Mogazy et al. [57] studied with strawberry plants to determine suppressive activities of *Thymus decussatus* derived Fe_2O_3 NPs on gray mold. This disease is caused by *Botrytis cinerea*. Spore germination and fungal growth were suppressed by Fe_2O_3 NPs for *in vitro* conditions. They reported that Fe_2O_3 NPs in 200 ppm concentration were more effective than low concentration (100 ppm) for both restriction of mycelial growth and spore germination. It was observed that high concentration of Fe_2O_3

NPs showed reduction of 61,1% for mycelial growth and 77% spore germination. For *in vivo* analyses, strawberry plants were treated with 200 ppm Fe_2O_3 NPs and disease severity of *B. cinerea* was reduced by 69,7% as compared the control. In addition to these, Fe_2O_3 NPs (200 ppm) remarkably increased defense-related enzymes (such as peroxidase, β-1,3-glucanase, phenylalanine ammonia lyase), phenolics, vitamin content (A, C, E) and cell wall component when compared to the control. Similar to this study, antifungal effect of Fe_2O_3 NPs obtained from *Calotropis procera* at different concentrations were also evaluated by Ali et al. [58]. The maximum growth inhibition (87.9%) on *A. alternata* was recorded at 1.0 mg mL^{-1} concentration. Furthermore, a chemical fungicide was also used as a control, exhibiting 82.2% growth inhibition against pathogen. Additionally, the lower concentration (0.25 mg mL^{-1}) of these NPs significantly supressed growth of pathogen. Moreover, Fe_2O_3 NPs synthesised from *Tridax procumbens* supressed *Sclerotium rolfsii* and *F. oxysporum* at 50 μg mL^{-1} concentration [59].

6. Green Synthesis TiO_2 NPs and Applications

Ti has crucial role in controlling pathogens, showing higher efficiency in nanoparticle form. Nowadays, titanium dioxide (TiO_2) has broadly been used as an eco-friendly and clean photocatalyst due to its non-toxic nature [60]. TiO_2 NPs have also utilised for controlling plant pathogens such as fungi and bacteria

[61, 62]. Hossain et al. [63] studied the effects of *Citrus limon*-based TiO_2 NPs on *Dickeya dadantii*. It causes root rot diseases on sweet potato. According to diffusion assays, growth of *D. dadantii* was inhibited at 50 μg mL^{-1} TiO_2 NPs concentration. In nutrient broth, it was observed that the extents of inhibition enhanced with the increase of the concentrations (12, 25, and 50 μg mL^{-1}). *In vivo* antibacterial activity test was also conducted with sweet potato tuber slices. As a result of *in vitro* assay, they observed that tissue maceration was obstructed about 60%.

In addition antibacterial effect, Irshad et al. [64] evaluated antifungal effects of *Trianthema portulacastrum* and *Chopodium quinoa*-derived TiO_2 NPs on wheat rust caused by *Ustilago tritici* with *in vitro* assay. TiO_2 NPs derived from both plant extracts showed antifungal activities. However, the synthesised TiO_2 NPs from *C. quinoa* were found to have the best antifungal activity towards *U. tritici.* It was also reported that percent inhibition of fungal growth was 79.8% at 75 μl mL^{-1} concentration. In another study, effects of TiO_2 NPs synthesised from *Moringa oleifera*-in wheat plants were determined on *Bipolaris sorokiniana* which is responsible for spot blotch disease. TiO_2 NPs at different concentrations (20, 40, 60 and 80 mg mL^{-1}) were applied on the plants in a glasshouse. It was also reported that disease severity was notably reduced at 40 mg mL^{-1} concentration [62]. Moreover, they reported that the biosythesised TiO_2NPs increased the total protein content at the same concentration. Similar to this, Jalill et

al. [65] studied with another phytopathogenic fungi in wheat, *Fusarium graminearum*. They produced TiO_2 NPs from *Curcuma longa* and analysed antifungal activities against this pathogen. The highest fungal growth and spore inhibition rate were determined at 20 mg mL^{-1} concentration. It was also mentioned that efficiency of green synthesised TiO_2 NPs was better than industrial synthetic ones. In addition, it has been demonstrated that TiO_2 NPs have important antifungal effects against fungal phytopathogens such as *Alternaria brassicae* [66] and *Penicillium expansum* [67].

7. Green Synthesis Ca NPs and Applications

Calcium phosphate (CaP) NPs have been widely used in various fields [68]. Nowadays, they are utilised in the field of agricultural and environmental sciences. On the other hand, there are limited investigation related to the effect of Ca NP in the field of plant pathology. A study performed by Das et al. [69] can be given as an example. For this investigation, CaP NPs derived from *Panax ginseng* extracts were conjugated with streptomycin and synthesised a new NPs were named CPG-S NPs. They used disc diffusion method to evaluate antibacterial effects of CPG-S NPs against eight strains of phytopathogenic bacteria (*Pseudomonas syringae* pv. *tobacci* (Pstab), *P. syringae* pv. *tobacci* (Pstab11528), *P. syringae* pv. *tomato* (PstT1), *P. syringae* pv. *tomato* DC3000 (virulent), *P. syringae* pv. *tomato* DC3000

(avirulent), *P. syringae* pv. *actinidiae* (Kyu-10), *P. syringae* pv. *actinidiae* (Kyu-16), *Xanthomonas smithii* pv. *citri* (Yu-1)), . The results of this study showed that CPG- S NPs were highly effective on *P. syringae* pv. *actinidiae* but less efficiency was observed on *X. smithii* pv. *citri*. They also reported that CPG-S NPs improved the antibacterial activity when compared to control and only streptomycin treatment.

In another study, Mogazy et al. [57] identified the inhibitory effects of $CaCO_3$ NPs against *B. cinerea* in strawberry plants. It was observed that high concentration of $CaCO_3$ NPs decreased by 62.8% and 78% for mycelial growth and spore germination, respectively. For *in vivo*, strawberry plants were treated with 200 ppm $CaCO_3$ NPs. According to pathogenicity test, the disease severity of *B. cinerea* reduced by 80% as compared the control. In addition to these, they reported that $CaCO_3$ NPs enhanced defense-related enzymes, phenolics, vitamin and cell wall component as compared to the control.

Conclusion and Future Perspectives

Global-scale problems in agriculture have led researchers to produce alternative products. Nanotechnology is one of new approaches in this manner. Especially, GS- NPs is important tool for reducing destructive effect of agrochemicals commonly used in agriculture and their usage is encouraging in terms of

increasing crop yield and quality. On the other hand, we need to enhance our knowledge regarding the future risk of excessive use of NPs. The detailed analyses not only for pathogens but also soil, water and plants have to be performed to examine accumulation for human healths after application of NPs. Therefore, future studies should be focused on biochemical and especially molecular analyses to reveal and minimise the potential applications of NPs in sustainable agriculture.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Singh M, Srivastava M, Kumar A, Pandey KD. Biosynthesis of nanoparticles and applications in agriculture. In Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology. Woodhead Publishing. 2019; p. 199-217. DOI: 10.1016/B978-0-12-817004-5.00012-9.

[2] Ul Haq I, Ijaz S. Use of metallic nanoparticles and nanoformulations as nanofungicides for sustainable disease management in plants. In: Prasad R, Kuma V, Kumar M, editors. Nanobiotechnology in Bioformulations,. Springer, Cham. 2019. p. 289-316. DOI: 10.1007/978-3-030-17061-5_12.

[3] Baweja P, Kumar S, Kumar G. Fertilizers and pesticides: their impact on soil health and environment. In Soil Health. Springer, Cham. 2020. p. 265-285. DOI: 10.1007/978-3-030-44364-1_15.

[4] Jat M, Dohling PNK, Ahuja A, Singh J. Effect of Pesticides on Soil Ecosystem Services and Processes. Indian Journal of Entomology. 2021; 1-10. DOI: 10.55446/IJE.2021.5.

[5] Singh B, Craswell E. Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. SN Applied Sciences. 2021; 3(4): 1-24. DOI: 10.1007/s42452-021-04521-8.

[6] Pahalvi HN, Rafiya L, Rashid S, Nisar B, Kamili AN. Chemical fertilizers and their impact on soil health. In Microbiota and Biofertilizers. 2nd ed. Springer, Cham. 2021. p. 1-20. DOI: 10.1007/978-3-030-61010-4_1.

[7] Mateo-Sagasta J, Turral H. More People, More Food, Worse Water? A Global Review of Water Pollution from Agriculture In: Mateo-Sagasta J, Zadeh SM, Turral H. editors. A Global Review of Water Pollution from Agriculture, FAO and International Water Management Institute (IWMI); Rome, 2018. p. 15-38.

[8] Rai M, Yadav A, Gade A. Current [corrected] trends in phytosynthesis of metal nanoparticles. Critical Reviews in Biotechnology. 2008; 28: 277-284. DOI: 10.1080/07388550802368903.

[9] Poole CP Jr, Owens FJ. Introduction to nanotechnology. New York: Wiley; 2003. 408 p.

[10] Kargozar S, Mozafari M. Nanotechnology and nanomedicine: start small, think big. Material Today Proceedings. 2018; 5:15492-15500. DOI: 10.1016/j.matpr.2018.04.155.

[11] Hou WC, Westerhoff P, Posner JD. Biological accumulation of engineered nanomaterials: a review of current knowledge. Environmental Science: Processes & Impacts. 2013; 15:103-122. DOI: 10.1039/C2EM30686G.

[12] Rafique M, Sadaf I, Rafique MS, Tahir MB. A review on green synthesis of silver nanoparticles and their applications. Artificial cells, nanomedicine, and biotechnology. 2017; 45:1272-1291. DOI: 10.1080/21691401.2016.1241792.

[13] Mughal SS, Hassan SM. Comparative Study of AgO Nanoparticles Synthesize Via Biological, Chemical and Physical Methods: A Review. American Journal of Materials Synthesis and Processing. 2022; 7(2): 15-28. DOI: 10.11648/j.ajmsp.20220702.11.

[14] Habibullah G, Viktorova J, Ruml T. Current Strategies for Noble Metal Nanoparticle Synthesis. Nanoscale Research Letters. 2021; 16(1): 1-12. DOI: 10.1186/s11671-021-03480-8.

[15] Salem SS, Fouda A. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. Biological Trace Element Research. 2021; 199(1): 344-370. DOI: 10.1007/s12011-020-02138-3.

[16] Elemike EE, Uzoh IM, Onwudiwe DC Babalola OO. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. Applied Science. 2019; 9:499. DOI: 10.3390/app9030499.

[17] Shilpa BM, Rashmi R, Manjula NG, Sreekantha A. Bioremediation of Heavy Metal Contaminated Sites Using Phytogenic Nanoparticles. In Phytonanotechnology. Singapore: Springer; 2022. p. 227-253. DOI: 10.1007/978-981-19-4811-4_11.

[18] Ali A, Zafar H, Zia M, ul Haq I, Phull AR, Ali JS, Hussain A. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnology, Science and Applications. 2016; 9: 49. DOI: 10.2147/NSA.S99986.

[19] Adeel M, Tingting J, Hussain T, He X, Ahmad MA, Irshad MK, Shakoor N, Zhang P, Changjian X, Hao Y, Zhiyong Z, Javed R, Rui Y. Bioaccumulation of ytterbium oxide nanoparticles insinuate oxidative stress, inflammatory, and pathological lesions in ICR mice. Environmental Science and Pollution Research. 2020: 27; 32944-32953. DOI: 10.1007/s11356-020-09565-8.

[20] Santo Pereira ADE, Oliveira HC, Fraceto LF, Santaella C. Nanotechnology potential in seed priming for sustainable agriculture. Nanomaterials. 2021;11(2); 267. DOI: 10.3390/nano11020267.

[21] Rónavári A, Igaz N, Adamecz DI, Szerencsés B, Molnar C, Kónya Z, Kiricsi M. Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical

applications. Molecules. 2021; 26(4): 844. DOI: 10.3390/molecules26040844.

[22] Egorov, E., Pieters, C., Korach-Rechtman, H. Robotics, microfluidics, nanotechnology and AI in the synthesis and evaluation of liposomes and polymeric drug delivery systems. Drug Delivery and Translational Research. 2021; 11: 345–352. DOI: 10.1007/s13346-021-00929.

[23] Dikshit PK, Kumar J, Das AK, Sadhu S, Sharma S, Singh S, Kim BS. Green synthesis of metallic nanoparticles: Applications and limitations. Catalysts. 2021; 11(8): 902. DOI: 10.3390/catal11080902.

[24] Mishra P, Kumari S, Saini P, Meena RK. Role of nanotechnology in management of plant viral diseases. Materials Today Proceedings. 2022; DOI: 10.1016/j.matpr.2022.06.355.

[25] Kaur P. Biosynthesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: a review. Biotechnology Research and Innovation. 2018; 2(1): 63-73. DOI: 10.1016/j.biori.2018.09.003.

[26] Hernández-Díaz JA, Garza-García JJ, Zamudio-Ojeda A, León-Morales JM, López-Velázquez JC, García-Morales S. Plant-mediated synthesis of nanoparticles and their antimicrobial activity against phytopathogens. Journal of the Science of Food and Agriculture. 2021; 101(4): 1270-1287. DOI: 10.1002/jsfa.10767.

[27] Kumar A, Choudhary A, Kaur H, Guha S, Mehta S, Husen A. Potential applications of engineered nanoparticles in plant

disease management: a critical update. Chemosphere, 2022a; 295: 133798. DOI: 10.1016/j.chemosphere.2022.133798.

[28] Tariq M, Mohammad KN, Ahmed B, Siddiqui MA, Lee J. Biological Synthesis of Silver Nanoparticles and Prospects in Plant Disease Management. Molecules. 2022; 27(15): 4754. DOI: 10.3390/molecules27154754.

[29] Kumar I, Bhattacharya J, Das BK. Efficacy of silver nanoparticles-based foliar spray application to control plant diseases, its effect on productivity, and risk assessment. Arabian Journal of Geosciences. 2022b; 15(5): 1-11. DOI: 10.1007/s12517-022-09652-9.

[30] Vanti GL, Nargund VB, Vanarchi R, Kurjogi M, Mulla SI, Tubaki S, Patil RR. Synthesis of *Gossypium hirsutum*-derived silver nanoparticles and their antibacterial efficacy against plant pathogens. Applied Organometallic Chemistry. 2019; 33(1): e4630. DOI: 10.1002/aoc.4630.

[31] Olfati A., Kahrizi D, Balaky STJ, Sharifi R, Tahir MB, Darvishi E.. Green synthesis of nanoparticles using *Calendula officinalis* extract from silver sulfate and their antibacterial effects on *Pectobacterium caratovorum*. Inorganic Chemistry Communications. 2021; 125: 108439. DOI: 10.1016/j.inoche.2020.108439.

[32] Dridi R, Essghaier B, Hannachi H, Khedher GB, Chaffei C, Zid MF. Biosynthesized silver nanoparticles using *Anagallis monelli*: Evaluation of antioxidant activity, antibacterial and

antifungal effects. Journal of Molecular Structure. 2022; 1251: 132076. DOI: 10.1016/j.molstruc.2021.132076.

[33] Mallaiah B, Muthamilan M, Muthu CR. Antifungal activity of organically synthesized silver nanoparticles against *Fusarium incarnatum* (Desm.) Sacc., incitant of wilt in Crossandra. Applied Biological Research. 2015; 17(2): 150-157. DOI: 10.5958/0974-4517.2015.00024.5.

[34] Ashraf H, Anjum T, Riaz S, Naseem S. Microwave-assisted green synthesis and characterization of silver nanoparticles using *Melia azedarach* for the management of *Fusarium* wilt in tomato. Frontiers in Microbiology. 2020; 11: 238. DOI: 10.3389/fmicb.2020.00238.

[35] Jebril S, Jenana RKB, Dridi C. Green synthesis of silver nanoparticles using *Melia azedarach* leaf extract and their antifungal activities: In vitro and in vivo. Materials Chemistry and Physics. 2020; 248: 122898. DOI: 10.1016/j.matchemphys.2020.122898.

[36] Lee KJ, Park SH, Govarthanan M, Hwang PH, Seo YS, Cho M, Lee WH, Lee JY, Kamala-Kannan S, Oh BT. Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens, Materials Letters. 2013; 105: 128-131. DOI: 10.1016/j.matlet.2013.04.076.

[37] Khan M, Khan AU, Bogdanchikova N, Garibo D. Antibacterial and antifungal studies of biosynthesized silver nanoparticles against plant parasitic nematode *Meloidogyne incognita*, plant pathogens *Ralstonia solanacearum* and

Fusarium oxysporum. Molecules. 2021a; 26(9): 2462. DOI: 10.3390/molecules26092462.

[38] Dhiman S, Singh S, Varma A, Goel A. Phytofabricated zinc oxide nanoparticles as a nanofungicide for management of Alternaria blight of Brassica. BioMetals, 2021; 34(6): 1275-1293. DOI: 10.1007/s10534-021-00342-9.

[39] Sri D, Yelamanda Rao K, Basha S, Lakshmi M, Reddy LV, Mannarapu, M, GangaiahDamu, A. Biosynthesis of zinc oxide nanoparticles using aqueous extract of *Andrographis alata*: characterization, optimization and assessment of their antibacterial, antioxidant, antidiabetic and anti-Alzheimer's properties. Journal of Molecular Structure, 2022; 134264. DOI: 10.1016/j.molstruc.2022.134264.

[40] Rajendran NK, George BP, Houreld NN, Abrahamse H. Synthesis of zinc oxide nanoparticles using *Rubus fairholmianus* root extract and their activity against pathogenic bacteria. Molecules. 2021; 26(10): 3029. DOI: 10.3390/molecules26103029.

[41] Khan RAA, Tang Y, Naz I, Alam SS, Wang W, Ahmad M, Li Y. Management of *Ralstonia solanacearum* in tomato using ZnO nanoparticles synthesized through *Matricaria chamomilla*. Plant Disease. 2021b; 105(10): 3224-3230. DOI: 10.1094/PDIS-08-20-1763-RE.

[42] Ahmad H, Venugopal K, Rajagopal K, De Britto S, Nandini B, Pushpalatha HG, Jogaiah S. Green synthesis and characterization of zinc oxide nanoparticles using *Eucalyptus*

globules and their fungicidal ability against pathogenic fungi of apple orchards. Biomolecules. 2020b;10(3): 425. DOI: 10.3390/biom10030425.

[43] Zhu W, Hu C, Ren Y, Lu Y, Song Y, Ji Y, He J. Green synthesis of zinc oxide nanoparticles using *Cinnamomum camphora* (L.) Presl leaf extracts and its antifungal activity. Journal of Environmental Chemical Engineering. 2021; 9(6): 106659. DOI: 10.1016/j.jece.2021.106659.

[44] Ali M, Wang X, Haroon U, Chaudhary HJ, Kamal A, Ali Q, Munis MFH. Antifungal activity of Zinc nitrate derived nano ZnO fungicide synthesized from *Trachyspermum ammi* to control fruit rot disease of grapefruit. Ecotoxicology and Environmental Safety.2022; 233: 113311. DOI: 10.1016/j.ecoenv.2022.113311.

[45] Khan AU, Khan M, Malik N, Parveen A, Sharma P, Min K, Alam M. Screening of biosynthesized zinc oxide nanoparticles for their effect on *Daucus carota* pathogen and molecular docking. Microscopy Research and Technique. 2022; 85(10): 3365-3373. DOI: 10.1002/jemt.24191.

[46] Abdelkhalek A, Al-Askar AA. Green synthesized ZnO nanoparticles mediated by *Mentha spicata* extract induce plant systemic resistance against Tobacco mosaic virus. Applied Sciences. 2020; 10(15): 5054. DOI: 10.3390/app10155054.

[47] Mehta MR, Mahajan HP, Hivrale AU. Green synthesis of chitosan capped-copper nano biocomposites: Synthesis, characterization, and biological activity against plant pathogens.

BioNanoScience, 2021; 11(2): 417-427. DOI: 10.1007/s12668-021-00823-8.

[48] Shende S, Gaikwad N, Bansod S. Synthesis and evaluation of antimicrobial potential of copper nanoparticle against agriculturally important phytopathogens. International Journal of Biology Research. 2016; 1(4): 41-47.

[49] Ahmad H., Venugopal K, Bhat AH, Kavitha K, Ramanan A., Rajagopal K, Manikandan E. Enhanced biosynthesis synthesis of copper oxide nanoparticles (CuO-NPs) for their antifungal activity toxicity against major phyto-pathogens of apple orchards. Pharmaceutical Research. 2020a; 37(12): 1-12. DOI: 10.1007/s11095-020-02966-x.

[50] Iliger KS, Sofi TA, Bhat NA, Ahanger FA, Sekhar JC, Elhendi AZ, Khan F. Copper nanoparticles: Green synthesis and managing fruit rot disease of chilli caused by *Colletotrichum capsici*. Saudi Journal of Biological Sciences. 2021; 28(2): 1477-1486. DOI: 10.1016/j.sjbs.2020.12.003.

[51] Sathiyabama M, Indhumathi M, Amutha T. Preparation and characterization of curcumin functionalized copper nanoparticles and their application enhances disease resistance in chickpea against wilt pathogen. Biocatalysis and Agricultural Biotechnology. 2020; 29: 101823. DOI: 10.1016/j.bcab.2020.101823.

[52] Rout GR, Sahoo S. Role of iron in plant growth and metabolism. Reviews in Agricultural Science. 2015; 3:1-24. DOI: 10.7831/ras.3.1.

[53] Ebrahiminezhad A, Zare-Hoseinabadi A,Sarmah A. Plant-mediated synthesis and applications of iron nanoparticles. Molecular Biotechnology. 2018; 60:154-168. DOI: 10.1007/s12033-017-0053-4.

[54] Ansari SA, Oves M, Satar R, Khan A, Ahmad SI, Jafri MA, Algahtani MH. Antibacterial activity of iron oxide nanoparticles synthesized by co-precipitation technology against *Bacillus cereus* and *Klebsiella pneumoniae*. Polish Journal of Chemical Technology. 2017; *19*(4). DOI: 10.1515/pjct-2017-0076.

[55] Alkhatib R, Alkhatib B, Abdo N, Al-Eitan L, Creamer R. Physio-biochemical and ultrastructural impact of (Fe_3O_4) nanoparticles on tobacco. BMC Plant Biology. 2019; 19(1):1-12. DOI: 10.1186/s12870-019-1864-1.

[56] Alam T, Khan RAA, Ali A, Sher H, Ullah Z, Ali M. Biogenic synthesis of iron oxide nanoparticles via *Skimmia laureola* and their antibacterial efficacy against bacterial wilt pathogen *Ralstonia solanacearum*. Materials Science and Engineering: C. 2019; 98: 101-108. DOI: 10.1016/j.msec.2018.12.117.

[57] Mogazy AM, Mohamed HI, El-Mahdy OM. Calcium and iron nanoparticles: A positive modulator of innate immune responses in strawberry against *Botrytis cinerea*. Process Biochemistry. 2022; 115: 128-145. DOI: 10.1016/j.procbio.2022.02.014.

[58] Ali M, Haroon U, Khizar M, Chaudhary HJ, Munis MFH. Facile single step preparations of phyto-nanoparticles of iron in

Calotropis procera leaf extract to evaluate their antifungal potential against *Alternaria alternata*. Current Plant Biology. 2020; 23: 100157. DOI: 10.1016/j.cpb.2020.100157.

[59] Rachael MK, Rajiv P. Production and characterization of green iron oxide nanoparticles with antifungal properties against fungal pathogens. International Journal of Research Pharmaceutical Science. 2020; 11: 2308-2314. DOI: 10.26452/IJRPS.V11I2.2206.

[60] Rodríguez-González V, Terashima C, Fujishima A. Applications of photocatalytic titanium dioxide-based nanomaterials in sustainable agriculture. Journal of Photochemistry and Photobiology C: Photochemistry Reviews. 2019; 40: 49-67. DOI: 10.1016/j.jphotochemrev.2019.06.001.

[61] Ahmad NS, Abdullah N, Yasin FM. Antifungal activity of titanium dioxide nanoparticles against *Candida albicans*. BioResources. 2019; 14(4): 8866-8878. DOI: 10.15376/biores.14.4.8866-8878.

[62] Satti SH, Raja NI, Javed B, Akram A, Mashwani ZUR, Ahmad MS, Ikram M. Titanium dioxide nanoparticles elicited agro-morphological and physicochemical modifications in wheat plants to control *Bipolaris sorokiniana*. Plos one. 2021; 16(2): e0246880. DOI: 10.1371/journal.pone.0246880.

[63] Hossain A, Abdallah Y, Ali MA, Masum MMI, Li B, Sun G, An Q. Lemon-fruit-based green synthesis of zinc oxide nanoparticles and titanium dioxide nanoparticles against soft rot

bacterial pathogen *Dickeya dadantii*. Biomolecules. 2019; 9(12), 863. DOI: 10.3390/biom9120863.

[64] Irshad MA, Nawaz ., ur Rehman MZ, Imran M, Ahmad J, Ahmad S, Ali, S. Synthesis and characterization of titanium dioxide nanoparticles by chemical and green methods and their antifungal activities against wheat rust. Chemosphere. 2020; 258: 127352. DOI: 10.1016/j.chemosphere.2020.127352.

[65] Jalill RDA, Nuaman RS, Abd AN. Biological synthesis of Titanium Dioxide nanoparticles by *Curcuma longa* plant extract and study its biological properties. World Scientific News. 2016; 49 (2): 204-222.

[66] Palmqvist NGM, Bejai S, Meijer J, Seisenbaeva GA Kessler VG. Nano titania aided clustering and adhesion of beneficial bacteria to plant roots to enhance crop growth and stress management. Scientific Reports. 2015; 5:10146. DOI: 10.1038/srep10146.

[67] Maneerat C, Hayata Y. Antifungal activity of TiO_2 photocatalysis against *Penicillium expansum* in vitro and in fruit tests. International Journal of Food Microbiology. 2006; 107:99-103. DOI: 10.1016/j.ijfoodmicro.2005.08.018.

[68] Lin K, Wu C, Chang J. Advances in synthesis of calcium phosphate crystals with controlled size and shape. Acta Biomaterialia. 2014; 10(10): 4071-102. DOI: 10.1016/j.actbio.2014.06.017.

[69] Das G, Baek KH, Patra JK. Biofabrication of streptomycin-conjugated calcium phosphate nanoparticles using red ginseng

extract and investigation of their antibacterial potential. Plos one. 2019; 14(6):e0217318. DOI: 10.1371/journal.pone.0217318.

CHAPTER 2

Transfer of Plasmid DNA Into Target Cell with Nanoparticles

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Transfer of Plasmid DNA Into Target Cell with Nanoparticles

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Abstract

The plasmid can be modified by recombinant DNA technology and used as a vector allowing gene transfer between cells. DNA carrying plasmid (pDNA) is effectively used for gene transfer, gene therapy and product protein. pDNA can be delivered to the target cell in various ways. With the development of nanotechnology, various molecules such as polymers are added to the nanoparticle structure and contribute to the transfer of genes. These molecules provide stability between DNA and nanoparticles by electrostatic interaction. Nanoparticles allow a non-viral method for pDNA delivery and also have the advantages of being easy to synthesize and effective compared to using other classic methods. New molecules added to nanoparticles make the transfer more effective in the treatment of diseases with DNA vaccines. This text, simple formation steps of the pDNA and the role of nanoparticles formed with helper

molecules in the transport of DNA to the target cell will be examined.

Keywords: Plasmid DNA, nanoparticle, gene transfer, recombinant DNA technology, cation polymers, nanotechnology

1. Introduction

Today, gene transfer is easy with various methods. Up to this stage, important studies have been carried out to ensure its development of gene transfer. Some of these studies, in 1971, the work of Canna and Nathan led them to discover restriction enzymes (RE), a defense mechanism that provides protection against foreign DNA in prokaryotes [1, 2]. In 1973, first recombinant DNA molecules were produced by Paul Berg, Annie Chang, Herbert Boyer, and Stanley Cohen of Stanford University and University of California San Francisco. In 1978, David Goeddel and colleagues produced the first recombinant protein, human insulin, in *Escherichia coli* (*E. coli*) cells [3, 4]. These events contributed to the acceleration of the development of recombinant DNA technology and gene transfer.

The plasmid resides in the bacterial cell and contains the genes that give the host bacterium the ability to survive. DNA carrying plasmid (pDNA) is a vector used for gene transfer created by recombinant DNA technology [5, 6]. Recombinant DNA

technology is a method of gene transfer from a different or same organism to another organism. Recombinant DNA allows the transfer of large and complex genomes and large quantities of DNA sequences are produced. In addition, this technology also allows for the modification of complex molecules [2, 7-9]. Recombinant DNA Technology includes the basic steps of cutting the desired gene with RE, transferring this gene to the vector and expression of the DNA fragment into the host cell. [1, 2].

Nanotechnology which started with Richard Feynman in 1959, continued with the synthesis and development of nanostructures in 1974 with the contributions of Norio Taniguchi. Recently, nanotechnology is used in different scientific fields [10]. Nanoparticles (NPs) defined their size between 1-100 nm. Nanotechnology offers a new non-viral transfer way and has the advantage of efficient, easy synthesis and reliability compared to conventional therapeutic and diagnostic methods. NPs are important for gene therapy and drug delivery to eukaryotic cells due to low immunity and safe delivery. Therefore, studies show that Nanoparticles with pDNA also have a potential for gene transfer[11-16].

1.1. Plasmid DNA

The circular molecule plasmid is found in bacterial cell and its structure includes genes associated with the ability to survive [5,

6]. The basic structure of plasmid has antibiotic resistance gene, origin of replication and multiple cloning sites (MCS). In addition, terminator region, inverted repeat (IR), promoter can be also added to the structure of the plasmids in accordance with the purpose (Figure 1) [17]. pDNA are effectively used for gene transfer, gene therapy and product protein. While DNA vaccines with pDNA has advantage than recombinant protein vaccination because of pDNA allows gene carry into the nucleus of target cell, it also has disadvantage as random integration into genome, toxicity [13-17].

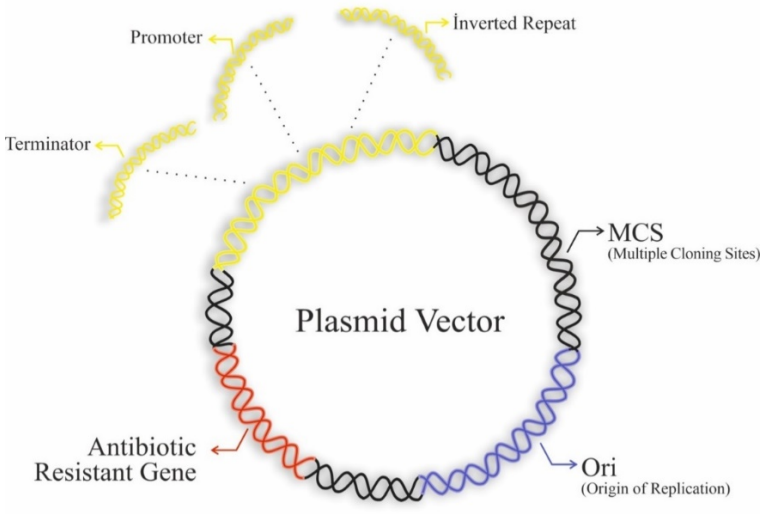

Figure 1. Basic plasmid structure [13-17].

pDNA, which is a vector, is created by recombinant DNA technique (Figure 2). The pDNA inserted into the target cell can replicate itself and create dozens of clones [2, 4, 7, 8]. There are certain stages in the preparation of the vector with Recombinant DNA Technology. DNA isolation is done from the cell for desired gene. DNA is isolated by separating from protein, RNA or another molecule. Pure DNA can extract various methods such as cetyldimethylethyl ammonium bromide (CTAB), salt target extraction [2, 5, 18-20]. The desired gene and vector are cut with same RE and the parts are completed with sticky ends by cutting [1, 2, 9, 21, 22].

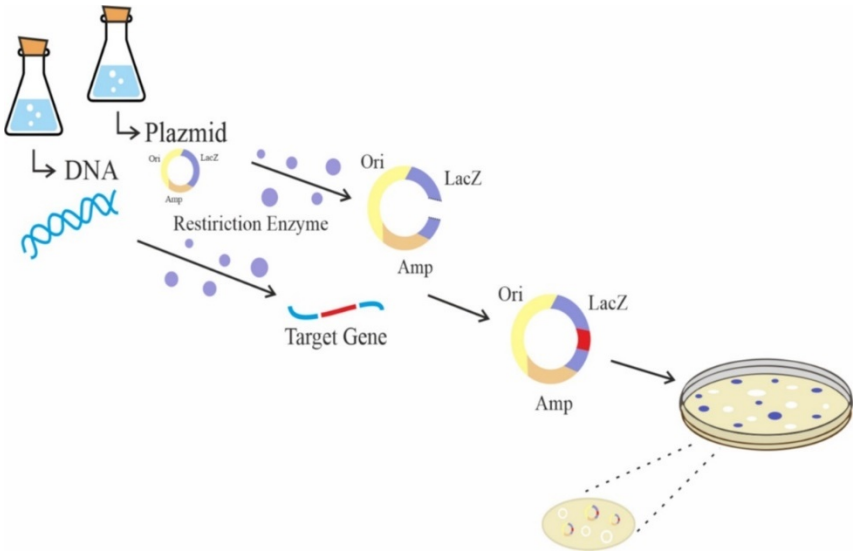

Figure 2. Transfer of target gene to plasmid [2, 4, 5, 7, 8, 18-20, 22].

Whether the gene is inserted into the vector is controlled by various methods. For this, the plasmid is transferred to an appropriate target cell. All host organisms have strengths and weaknesses. Therefore, selection of the right host cell may depend on the protein of interest. However, if a protein needs post-translational modification, bacteria can't be selected for transfer because your modification may not be present in the bacteria [8, 23, 24]. The Chinese hamster ovary (CHO) is used as a standard for recombinant protein production. While CHO has some advantages such as rapid growth and adapted culture, it also has the disadvantage as the process can take several months [24-27]. In addition, yeast cells also have a higher yield of recombinant proteins with compared mammalian cells and can secrete recombinant proteins into the extracellular environment [24, 28, 29]. After cell selection, various methods such as electroporation, calcium chloride (Ca/Cl_2), microinjection, gene gun, nanoparticle can deliver the plasmid to the target cell (Figure 3) [17, 18, 28, 30-33]. In addition nanoparticle as a new technology can use for transfer into target cell [34, 35]. After plasmid transfer into the cell, it must be confirmed that the correct vector has entered the cell. Various types of methods are available for this, such as blue-white scanning. The blue-write scanning technique is the most widely used technique to detect recombinant bacteria (Figure 2) [36-39].

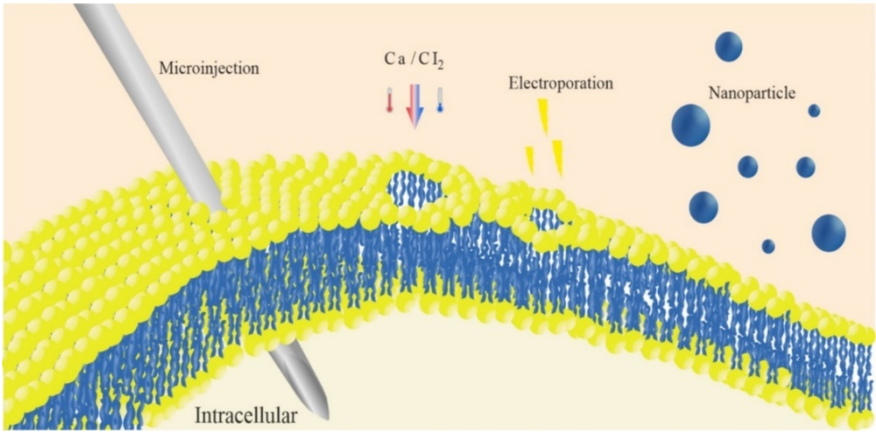

Figure 3. Various delivery of plasmid into intracellular [17, 18, 28, 30-33].

1.2. Nanoparticles

NPs have been widely used in medicine therapeutic agent, energy or industrial. Each of these NPs have its own characteristics. For instance, silver nanoparticle can be used as sensor material and electronic components [39-42]. NPs can target specific membrane protein of target cell. Some research determined that they can create complex with plasmid and supply DNA transferring as rapid and simple into cell [18, 32, 44-47]. Nanoparticle has various structure such as spherocylindrical, hemispherical, spherical can be used for transfer [11, 47, 48]. NPs can carry not only DNA but also various molecule such us messenger RNA (mRNA), small interfering RNA (siRNA) or proteins [48]. Recent research reveal that NPs are effective for

transfer of pDNA. This transport is a non-viral type of delivery [49].

Characteristics of nanoparticle is associated with various parameter such us structure, size range, distribution or morphology [11, 49, 50]. NPs have low cytotoxicity and strong protect the plasmid against enzymatic degradation [34]. There are the methods used such as Dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR) for characteristic of NPs [51].

1.3. Interaction of nanoparticles

NPs can be designed with various molecules. Some polymers can combine with nanoparticle and this structure support binding of nanoparticle to pDNA. For instance, Polyethyleneimine (PEI) and poly-L-lysine (PLL) are polycationic polymers and help the nanoparticle for combined with polyanion pDNA. The NPs form a polymer interaction using of advantage of electrostatic interactions between positively and negatively charge with pDNA [11, 34, 48, 49, 52].

1.4. Cellular uptake of nanocomplexes

NPs can enter through the cell membrane and nucleus. There are some features such as surface charge and concentration of

nanoparticle can affect cellular uptake [53, 54]. Cellular uptake of NPs can be ways through endocytic partway such as caveolin-mediated endocytosis, lipid rafts-mediated endocytosis, clathrin-mediated endocytosis, phagocytosis and macropinocytosis. Their uptake into cells occurs in different ways (Figure 4).

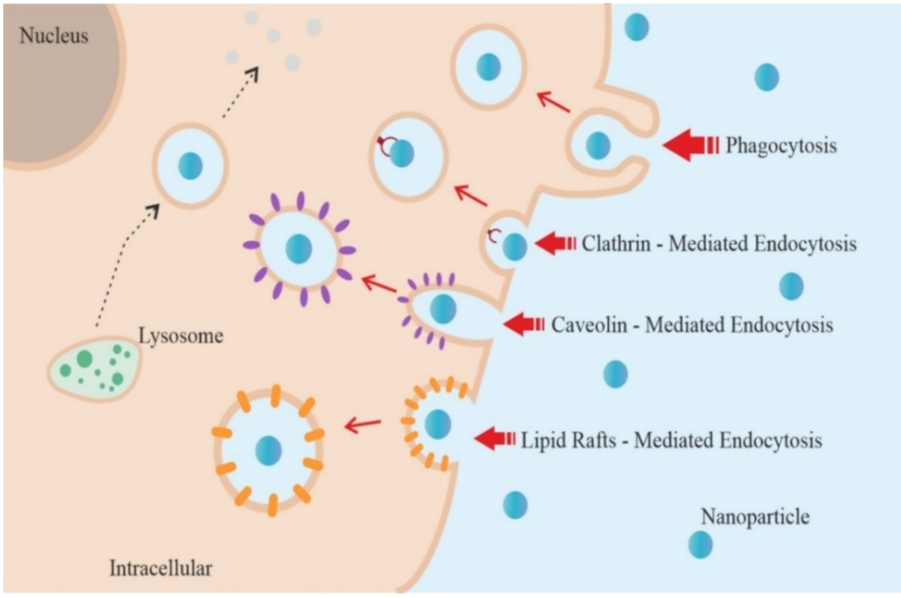

Figure 4. Cellular uptake of nanoparticles with endocytic pathway [11,12, 34, 35, 49, 55-57].

For instance, phagocytosis is the process of uptake of pathogens and molecules into the cell, while macropinocytosis is an uptake process for extracellular fluid by actin-regulated process. Clathrin-mediated endocytosis occurs via receptor-ligand binding. Caveolin-dependent endocytosis takes by creating hairpin-like caveolin coats. Another partway, this is non-

endocytic cellular uptake mechanism such as fusion and penetration. Endocytic partway has disadvantage than non-endocytic. When nanoparticle pass into cell via endocytic partway, this particle can take via lysosome and recently digested by hydrolase. As a result, entry into the cell fails [11, 12, 34, 35, 49, 55-57].

1.5. Transfer of pDNA into target cell by nano-complexes

There are several ways for plasmid transfer via nanoparticle. For instance, cationic lipids, cationic polymers, exosomes or nano-liposomes can transfer DNA into the host cell. [11, 22, 47, 58-60]. Various molecules such as PEI, PLL, polyethylene glycol (PEG) or chitosan can be combined with the nanoparticle to form a complex. These complexes are suitable components for drug and gene delivery to target cells and tissues [51, 61, 62]. In addition, the nanoparticle with these components is taken into the cell by though endocytosis and protects pDNA from enzymatic degradation [11, 48, 52, 63].

Prepared NPs have different feature own. For instance, poly (hexamethylene biguanide) (PHMB) shows different characteristics in size, charge, gene expression efficiency compared with PEI analogues. In addition, PHMB particle at pH 12 is slightly smaller than PEI particle at pH 7.4 [52]. PLL used for gene delivery, although more researched and optimized, has

low transfection efficiency [64, 65]. Cationic polymer PEI, has widely efficient transfection both in vitro and in vivo [64, 66]. PEG is an effective polymer for drug delivery. PEG can decrease the immunogenicity and increase the systemic circulation time [61]. Chitosan nanoparticle (CSNP) is a popular for gene therapy and has high transfer efficiency. Chitosan has properties such as lower molecular weight and less cytotoxicity [51, 61, 66]. Although high molecular weight chitosan forms a compact structure with pDNA and low-molecular weight chitosan can easily endocytosis, some studies show that high molecular wight chitosan also has better in vitro transfection [63, 67-71]. Polyamidoamine (PAMAM) has a similar structure to histone protein and great potential as a gene delivery vector. PAMAM, like others, is used in gene and drug delivery [66, 71, 72]. Some research demonstrate that nano-complexes are safety, no adverse reaction and no serious cytotoxicity. Nanoparticle with PAMAM can combined with pDNA and this complex is efficiently transferred to human cell culture and mice [44, 66, 74]. Some molecule can preparation together for nanoparticle. For instance, TPP nanoparticle consist three molecule these α-tocopherol, PEI, PEG. TPP strongly complexes with the plasmid. In addition, TPP has strongest transfection at 48 and 72 h compared with PEI [34].

Conclusion

New methods developed in recent years create curative effects in the nanoparticle transfer system. pDNA can be delivered reliably and easily to the target cell via nanoparticles. The properties of the components used in the connection of the nanoparticle with the pDNA are different from each other. As these properties develop, new syntheses emerge that will increase the transfer quality. So, developing delivery systems will create more efficient and precise results for future gene transfers.

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References

[1] Danna K, Nathans D. Specific cleavage of simian virus 40 DNA by restriction endonuclease of Hemophilus influenzae. Proceedings of the National Academy of Sciences. 1971;68(12):2913-7. DOI: 10.1073/pnas.68.12.2913.

[2] Karataş M. Moleküler biyoloji: Nobel Akademik Yayıncılık; 2012.

[3] Johnson IS. Human insulin from recombinant DNA technology. Science. 1983; 219(4585): 632-7. DOI: 10.1126/science.6337396.

[4] Goeddel DV, Kleid DG, Bolivar F, Heyneker HL, Yansura DG, Crea R, et al. Expression in Escherichia coli of chemically synthesized genes for human insulin. Proceedings of the National Academy of Sciences. 1979;76(1):106-10. DOI: 10.1073%2Fpnas.76.1.106.

[5] Brown TA. Gene cloning and DNA analysis: an introduction: John Wiley & Sons; 2020.

[6] Rotchanapreeda T, Ngonsawan W, Klomtun M, Somana J. The plasmid vectors, pBS2ndd and pBS3ndd, for versatile cloning with low background in Escherichia coli. World Journal of Microbiology and Biotechnology. 2018; 34(6): 1-27. DOI: 10.1007/s11274-018-2466-z.

[7] Krebs JE, Goldstein ES, Kilpatrick ST. Lewin's genes XII: Jones & Bartlett Learning; 2017.

[8] Rosano GL, Ceccarelli EA. Recombinant protein expression in Escherichia coli: advances and challenges. Frontiers in Microbiology. 2014;5:172.

[9] Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, et al. Role of recombinant DNA technology to improve life. International journal of genomics. 2016. DOI: 10.1155/2016/2405954.

[10] Atakan, A., Özgönen Özkaya, H., 2018. Fitopatolojide nanoteknoloji. Harran Tarim ve Gida Bilimleri Dergisi, 22(2): 296-303. DOI: 10.29050/harranziraat.287483. DOI: 10.29050/harranziraat.287483.

[11] Shi B, Zheng M, Tao W, Chung R, Jin D, Ghaffari D, et al. Challenges in DNA delivery and recent advances in multifunctional polymeric DNA delivery systems. Biomacromolecules.2017; 18(8): 2231-2246. DOI: 10.1021/acs.biomac.7b00803.

[12] Ma P, Li J, Gao Y, Wu J, Men K, Li C, et al. Local and Systemic Delivery of the BimS Gene Nano-Complex for Efficient Oral Squamous Cell Carcinoma Therapy. International Journal of Nanomedicine. 2022: 2925-41. DOI: 10.2147/IJN.S357702.

[13] Faria R, Vivés E, Boisguerin P, Sousa A, Costa D. Development of peptide-based nanoparticles for mitochondrial plasmid dna delivery. Polymers. 2021; 13(11):1 836. DOI: 10.3390/polym13111836.

[14] Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. Molecules. 2019; 25(1): 112. DOI: 10.3390/molecules25010112.

[15] Knoll M, Ruska E. Das elektronenmikroskop. Zeitschrift für physik. 1932; 78(5): 318-39. DOI: 10.1007/BF01342199.

[16] Keelan JA, Leong JW, Ho D, Iyer KS. Therapeutic and safety considerations of nanoparticle-mediated drug delivery in

pregnancy. Nanomedicine. 2015; 10(14): 2229-47. DOI: 10.2217/nnm.15.48.

[17] Duarte SO, Monteiro GA. Plasmid replicons for the production of pharmaceutical-grade pDNA, proteins and antigens by Lactococcus lactis cell factories. International Journal of Molecular Sciences. 2021; 22(3): 1379. DOI: 10.3390/ijms22031379.

[18] Tan H, Fu L, Seno M. Optimization of bacterial plasmid transformation using nanomaterials based on the Yoshida effect. International journal of molecular sciences. 2010; 11(12): 4962-72. DOI: 10.3390/ijms11124962.

[19] Wang T, Wang L, Zhang J, Dong W. A simplified universal genomic DNA extraction protocol suitable for PCR. Genet Mol Res. 2011; 10(1): 519-25. DOI: 10.4238/vol10-1gmr1055.

[20] Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. Journal of Biomedicine and Biotechnology. 2009; 2009. DOI: 10.1155/2009/574398.

[21] Datta N. Escherichia coli as a genetic tool. Epidemiology & Infection. 1985; 95(3): 611-8. DOI: 10.1017/s0022172400060708.

[22] Zha Y, Li Y, Lin T, Chen J, Zhang S, Wang J. Progenitor cell-derived exosomes endowed with VEGF plasmids enhance osteogenic induction and vascular remodeling in large segmental bone defects. Theranostics. 2021; 11(1): 397. DOI: 10.7150/thno.50741.

[23] Sahdev S, Khattar SK, Saini KS. Production of active eukaryotic proteins through bacterial expression systems: a review of the existing biotechnology strategies. Molecular and cellular biochemistry. 2008; 307(1): 249-64. DOI: 10.1007/s11010-007-9603-6.

[24] Ferrer-Miralles N, Saccardo P, Corchero JL, Xu Z, García-Fruitós E. General introduction: recombinant protein production and purification of insoluble proteins. Insoluble Proteins. 2015: 1-24. DOI: 10.1007/978-1-4939-2205-5_1.

[25] Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nature Biotechnology. 2004; 22(11): 1393-8. DOI: 10.1038/nbt1026.

[26] Figueroa Jr B, Ailor E, Osborne D, Hardwick JM, Reff M, Betenbaugh MJ. Enhanced cell culture performance using inducible anti-apoptotic genes E1B-19K and Aven in the production of a monoclonal antibody with Chinese hamster ovary cells. Biotechnology and bioengineering. 2007; 97(4): 877-92. DOI: 10.1002/bit.21222.

[27] Rosser MP, Xia W, Hartsell S, McCAMAN M, Zhu Y, Wang S, et al. Transient transfection of CHO-K1-S using serum-free medium in suspension: a rapid mammalian protein expression system. Protein Expression and Purification. 2005; 40(2): 237-43. DOI: 10.1016/j.pep.2004.07.015.

[28] Ardiani A, Higgins JP, Hodge JW. Vaccines based on whole recombinant Saccharomyces cerevisiae cells. FEMS yeast

research. 2010; 10(8): 1060-9. DOI: 10.1111/j.1567-1364.2010.00665.x.

[29] Ferrer-Miralles N, Domingo-Espín J, Corchero JL, Vázquez E, Villaverde A. Microbial factories for recombinant pharmaceuticals. Microbial cell factories. 2009; 8(1): 1-8. DOI: 10.1186/1475-2859-8-17.

[30] Jablonski L, Sriranganathan N, Boyle S, Carter G. Conditions for transformation of Pasteurella multocida by electroporation. Microbial pathogenesis. 1992; 12(1): 63-8. DOI: 10.1016/0882-4010(92)90066-w.

[31] Zhang D, Lee H, Zhu Z, Minhas JK, Jin Y. Enrichment of selective miRNAs in exosomes and delivery of exosomal miRNAs in vitro and in vivo. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2017; 312(1): L110-L21. DOI: 10.1152/ajplung.00423.2016.

[32] Tay A, Melosh N. Mechanical Stimulation after Centrifuge-Free Nano-Electroporative Transfection Is Efficient and Maintains Long-Term T Cell Functionalities. Small. 2021; 17(38): 2103198. DOI: 10.1002/smll.202103198.

[33] Koga Y, Yasunaga M, Moriya Y, Akasu T, Fujita S, Yamamoto S, et al. Exosome can prevent RNase from degrading microRNA in feces. Journal of gastrointestinal oncology. 2011; 2(4): 215. DOI: 10.3978/j.issn.2078-6891.2011.015.

[34] Hossian AN, Jois SD, Jonnalagadda SC, Mattheolabakis G. Nucleic Acid Delivery with α-Tocopherol-Polyethyleneimine-

Polyethylene Glycol Nanocarrier System. International journal of nanomedicine. 2020; 15: 6689. DOI: 10.2147/IJN.S259724

[35] Zhang S, Gao H, Bao G. Physical principles of nanoparticle cellular endocytosis. ACS nano. 2015; 9(9): 8655-71. DOI: 10.1021/acsnano.5b03184.

[36] Brandt ME, Gabrik AH, Vickery LE. A vector for directional cloning and expression of polymerase chain reaction products in Escherichia coli. Gene. 1991; 97(1): 113-7. DOI: 10.1016/0378-1119(91)90017-6.

[37] Juers DH, Matthews BW, Huber RE. LacZ β-galactosidase: structure and function of an enzyme of historical and molecular biological importance. Protein Science. 2012; 21(12): 1792-807. DOI: 10.1002/pro.2165.

[38] Ullmann A, Jacob F, Monod J. Characterization by in vitro complementation of a peptide corresponding to an operator-proximal segment of the β-galactosidase structural gene of Escherichia coli. Journal of molecular biology. 1967; 24(2): 339-43. DOI: 10.1016/0022-2836(67)90341-5.

[39] Riggs AD. Making, cloning, and the expression of human insulin genes in bacteria: the path to Humulin. Endocrine Reviews. 2021; 42(3): 374-80. DOI: 10.1210/endrev/bnaa029.

[40] Tan P, Li H, Wang J, Gopinath SC. Silver nanoparticle in biosensor and bioimaging: Clinical perspectives. Biotechnology and Applied Biochemistry. 2021; 68(6): 1236-42. DOI: 10.1002/bab.2045.

[41] Ramanathan S, Gopinath SC. Potentials in synthesizing nanostructured silver particles. Microsystem Technologies. 2017; 23(10): 4345-57. DOI: 10.1007/s00542-017-3382-0

[42] Logaranjan K, Raiza AJ, Gopinath SC, Chen Y, Pandian K. Shape-and size-controlled synthesis of silver nanoparticles using Aloe vera plant extract and their antimicrobial activity. Nanoscale research letters. 2016; 11(1): 1-9. DOI: 10.1186/s11671-016-1725-x.

[43] Velusamy P, Su C-H, Venkat Kumar G, Adhikary S, Pandian K, Gopinath SC, et al. Biopolymers regulate silver nanoparticle under microwave irradiation for effective antibacterial and antibiofilm activities. PLoS One. 2016; 11(6): e0157612. DOI: 10.1371/journal.pone.0157612

[44] Liu M, Guo Z, Liu J, Ren H, Guo J, Liao S, et al. Preparing PAMAM-NK4 nano complexes and examining their in vitro growth suppression effects in breast cancer. Gland Surgery. 2021; 10(9): 2695. DOI: 10.21037/gs-21-443.

[45] Pu Q, Fan X-T, Sun A-Q, Pan T, Li H, Lassen SB, et al. Co-effect of cadmium and iron oxide nanoparticles on plasmid-mediated conjugative transfer of antibiotic resistance genes. Environment International. 2021; 152: 106453. DOI: 10.1016/j.envint.2021.106453.

[46] Chen Z, Deng S, Yuan D-c, Liu K, Xiang X-c, Cheng L, et al. Novel nano-microspheres containing chitosan, hyaluronic acid, and chondroitin sulfate deliver growth and differentiation factor-5 plasmid for osteoarthritis gene therapy. Journal of

Zhejiang University-Science B. 2018; 19(12): 910-23. DOI: 10.1631/jzus.B1800095.

[47] Sun N-f, Tian A-l, Hua Y-x, Hu S-y, Hu A-b. Preparation and characterization of nano-liposome-mediated FL gene in the Lovo cells. Cancer Biotherapy and Radiopharmaceuticals. 2012; 27(8): 490-494. DOI: 10.1089/cbr.2012.1189.

[48] Hu Y, He Z, Hao Y, Gong L, Pang M, Howard GP, et al. Kinetic control in assembly of plasmid DNA/polycation complex nanoparticles. ACS nano. 2019; 13(9): 10161-78. DOI:10.1021/acsnano.9b03334.

[49] Fröhlich E. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. International journal of nanomedicine. 2012; 7: 5577. DOI: 10.2147/IJN.S36111.

[50] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature biotechnology. 2015; 33(9): 941-51. DOI: 10.1038/nbt.3330.

[51] Piri-Gharaghie T, Doosti A, Mirzaei SA. Fabrication and characterization of pcDNA3. 1 (+) location within chitosan/nanoparticles complexes for enhanced gene delivery. Iranian Journal of Biotechnology. 2022; 20(3): 88-100. DOI: 10.30498/ijb.2022.297534.3110.

[52] Chivu A, Chindera K, Mendes G, An A, Davidson B, Good L, et al. Cellular gene delivery via poly (hexamethylene biguanide)/pDNA self-assembled nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics. 2021; 158: 62-71. DOI: 10.1016/j.ejpb.2020.11.001.

[53] LaManna CM, Lusic H, Camplo M, McIntosh TJ, Barthélémy P, Grinstaff MW. Charge-reversal lipids, peptide-based lipids, and nucleoside-based lipids for gene delivery. Accounts of chemical research. 2012; 45(7): 1026-38. DOI: 10.1021/ar200228y.

[54] Shi B, Zhang H, Shen Z, Bi J, Dai S. Developing a chitosan supported imidazole Schiff-base for high-efficiency gene delivery. Polymer Chemistry. 2013; 4(3): 840-50. DOI: 10.1039/C2PY20494K.

[55] Bae Y-U, Kim B-K, Park J-W, Seu Y-B, Doh K-O. Endocytic pathway and resistance to cholesterol depletion of cholesterol derived cationic lipids for gene delivery. Molecular pharmaceutics. 2012; 9(12): 3579-85. DOI: 10.1021/mp300458h.

[56] Yameen B, Choi WI, Vilos C, Swami A, Shi J, Farokhzad OC. Insight into nanoparticle cellular uptake and intracellular targeting. Journal of controlled release. 2014; 190: 485-99. DOI: 10.1016/j.jconrel.2014.06.038.

[57] Xiang S, Tong H, Shi Q, Fernandes JC, Jin T, Dai K, et al. Uptake mechanisms of non-viral gene delivery. Journal of controlled release. 2012; 158(3): 371-8. DOI: 10.1016/j.jconrel.2011.09.093.

[58] Lindell J, Girard P, Müller N, Jordan M, Wurm F. Calfection: a novel gene transfer method for suspension cells. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression. 2004; 1676(2): 155-61. DOI: 10.1016/j.bbaexp.2003.11.016.

[59] Thomas M, Klibanov A. Non-viral gene therapy: polycation-mediated DNA delivery. Applied microbiology and biotechnology. 2003; 62(1): 27-34. DOI: 10.1007/s00253-003-1321-8.

[60] Kircheis R, Wightman L, Wagner E. Design and gene delivery activity of modified polyethylenimines. Advanced drug delivery reviews. 2001; 53(3): 341-58. DOI: 10.1016/s0169-409x(01)00202-2.

[61] Rai R, Alwani S, Badea I. Polymeric nanoparticles in gene therapy: new avenues of design and optimization for delivery applications. Polymers. 2019; 11(4): 745. DOI: 10.3390/polym11040745.

[62] Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Advanced drug delivery reviews. 2016; 99: 28-51. DOI: 10.1016/j.addr.2015.09.012.

[63] Nie H, Wang C-H. Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA. Journal of controlled release. 2007; 120(1-2): 111-21. DOI: 10.1016/j.jconrel.2007.03.018.

[64] Klemm P, Behnke M, Solomun JI, Bonduelle C, Lecommandoux S, Traeger A, et al. Self-assembled PEGylated amphiphilic polypeptides for gene transfection. Journal of Materials Chemistry B. 2021; 9(39): 8224-36. DOI: 10.1039/d1tb01495a.

[65] Merdan T, Kunath K, Fischer D, Kopecek J, Kissel T. Intracellular processing of PEI/ribozym complexes can be observed in living cells using CSLM and inhibitor experiments. Pharm Res. 2002; 19: 140-6. DOI: 10.1023/a:1014212630566.

[66] Cao D, Qin L, Huang H, Feng M, Pan S, Chen J. Transfection activity and the mechanism of pDNA-complexes based on the hybrid of low-generation PAMAM and branched PEI-1.8 k. Molecular BioSystems. 2013; 9(12): 3175-86. DOI: 10.1039/C3MB70261H.

[67] Malakooty E, Eslaminejad MB, Gheibi N, Bagheri F. Chitosan/DNA nanoparticles characteristics determine the transfection efficacy of gene delivery to human mesenchymal stem cells. Experimental Hematology. 2013; 41(8): S65. DOI: 10.3109/21691401.2013.832685.

[68] MacLaughlin FC, Mumper RJ, Wang J, Tagliaferri JM, Gill I, Hinchcliffe M, et al. Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. Journal of controlled release. 1998; 56(1-3): 259-72. DOI: 10.1016/s0168-3659(98)00097-2.

[69] Kiang T, Wen J, Lim HW, Leong KW. The effect of the degree of chitosan deacetylation on the efficiency of gene transfection. Biomaterials. 2004; 25(22): 5293-301. DOI: 10.1016/j.biomaterials.2003.12.036.

[70] Sato T, Ishii T, Okahata Y. In vitro gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan

on the transfection efficiency. Biomaterials. 2001; 22(15): 2075-80. DOI: 10.1016/s0142-9612(00)00385-9.

[71] Köping-Höggård M, Vårum K, Issa M, Danielsen S, Christensen B, Stokke B, et al. Improved chitosan-mediated gene delivery based on easily dissociated chitosan polyplexes of highly defined chitosan oligomers. Gene therapy. 2004; 11(19): 1441-52. DOI: 10.1038/sj.gt.3302312.

[72] Navarro G, de ILarduya CT. Activated and non-activated PAMAM dendrimers for gene delivery in vitro and in vivo. Nanomedicine: Nanotechnology, Biology and Medicine. 2009; 5(3): 287-97. DOI: 10.1016/j.nano.2008.12.007.

[73] Kono K, Kojima C, Hayashi N, Nishisaka E, Kiura K, Watarai S, et al. Preparation and cytotoxic activity of poly (ethylene glycol)-modified poly (amidoamine) dendrimers bearing adriamycin. Biomaterials. 2008; 29(11): 1664-75. DOI: 10.1016/j.biomaterials.2007.12.017.

[74] Majoros IJ, Myc A, Thomas T, Mehta CB, Baker JR. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules. 2006; 7(2): 572-9. DOI: 10.1021/bm0506142.

CHAPTER 3

Recombinant Protein Production Methods in Model Organisms

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Recombinant Protein Production Methods in Model Organisms

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Abstract

Model organisms are basic materials for examination cause of definite genomic mapping and can differentiate genomic DNA fragments. The fundament of their system's logic is put on DNA which we need on the mansion organism's nucleus DNA. For this combination, used gen systems include vectors which have antibiotic resistance and promotor sites and linkers. Choose the suitable mansion, vector and carrier organism to complete the interaction. When the finding perfect match can be product protein mediated to gene.

Keywords: recombinant protein, cloning, model organisms, *Escherichia coli*, DNA.

1. Introduction

Model organisms term join our life with large-scale genomic sequencing in Human Genome Project. Human Genome Project's

target sequence of the genomics of different organisms and attach the database of these consequences for using the future projects. The consequences of the Human Genome Project showed model organisms used as the reference point for different variant and genetic disorder research [1].

Human Genome Project uses yeast cell lines to discover the relationship between mammalian cells and yeast cells. This study examines a and α factor secretion pathways and coding sequences. Yeast cells secrete a factor out of the cell membrane and code with the *STE6* gene and mammalian cells secrete α factor out of the cell membrane similar pathway of a factor and coding with *mdrI. STE6* and *mdrI* sequences show us the relation and their relatedness. When discovered human metabolism for a factor secretion, it had a similar pathway to yeast cell secretion. Hence examination of that pathway found the *KEX2* gene coding this factor. Fundamental different behind the organisms to Arg-Lys linkage and this linkage use milestones for future research. This examines which included in Human Genome Project, unfolds the relation between model organisms and human cells [2].

When the researchers choose the model organisms, they want the two elements to this organism. First, model organisms present the practice chance for experiments to impractical organism research. Human or mammalian experiments not to be able

practical much but in these experiments can use model organisms for the begin of the research. Second advantage of the model organisms, when the researchers work with the model organisms, they understand fundamental of genetic and biology easier than other organisms [3].

2. Recombinant Protein Production Methods Applications in Model Organisms

2.1. Recombinant protein production in *Escherichia coli*

E. coli is highly preferred for recombinant protein production due to its known to all genetics, culture basically, low time life cycle and favourable genetic manipulation [4]. It is also used to minimize production costs. In addition, E. coli was the first host used to produce recombinant DNA (rDNA) biopharmaceuticals, which enabled the validation of Eli Lilly's rDNA human insulin in 1982 [5]. Theoretically, the following steps are applied to produce recombinant protein; The gene of interest is retrieved, cloned into an appropriate expression vector, transformed into the host of choice, induced, and then ready for protein purification and characterization. In practice, however, setbacks can occur, such as insufficient growth of the host, no protein production, or formation of inclusion bodies [6].

To prevent these conditions; vector, host strain, culture parameters of recombinant host strain can be change and co-expression can be done [4]. The promoter choose different from which the gene of need, will be cloned or changing the fusion tag, which affects the solubility of the recombinant protein in the host, means changing the vector. For obtaining recombinant proteins the pET vector include 6-Histidine tag (His-tag). For this property usually the first choice the pET vector for recombinant protein production *E.coli*-mediated. His-tag can be highly expressed with strong promoter. When the histidine-tagged gene does not express the protein or comprise of recombinant protein forms inclusion bodies in cytoplasm, the gene of interest should be cloned into different system such as the pGEX system or the GST-Tag or pMAL system. It is well known that maltose binding protein and GST tags increase fusion protein solubility and gene expression. [7-10]. The disadvantage of the pMAL system and the pGEX vector is the large sizes of the fusion tag. Provision tags should be removed after protein expression and purification, as tags can disrupt target protein structure and function [11].

So many different genetically altered *E. coli* strain are provided from biotech companies. pMAL and pGEX system which genes cloned with ‘tac’ promoter, ensure to expressed in the cloning host directly. The BL21 strain is commonly choice for production systems due to the absence of two major protease genes cloned in the pET system with T7 promoter.[4] C43(DE3)

strain can be used for the expression for gene such as coding globular or membrane protein [12].

Recombinant *E. coli* culture status affects gene expression and resolution of expressed gene. Recombinant protein solubility is increased by reducing the amount of IPTG at low temperatures and prolonging induction. Also adding chemical solutions in culture medium can induce chaperon expression and thus expression of the gene of interest increase [13]. Among the inducers that increase the expression and production of proteins, factors such as ethanol, heat shock, benzyl alcohol, osmolytes and the ionic strength of the buffer can be listed [4].

Few of the proteins need their counterparts for their stability. For this reason, they are either not expressed or immediately degrade their counterparts. In this case, proteins that interact with or stabilize the protein of interest must be identified. Genes encoding these proteins processing include co-expressed which compatible the protein of interest [14]. Removal of the signal peptide coding sequence also enhances recombinant protein expression and stability [15].

2.2. Recombinant protein production in *Escherichia virus T4*

Bacteriophages interact can human cells and other organism cells on different immune system pathways. The interaction of

bacteriophage and immune system present to explain chance viral interactions during the experiments. Broadly choose the T4 bacteriophage for recombinant protein cloning and infection which is *E. coli* mediated bacteriophage to T4. T4 bacteriophage, grow up *E. coli* and purification with affinity chromatography and size exclusion chromatography methods which is cheapest way for bacteriophage experiments. T4 bacteriophage structure include 930 major capsid (gp23) and the centre of main capsids around highly immunogenic outer capsids (gphoc) with per 155 molecules for every gphoc. These two proteins use for purification T4 to *E. coli* [16-17].

Recombinant protein production strategies mediated by T4, basic cloning and recombining technics in literature. Gateway cloning strategy using DNA fragments into the cloning vector. It helps during the infection of *E. coli*, carrying inside related gene fragments. Inside, have an expression vector for integrating our DNA fragment with the *E. coli* genome. Differently hosted *E. coli* recombinant protein production can't touch directly bacterial genome through this strategy. When they infect bacteria, virus particles lysis themselves and integrate the virus genome into the bacterial genome which is called bacteriophage. Must have all conditions and vectors optimised for *E. coli*. End of the integration successfully, processes going look like *E. coli* protein metabolism pathways and production mediated by *E. coli* [16].

2.3. Recombinant protein production in *Drosophila melanogaster*

Recombinant protein production in *Drosophila melanogaster* process with cell lines of *Drosophila melanogaster*. They have different type of cell lines and choose for your protein stabilization optimized cell line. Animal cell culture lines, very sensitive and easy contaminated cell lines like other mammalian lines. They need special laboratory conditions which call good manufacturing practice for avoid contamination with cross cell-lines and other microorganisms. In this laboratory has special conditions and precautions as researchers as cell lines. These precautions include particulars in air then oxygen and pH levels. Laboratory conditions optimize for different research and different cell- lines growth types [18].

2.4. Recombinant protein production in *Pichia pastoris*

Among the heterologous protein production systems, *Pichia pastoris* is a widely used system [19]. In order to overexpress a heterologous protein in *P. pastoris*, homologous recombination (HR) is the most used technique. In addition, electroporation is a frequently preferred transformation technique [20]. A number of *P. pastoris* promoters are currently associated with its methanol utilization pathway supressed by ethanol and glucose, and strongly stimulated by methanol [21]. For production aims the

most often used promoter is P_{Aox1}. Aox1 is encoded by the syrong methanol-inducible gene, which naturally occurs at high levels, constituting 30% of the total cell protein. Also Aox1 catalyzes the oxidation of methanol to formaldehyde. P_{Aox2} which is weaker homolog of P_{Aox1}, can be used for protein production [22].

2.5. Recombinant protein production in *Nicotiana benthamiana*

Plant systems present to low maintenance cost and avoid human pathogen contamination for protein research. Recombination for plant system the human gene must be codon optimization cause of different amino acid expression ways. Target gene regulated optimize to plant amino acid codon expression [23]. Basic cloning and increase processes can be mediated *E. coli* but *E. coli* can't into the plant cell for recombination. Generally using for recombination *Agrobacterium tumafaciens* into the target gene to plant gene. *Agrobacterium tumafaciens* have T-DNA which can integrated plant gene. Later, integration of T-DNA, bacteria create nodule for through into the plant cell same as virus genome [24].

Agrobacterium tumafaciens integration mechanism running on step by step. Nodule where on the stem, triggered pathogen defense system and secretes phytohormones. Phytohormones regulated to gravitation of T-DNAs into the plant cells and

optimize to conditions for better integration and recombinant to DNA fragments. Care T-DNAs different functional particules and support the *Agrobacterium Tumafaciens* into. During the integration, Agrobacterium able supress to Hypersensitive Responses (HR) [24].

2.6. Recombinant protein production in *Arabidopsis thaliana*

Arabidopsis thaliana is a most used organism for developmental and plant biology [25]. The studies related about gene expression, post-translational modifications, and protein targeting in this organism are well studied. An *Arabidopsis*-mediated recombinant protein production systems call as super-expression system, suitable for biochemical and structural studies has been reported by Jeong et al. With this platform, 0.4 mg of purified protein could be obtained per gram of fresh weight (FW) using a heterologous model protein (mCherry). It has been shown that homologous overexpression is beneficial for integral-membrane proteins, such as oligosaccharyltransferases (OT), that contain seven or more subunits that can be assembled on a single overexpressed central STT3a subunit [26].

Arabidopsis provides a number of convenience as a protein expression systems especially for *Arabidopsis* research and it is easy to do *Agrobacterium*-mediated floral transformation [27]. All transformed process for produced using this protocol being

that represents an independent transformation event.[28] Therefore, individual transformants are physically separated from each other, and transformants with high expression levels do not suffer a competitive growth disadvantage compared to tissue-culture-based transformation protocols. The cells can be maintained in the dark at ambient temperature (25 °C) in petri dish-based cell culture systems without the need for a large growth space or a sophisticated incubator once they have been identified. Prepared cell lines characteristically amount 2 times their mass in one week and 20-30 g can be harvested for production in laboratory experiments. Secondly, *Arabidopsis* proteins produced in *Arabidopsis* cells occur proper post-translational modifications and form active complexes with support their native partners, unlike in heterologous systems. However, it should not be ignored that these advantages can sometimes be disadvantages [26, 29].

2.7. Recombinant protein production in *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is an eukaryote model organism. It is well-understood and allows production of heterologous proteins. *Saccharomyces cerevisiae* present to low cost modification and in vitro purification after fermentation with ability of post translational modification and secretion [30]. *S. cerevisiae* is suitable for industrial fermentations due to its

tolerance to acidic pH, high sugar and ethanol include environment, and osmotic pressure [31]. During secretion, *Saccharomyces cerevisiae* frequently hyperglycosylates proteins. Leader sequences are frequently mutated and selected to reduce unprocessed and hyperglycosylated proteins and to direct proteins more effectively into secretory systems. The leader sequence can be a native signal peptide, a heterologous secretory peptide or a synthetic (designed) leader. It has been confirmed in several instances that the alpha factor leader of *Saccharomyces cerevisiae*, which has three glycosylation sites, increases protein secretion levels [32].

2.8. Recombinant protein production in *Caenorhabditis elegans*

Caenorhabditis elegans is a good model organism for learning the basics of nematode biology, thanks to its wealth of open access informative and experimental resources [33]. *C. elegans* has proved a strong model for the discovery of nematocidal drugs, the identification of drug destinations and the identification of resistance mechanisms because there are few efficient molecular instruments for parasitic nematodes. Compared with other eukaryotic organisms that express parasitic proteins heterologously, *C. elegans* has a closer relationship with parasitic nematodes. *C. elegans* has been used in several studies as a proxy expression system for study parasite gene function and regulation.

Furthermore, post-translationally modified proteins were obtained in sufficient quantities in transgenic *C. elegans* by expressing several *Haemonchus contortus* genes. It has been proposed that *C. elegans* is capable of posttranslationally modify proteins using N-glycans that are paucimannosidic, oligomannosidic, truncated complex N-glycans, phosphorylcholine (PC)-containing and fucose-rich. In spite of the fact that some N-glycans are individual to *C. elegans*, most are found in parasitic nematodes, which have antennal modifications that have not been detected in *C. elegans* [34].

Conclusion

In this book chapter, we mention recombinant protein production of different model organisms. Fundamental of used E. coli mediated transformation system for different organisms. After this section, every organism has different conditions and vectorial optimizations to comply with the target DNA and recombinant DNA. The integrated DNA fragments are helped by restriction enzymes or primers for association target DNA fragments and recombinant fragments. In bacterial systems, grows up bacteria different mediums depending on the bacteria's needs. On the other side, using the recombinant systems for animal systems we must use animal cell lines with special condition laboratory conditions. The only matter in these systems

is protection from recombinant fragments, optimize with target DNA and comfortable conditions for the mansion.

References

[1] Ankeny RA, Leonelli S. Model Organisms. Cambridge: Cambridge University Press; 2021. doi:10.1017/9781108593014.

[2]Olson, M. V. The human genome project. Proceedings of the National Academy of Sciences, 1993; 90(10), 4338–4344. DOI:10.1073/pnas.90.10.4338.

[3] Reed, L. K., Baer, C. F., & Edison, A. S. (2017). Considerations when choosing a genetic model organism for metabolomics studies. Current Opinion in Chemical Biology, 36, 7. DOI: 10.1016/j.cbpa.2016.12.005.

[4] Gopal GJ, Kumar A. Strategies for the production of recombinant protein in *Escherichia coli*. Protein J. 2013; 32(6): 419-25. DOI: 10.1007/s10930-013-9502-5.

[5] Redwan, E. M. The optimal gene sequence for optimal protein expression in *Escherichia coli*: principle requirements. Arab. J. Biotechnol, 2006, 11: 493-510.

[6] Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. Front Microbiol. 2014; 17: 5: 172. DOI: 10.3389/fmicb.2014.00172.

[7] Collins-Racie, L. A., McColgan, J. M., Grant, K. L., DiBlasio-Smith, E. A., McCoy, J. M., & LaVallie, E. R.

Production of recombinant bovine enterokinase catalytic subunit in *Escherichia coli* using the novel secretory fusion partner DsbA. Bio/technology. 1995; 13(9): 982-987.

[8] di Guana, C., Lib, P., Riggsa, P. D., & Inouyeb, H. Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. Gene. 1998; 67(1): 21-30.

[9] Hu, J., Qin, H., Gao, F. P., & Cross, T. A. A systematic assessment of mature MBP in membrane protein production: overexpression, membrane targeting and purification. Protein Expression and Purification. 2011; 80(1): 34-40.

[10] Smith, D. B., & Johnson, K. S. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene. 1998; 67(1): 31-40.

[11] Butt, T. R., Edavettal, S. C., Hall, J. P., & Mattern, M. R. SUMO fusion technology for difficult-to-express proteins. Protein Expression and Purification. 2005; 43(1): 1-9.

[12] Miroux, B., & Walker, J. E. Over-production of proteins in *Escherichia coli:* mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. Journal of Molecular Biology, 1996; 260(3): 289-298.

[13] Structural Genomics Consortium., Architecture et Fonction des Macromolécules Biologiques., Berkeley Structural Genomics Center. *et al.* Protein production and purification. Nat Methods**.** 2008; 5: 135-146. DOI: 10.1038/nmeth.f.202.

[14] De Marco, A., Vigh, L., Diamant, S., & Goloubinoff, P. Native folding of aggregation-prone recombinant proteins in Escherichia coli by osmolytes, plasmid-or benzyl alcohol–overexpressed molecular chaperones. Cell Stress & Chaperones. 2005; 10(4): 329-339.

[15] Raina, S., & Missiakas, D. Making and breaking disulfide bonds. Annual Review of Microbiology. 1997; 51, 179.

[16] Miernikiewicz, P., Owczarek, B., Piotrowicz, A., Boczkowska, B., Rzewucka, K., Figura, G., Letarov, A., Kulikov, E., Kopciuch, A., Świtała-Jeleń, K., Oślizło, A., Hodyra, K., Gubernator, J., & Dąbrowska, K. (2012). Recombinant expression and purification of T4 Phage Hoc, Soc, gp23, gp24 Proteins in Native Conformations with Stability Studies. PLoS One, 2012; 7(7). DOI: 10.1371/journal.pone.0038902.

[17] Fokine, A., Chipman, P. R., Leiman, P. G., Mesyanzhinov, V. V., Rao, V. B., & Rossmann, M. G. Molecular architecture of the prolate head of bacteriophage T4. Proceedings of the National Academy of Sciences of the United States of America, 2004; 101(16): 6003-6008. DOI: 10.1073/pnas.0400444101.

[18] Zitzmann, J., Weidner, T., Eichner, G., Salzig, D., & Czermak, P. Dielectric spectroscopy and optical density measurement for the online monitoring and control of recombinant protein production in stably transformed *Drosophila melanogaster* S2 Cells. Sensors. 2018; 18(3): 900. DOI:10.3390/s18030900

[19] Macauley-Patrick S, Fazenda ML, McNeil B, Harvey LM. Heterologous protein production using the *Pichia pastoris* expression system. Yeast. 2005; 22(4): 249–270.

[20] Cregg JM, Russell KA. Transformation. Methods in Molecular Biology. 1998; 103: 27–39.

[21] Cereghino J, Cregg J. Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. FEMS Microbiology Reviews, 2000; 24(1): 45–66.

[22] Mochizuki S, Hamato N, Hirose M et al. Expression and characterization of recombinant human antithrombin III in *Pichia pastoris*. Protein Expression and Purification. 2001; 23(1): 55–65.

[23] Soni, A. P., Lee, J., Shin, K., Koiwa, H., & Hwang, I. Production of Recombinant Active Human TGFβ1 in *Nicotiana benthamiana*. Frontiers in Plant Science. 2022. DOI: 10.3389/fpls.2022.922694.

[24] Gohlke, J., & Deeken, R. Plant responses to *Agrobacterium tumefaciens* and crown gall development. Frontiers in Plant Science. 2014. DOI: 10.3389/fpls.2014.00155.

[25] Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M. *Arabidopsis thaliana*: a model plant for genome analysis. Science. 1998; 282(662): 79–82.

[26] Jeong IS, Lee S, Bonkhofer F, Tolley J, Fukudome A, Nagashima Y, May K, Rips S, Lee SY, Gallois P, et al. Purification and characterization of *Arabidopsis thaliana* oligosaccharyltransferase complexes from the native host: a

protein super-expression system for structural studies. Plant Journal. 2018. DOI: 10.1111/tpj.13847.

[27] Clough SJ, Bent AF. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant Journal. 1998; 16735–743.

[28] Defeauz C, Clough SJ, Bent AF. Female reproductive tissues are the primary target of *Agrobacterium*-mediated transformation by the *Arabidopsis* floral dip. Plant Physiology 2000; 123895–904.

[29] Butaye KM, Goderis IJ, Wouters PF, Pues JM, Delaure SL, Broekaert WF, Depicker A, Cammue BP, De Bolle MF. Stable high-level transgene expression in *Arabidopsis thaliana* using gene silencing mutants and matrix attachment regions. Plant Journal. 2004; 39440–449. DOI: 10.1111/j.1365-313X.2004.02144.x.

[30] Schmidt F. Recombinant expression systems in the pharmaceutical industry. Applied Microbiology and Biotechnology. 2004; 65(4): 363–372.

[31] Hahn-Hägerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, GorwaGrauslund M. Towards industrial pentose-fermenting yeast strains. Applied Microbiology and Biotechnology. 2007; 74(5): 937–953.

[32] Liu, Z., Tyo, K. E. J., Martínez, J. L., Petranovic, D., & Nielsen, J. Different expression systems for production of recombinant proteins in Saccharomyces cerevisiae. Biotechnology and Bioengineering. 2012; 109(5): 1259–1268.

[33] Salinas, G., & Risi, G. *Caenorhabditis elegans*: nature and nurture gift to nematode parasitologists. Parasitology. 2018; 145(8): 979-987.

[34] Lubisch, M., Moyzio, S., Kaiser, C. S., Krafeld, I., Leusder, D., Scholz, M., Hoepfner, L., Hippler, M., Liebau, E., & Kahl, J. Using *Caenorhabditis elegans* to produce functional secretory proteins of parasitic nematodes. Acta Tropica. 2022; 225: 106176.

CHAPTER 4

Evaluation of the Anti-Cancer Effects of Naphthoquinones

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Evaluation of the Anti-Cancer Effects of Naphthoquinones

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Abstract

Cancer is a common non-infectious disease characterized by uncontrolled cell growth. Today, cancer is one of the leading causes of death worldwide, and its prevalence is predicted to increase significantly in the near future. It puts not only tremendous pressure on the health systems but also causes significant damage to the country's economies. Today, applications such as surgical intervention, radiation, and chemotherapy are used in cancer treatment, but alternative methods have started to be sought due to the many disadvantages of these methods. Various synthetic and natural quinones have been shown to have anticancer activity in studies conducted by the National Cancer Institute (NCI-USA). These quinones have chemical structures with many functional groups and the potential for cytotoxic activity. The mechanism of action of naphthoquinones on cancer cells can be pretty diverse. Some of these mechanisms are reactive oxygen species (ROS) production, p53 regulation, topoisomerase inhibition, induction of

endoplasmic reticulum (ER) -mediated apoptosis, regulation of tumor-associated inflammation, and suppression of telomerase activity. However, the mechanism underlying the anticancer effect of naphthoquinones has not yet been entirely elucidated. This review aims to evaluate the anticancer properties of naphthoquinones and examine the possible mechanisms of this relationship.

Keywords: Anti-cancer effect, cancer, naphthoquinone, reactive oxygen species

1. Introduction

Quinones are an essential group of compounds naturally found in plants, fungi, and bacteria. It is not found in animals because it is synthesized through the shikimate pathway [1]. Quinones can be defined as -ortho or -para dione conjugated to an aromatic structure. The chemical design of quinones allows them to act as electron scavenger agents by interacting with various biological molecules in oxidation-reduction reactions [2]. The majority of quinones are found in plants as benzoquinone, naphthoquinone, and anthraquinone. Naphthoquinones is the most exciting group among these groups due to their biological activity and structural properties. Naphthoquinones play an essential role as an oxidizing agent in many plant families, electron transport systems, and oxidative processes due to their easy reduction feature [3].

Naphthoquinones have physiological roles in many biological systems. Ubiquinone, plastoquinone, and vitamin K are some crucial naphthoquinones [4].

Balsaminaceae [5] , *Boraginaceae* [6], *Droseraceae* [7], *Ebenaceae* [8], *Juglandaceae* [9], *Lythraceae* [10], *Nepenthaceae* [11], *Plumbaginaceae* [12] plant families contain considerable and diverse naphthoquinone. Throughout history, naphthoquinones have been used for many years as a dye because of their yellow-brown color and in treating various diseases in folk medicine. Today, researchers show great interest in this chemical group due to the broad-spectrum biological effects such as antibacterial, antifungal, and antiparasitic [4].

2. ROS Production and Oxidative Stress

Reactive oxygen species (ROS) emerge as by-products of metabolism as a result of various biochemical reactions and the activities of organelles that use high levels of oxygen, especially mitochondria. ROS are partially reduced or excited forms of atmospheric oxygen called free radicals due to the unpaired electron in their final orbital. [13]. Superoxide anion radical (O^{-2}_2), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) are some of the intracellular ROS. The superoxide anion is produced by complexes I and III in the electron transport chain. Since it is highly reactive, it can easily pass through the inner membrane of

the mitochondria, where it is reduced to H_2O_2. Peroxisomes contain catalase enzyme, this enzyme decomposed H_2O_2 into water and oxygen and rendered ineffective. However, when peroxisomes are damaged or the activation of their related enzymes is reduced, H_2O_2 enters the cytoplasm. Here, in the presence of reduced metals such as Fe^{+2}, it is transformed into the most harmful ROS, OH^- radical, by Fenton reaction [14]. ROS can also be produced indirectly from the direct interaction between active metals and oxygen species by Fenton and Haber-Weiss reactions or indirectly by activation of enzymes such as nitric oxide synthase and NADPH oxidase [15].

Low ROS concentrations are obligatory for specific functions in the cell. Protein folding in the endoplasmic reticulum (ER) and controlling caspase activity during apoptosis are examples of these functions. However, in some cases, ROS can harm the cell. This adverse effect occurs when the balance between intracellular concentrations of ROS and endogenous antioxidant capacity is disturbed. When this balance is disturbed, molecules such as proteins and nucleic acids are damaged [16]. This situation, which occurs as a result of the disruption of the balance between the formation of ROS and its detoxification, is called oxidative stress [17].

Cancer cells have more metabolic activity and produce more ATP than normal cells. As a result of this fact, it has the capacity

to produce more endogenous ROS. This high but non-lethal concentration of ROS is thought to increase cancer cell survival chances and promote tumor growth even while damaging DNA [18]. Studies have shown that oxidative balance in cancer cells can be used for chemotherapeutic selectivity. Increasing ROS in cancer cells can disrupt the oxidative balance, which is already at a critical level. Thus, increased ROS level can cause cell death [13].

Quinones can take up a varying number of electrons to form semi-quinones and hydroquinone, both of which are highly reactive molecules. A reduction of one electron forms a semiquinone, while a reduction of two electrons forms a hydroquinone [19]. Under aerobic conditions, naphthoquinones have single-electron reductions, and the enzyme cytochrome P450 reductase catalyzes this process. This reaction is reversible, after which the semiquinone can be converted to the quinone. In this process, O_2 is reduced to form a superoxide anion radical (O^{-2}_2). In anaerobic conditions, two-electron reductions can occur [20]. The enzyme responsible for these reductions is NQO1 (NADPH: quinone oxidoreductase 1), which is abundantly expressed in cancer cells [21] These mechanisms are shown in Figure 1 [22].

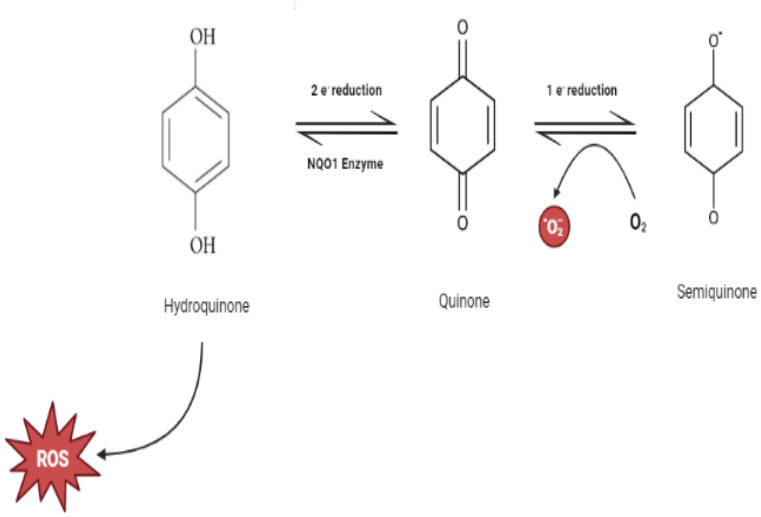

Figure 1. General reduction reactions of quinones and NQO1-mediated ROS production.

This is one of the mechanisms by which naphthoquinones may have an anti-cancer effect. Cytotoxic hydroquinones are formed by the reduction of quinones with the NQO1 enzyme. Activation of quinones with the NQO1 enzyme has been used to target cancer cells [23]. The selectivity of naphthoquinones towards cancer cells may be due to the overexpression of the NQO1 enzyme in cancer cells because naphthoquinones require this enzyme to be biochemically active [24].

2.1. The Role of NQO1 in cancer as a quinone detoxifier

NQO1 enzyme is primarily found in the cytosol that can use reduced nicotinamide adenine dinucleotide (NADH) or reduced

nicotinamide adenine dinucleotide phosphate (NADPH) as electron source. The NQO1 has a vital role in protecting the cell against harmful effects of quinones. Quinones are thought to react as substrates of this enzyme [25]. Hydroquinones formed as a result of NQO1 activity are not always stable compounds. They can react with molecular oxygen to form semi-quinones and ROS that can cause oxidative stress. [26]. Hydroquinones produced by these mechanisms can perform as an alkylating agent that can cause irreparable damage to nucleophilic sites, including DNA. Anti-cancer agents for NQO1 have been designed to target this problem [27].

NQO1 enzyme is mainly expressed in tissues that require a high antioxidant defense. In addition, studies have shown that this enzyme is higher in cancer cells than in normal cells. This situation suggested that this enzyme could be targeted to provide selectivity [28,29]. NQO1 enzyme may have a function for cancer therapy with various mechanisms. The primary function of this enzyme is to protect cells from quinone-induced mutagenic and cytotoxic effects. It has been observed that any inhibition of this enzyme suppresses cell growth. Moreover, NQO1 can also activate quinone-like compounds through reduction reactions [30].

The compounds with the naphthoquinone moiety consistently display powerful anticancer activity, closely associated with ROS

formation and alkylation. Derivatives of shikonin, a natural naphthoquinone, exhibit a remarkable capacity to generate ROS and increase alkylation levels in biological systems [31]. Nazeem et al. have shown that human skin carcinoma cells (α-431 cell line) are susceptible to plumbagin, a natural naphthoquinone, as it induces ROS production via the cooper redox cycle mechanism [32]. Menadione, a polycyclic aromatic ketone, has been shown to cause cell damage by increasing oxidative stress and may be a promising chemotherapeutic agent in cancer treatment. The efficacy of menadione against cancer is partially due to oxidative stress via the redox cycle of quinone to generate reactive oxygen species [33].

The action of lapacol is thought to be related to the production of ROS by damaging DNA and subsequently inducing apoptosis [34]. A study found that lapacol activates caspase-3 and caspase -7 and induces mitochondria-mediated cellular apoptosis. In conclusion, it has been shown that lapacol might be a potent RSK2 inhibitor [35].

2.2. Regulation of tumor suppressor factors

The p53 factor has been considered a potential target in anti-cancer therapy studies as it modulates apoptotic pathways. Another tumor suppressor, p73, such as p53, acts on the targets, p21, and $p16^{inka4}$ [36]. These factors are usually held at low levels

under basal conditions, and their concentration increases when the cell is stressed [37]. Moreover, there is the CCAAT box-binding protein (ICBP90), a nuclear protein that binds to a portion of a known sequence of the gene promoter and promotes the activity of topoisomerase IIa [38]. This protein is regulated by the p53 and p73 tumor suppressor factors and is thought to be overexpressed in several types of cancer [36].

Ishteyaque et al. observed that lawsone showed cytotoxic and anti-cancer activities when studied on human lung carcinoma, hepatocellular carcinoma cancer cell lines [39]. In another study, Lawsone inhibited SKOV-3 cells by arresting the G1/G0 phase of the cell cycle, increasing the expression of p53 and Cip1/p21 and subsequently reducing the levels of two essential proteins cyclin E and cyclin D1 and inhibited Bcl-2. caused the apoptosis of SKOV-3 cells by suppressing it [40].

In a study, plumbagin promotes cellular apoptosis and autophagy in human tongue squamous carcinoma cell (TSCC) lines, including P38 MAPK and PI3K/Akt/mTOR mediated pathways with the contribution of GSK3ß and ROS mediated pathways [41]. Induction of autophagy and apoptosis by juglone has been associated with the activation of MAPK family members (p38 and JNK) and ROS production [42].

Jang et al. have shown that shikonin up-regulates factor p73 in human cervical and breast cancer cell lines [36]. Moreover, it has also been revealed that shikonin activates the p53 factor as a result of DNA damage, decreases the expression of cdk4, and causes apoptosis in human malignant melanoma A375-S2 cells [43]. It has been reported that plumbagin up-regulates p53 expression, causes cell cycle arrest at the G2/M stage and changes the Bax/Bcl-2 ratio [44]. In human hepatocellular carcinoma cells plumbagin increases the expression of apoptosis markers such as caspase-3 and -7 [45]. In another very recent study, a natural derivative of juglone was found to induce apoptosis and necrosis in some human cancer cell lines in a caspase-dependent manner [46].

2.3. Inhibition of DNA topoisomerases

DNA topoisomerase enzymes are involved in DNA replication, transcription, recombination, and chromosome separation. All cells contain two major forms of the topoisomerase enzyme: Type I cuts single strands of DNA, and Type II cuts double-stranded DNA [47]. In a previous study, it was found that naphthoquinones inhibited both type I and type II topoisomerase enzymes found in eukaryotic cells. Type II topoisomerase enzyme is associated with cytotoxicity and has become the target of many anti-cancer agents. Topoisomerase enzymes are crucial, the balance in its activities must not be

disturbed for the cell to be healthy. Disturbance of balance induces apoptosis [48]. Especially type II topoisomerase provides the protection of the DNA helix by creating temporary breaks in DNA in DNA replication, transcription and recombination events. Krishnan and Bastow also demonstrated in vitro inhibition of type II topoisomerase by α and β-lapachone and identified them as irreversible catalytic inhibitors [49]. Although the effect of naphthoquinones on type I topoisomerases has received relatively little attention, Zhang et al. discovered that shikonin exerted an inhibitory influence on this enzyme at low concentrations [50].

Recent studies have revealed that alkannin and shikonin derivatives exhibit antineoplastic effects by inhibiting cancer cell growth, inducing apoptosis, inhibiting DNA topoisomerases, having antimitogenic effects, and reducing carcinogenesis and angiogenesis [51,52,53].

2.4. ER (Endoplasmic Reticulum) stress and ınduction of apoptosis

ER stress is a chronic disruption of the homeostasis of the ER. This is caused by the accumulation of abnormal proteins that prevent the ER from folding the proteins needed by the cell [54,55]. Changes in the Ca^{+2} level and ROS production in the cytoplasm cause ER stress. This can cause mitochondrial activity, leading to apoptosis. Shikonin, a naphthoquinone, was found to

cause cell death via ER stress in prostate cancer cell lines. It has been proven that it does by affecting the production of ROS and increasing the Ca^{+2} levels in the cell [56]. In addition, in vitro and in vivo studies have shown that this naphthoquinone inhibits the tumor proteasome, which degrade damaged or unfolded proteins [57]. As a result, it is thought that abnormal proteins accumulated in the cell may cause ER stress in the cell [56]. In addition to all these, another important study pointed out that ER stress poses a risk in terms of cytotoxicity because it induces up-regulation of the JNK/c-Jun pathway in addition to its anti-cancer effect [58].

Jin et al. evaluated a novel naphthoquinone derivative PPE8's capacity to induce ER stress in p53 null H1299 and p53 wild-type A549 cells. They showed that PPE8 induced ER stress in p53-null H1299 cells. Their outcomes indicate PPE8 as ER stress inducer in cells lacking p53, which could be a candidate to treat p53 null lung cancer [59].

Hui et al. demonstrated that shikonin significantly restricted the activity of colorectal cancer cells in a time- and dose-dependent manner. They proposed shikonin could inhibit the proliferation of the colorectal cancer cell through the activation of ROS mediated-ER stress [60].

Plumbagin, which also affects osteosarcoma cells; ROS, ER stress have been found to activate the apoptosis signaling

pathway, eliciting mitochondrial dysfunction and finally causing caspase activation [61].

3. Reported Targets Mechanisms: Recent Developments in Anti-cancer Pharmacology of Naphthoquinones

Inflammation is a critical factor in the initiation and progression of tumors and leads to tumor metastasis. Genetic and epigenetic changes in cancerous cells create an inflammatory environment that enables tumor survival and progression [62]. It has been suggested that signal transducer and activator of transcription (STAT) family proteins, particularly STAT3, selectively induce and maintain the inflammatory microenvironment during tumor initiation as well as during tumor progression [63]. A study suggested that STAT3 may be a target in the treatment of cancer even in its chronic inflammatory conditions. At the same time, they reported that STAT3 is permanently activated in tumor-associated immune cells, resulting in the suppression of innate and acquired immune responses [64].

Bhasin et al. studied the synthesis of anthraquinone and naphthoquinone derivatives using STA-$_{21}$ an inhibitor of the SH2 domain of STAT3, as a model [65]. They tested their antiproliferative activity in prostate and colon human cancer cell

lines and obtained promising results, suggesting that STAT3 may be one of many targets of the quinone class of compounds [66].

Joo et al. demonstrated the effect of plumbagin on the STAT3 signaling pathway in gastric cancer cell lines. They suggested that it negatively modulates its activity through phosphorylation rather than protein degradation. They also noted that there was plumbagin-induced apoptosis in cancer cells [67].

Tian et al. demonstrated shikonin's role in inhibiting the epidermal growth factor receptor (EGFR)- the nuclear factor kappa B (NF-κB) signaling pathway on A431 in epidermoid carcinoma cells. And shikonin has been reported to reduce the phosphorylation of EGFR and STAT3 in a concentration-dependent manner [68]. In another study in human colon cancer cells, Lawsone, a natural naphthoquinone, was shown to delay cell cycle progression by inactivating NF-kB and reducing cyclin B1 and cdk1 expression without inducing apoptosis [69].

In a study, Khaw et al. evaluated plumbagin and telomerase activity in human glioblastoma multiforme and medulloblastoma cells. Plumbagin was confirmed to perform mechanisms such as cell cycle arrest in the G2/M phase, suppressing telomerase activity, and shortening of telomeres after 15 days of incubation with the compound [44].

Lim et al. started researching mucosa-associated lymphoid tissue lymphoma translocation protein (MALT1) inhibitors to find new drugs to treat diffuse large B-cell lymphoma, one of the cancer types. They discovered that 1,2-amino-naphthoquinones were suitable inhibitors but could not inhibit the proliferation of OCI-LY3 cells (human B-cell lymphoma) in vitro. While testing β-lapachone, they found that it is a highly potent MALT1 inhibitor and a significant cell growth inhibitor [70].

In a study, plumbagin showed anticancer effects by inhibiting proliferation and inducing apoptosis in human esophageal squamous cell carcinoma (ESCC) cells in vitro and in vivo. It is thought that the reason why Plumbagin exhibits these effects is due to the cancellation of STAT3-PLK1-AKT signaling [71]. In another study plumbagin significantly inhibited the proliferation and invasion of L9981 and NL9980 cells and could be an effective treatment for LCLC (large cell lung cancer) by targeting the IL-6/STAT3 signaling pathway [72].

Conclusion

Naphthoquinones are a plant-derived biochemically highly active chemical group. In this review, the anti-cancer activity and mechanisms of naphthoquinones are summarized. In our review, the anti-cancer effects of naphthoquinones were discussed by increasing the amount of ROS mediated by the NQO1 enzyme,

creating ER stress, inhibiting DNA topoisomerase enzymes, and regulating tumor suppressor factors. However, its anti-cancer activities still need to be fully elucidated. These mechanisms might be investigated profoundly in future studies, and a new approach to cancer treatment might be developed.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Monks TJ, Jones DC. The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. Curr Drug Metab. 2002; 3:425–438. DOI: 10.2174/1389200023337388.

[2] El-Najjar N, Gali-Muhtasib H, Ketola RA, Vuorela P, Urtti A, Vuorela H. The chemical and biological activities of quinones: overview and implications in analytical detection. Phytochem Rev. 2011; 10:353–370. DOI: 10.1007/s11101-011-9209-1.

[3] Ventura Pinto A, Lisboa de Castro S. The trypanocidal activity of naphthoquinones: a review. Molecules. 2009; 14:4570–4590. DOI: 10.3390/molecules14114570.

[4] Babula P, Adam V, Havel L, Kizek R. Noteworthy secondary metabolites naphthoquinones-their occurrence, pharmacological properties and analysis. Curr Pharm Anal. 2009; 5:47–68. DOI: 10.3390/jof7050370.

[5] Sakunphueak A, Panichayupakaranant P. Simultaneous determination of three naphthoquinones in the leaves of Impatiens balsamina L. by reversed-phase high-performance liquid chromatography. Phytochem Anal. 2010; 21:444–450. DOI: 10.1002/pca.1216.

[6] Assimopoulou AN, Karapanagiotis I, Vasiliou A, Kokkini S, Papageorgiou VP. Analysis of alkannin derivatives from Alkanna species by high-performance liquid chromatography/photodiode array/mass spectrometry. Biomed Chromatogr. 2006; 20:1359–1374. DOI: 10.1002/bmc.705.

[7] Zenk MH, Fürbringer M, Steglich W. Occurrence and distribution of 7-methyljuglone and plumbagin in the Droseraceae. Phytochemistry. 1969; 8:2199–2200. DOI: 10.1016/S0031-9422(00)88181-9.

[8] Kuete V, Tangmouo JG, Meyer JJM, Lall N. Diospyrone, crassiflorone and plumbagin: three antimycobacterial and antigonorrhoeal naphthoquinones from two Diospyros spp. Int J Antimicrob Agents. 2009; 34:322–325. DOI: 10.1016/j.ijantimicag.2009.04.008.

[9] Hirakawa K, Ogiue E, Motoyoshiya J, Yajima M. Naphthoquinones from juglandaceae. Phytochemistry. 1986; 25:1494–1495. DOI: 10.1177/1934578X1601100643.

[10] Mahkam M, Nabati M, Rahbar Kafshboran H. Isolation, identification and characterization of lawsone from henna leaves powder with soxhlet technique. Q J Iran Chem Commun. 2014; 2:34–38.

[11] Raj G, Kurup R, Hussain AA, Baby S. Distribution of naphthoquinones, plumbagin, droserone, and 5-O-methyl droserone in chitin-induced and uninduced Nepenthes khasiana: molecular events in prey capture. J Exp Bot. 2011; 62:5429–5436. DOI: 10.1093/jxb/err219.

[12] Hook I, Mills C, Sheridan H. Bioactive naphthoquinones from higher plants. Stud Nat Prod Chem. 2014; 41:119–160. DOI: 10.1021/np100358a.

[13] Wellington KW. Understanding cancer and the anticancer activities of naphthoquinones–a review. RSC Adv. 2015; 5:20309–20338. DOI: 10.1039/C4RA13547D.

[14] Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinsons Dis. 2013; 3:461–491. DOI: 10.3233/JPD-130230.

[15] Smith DG, Cappai R, Barnham KJ. The redox chemistry of the Alzheimer's disease amyloid β peptide. Biochim Biophys Acta (BBA)-Biomembranes. 2007; 1768:1976–1990. DOI: 10.1016/j.bbamem.2007.02.002.

[16] Veskoukis AS, Tsatsakis AM, Kouretas D. Dietary oxidative stress and antioxidant defense with an emphasis on plant extract administration. Cell Stress Chaperones. 2012; 17:11–21. DOI: 10.1007/s12192-011-0293-3.

[17] Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. Front Aging Neurosci. 2010; 2:12. DOI: 10.3389/fnagi.2010.00012.

[18] Poljšak B, Fink R. The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. Oxid Med Cell Longev. 2014; 2014. DOI: 10.1155/2014/671539.

[19] Stangel M, Zettl UK, Mix E, Zielasek J, Toyka K V, Hartung H-P, Gold R. H2O2 and nitric oxide-mediated oxidative stress induce apoptosis in rat skeletal muscle myoblasts. J Neuropathol Exp Neurol. 1996; 55:36–43. DOI: 10.1097/00005072-199601000-00004.

[20] Abreu FC de, Ferraz PA de L, Goulart MOF. Some applications of electrochemistry in biomedical chemistry. Emphasis on the correlation of electrochemical and bioactive properties. J Braz Chem Soc. 2002; 13:19–35. DOI: 10.1590/S0103-50532002000100004.

[21] Lee H, Oh E-T, Choi B-H, Park M-T, Lee J-K, Lee J-S, Park HJ. NQO1-induced activation of AMPK contributes to cancer cell death by oxygen-glucose deprivation. Sci Rep. 2015; 5:1–11. DOI: 10.1038/srep07769.

[22] Tandon VK, Kumar S. Recent development on naphthoquinone derivatives and their therapeutic applications as

anticancer agents. Expert Opin Ther Pat. 2013; 23:1087–1108. DOI: 10.1517/13543776.2013.798303.

[23] Siegel D, Yan C, Ross D. NAD (P) H: quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones. Biochem Pharmacol. 2012; 83:1033–1040. DOI: 10.1016/j.bcp.2011.12.017.

[24] Pink JJ, Planchon SM, Tagliarino C, Varnes ME, Siegel D, Boothman DA. NAD (P) H: quinone oxidoreductase activity is the principal determinant of β-lapachone cytotoxicity. J Biol Chem. 2000; 275:5416–5424. DOI: 10.1074/jbc.275.8.5416.

[25] Segura-Aguilar J, Lind C. On the mechanism of the Mn3+-induced neurotoxicity of dopamine: Prevention of quinone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. Chem Biol Interact. 1989; 72:309–324. DOI: 10.1016/0009-2797(89)90006-9.

[26] Cadenas E. Antioxidant and prooxidant functions of DT-diaphorase in quinone metabolism. Biochem Pharmacol. 1995; 49:127–140. DOI: 10.1016/s0006-2952(94)00333-5.

[27] Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD (P) H: quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact. 2000; 129:77–97. DOI: 10.1016/s0009-2797(00)00199-x.

[28] Ross D, Beall HD, Siegel D, Traver RD, Gustafson DL. Enzymology of bioreductive drug activation. Br J Cancer Suppl. 1996; 27:S1.

[29] Awadallah NS, Dehn D, Shah RJ, Nash SR, Chen YK, Ross D, Bentz JS, Shroyer KR. NQO1 expression in pancreatic cancer and its potential use as a biomarker. Appl Immunohistochem Mol Morphol. 2008; 16:24–31. DOI: 10.1097/PAI.0b013e31802e91d0.

[30] Pidugu LSM, Mbimba JC, Ahmad M, Pozharski E, Sausville EA, Emadi A, Toth EA. A direct interaction between NQO1 and a chemotherapeutic dimeric naphthoquinone. BMC Struct Biol. 2016; 16:1–10. DOI 10.1186/s12900-016-0052-x.

[31] Wang R, Zhou W, Meng Q, Zhang X, Ding J, Xu Y, Song H, Yang K, Cui J, Li S. Design, synthesis, and biological evaluation of shikonin and alkannin derivatives as potential anticancer agents via a prodrug approach. ChemMedChem. 2014; 9:2798–2808. DOI: 10.1002/cmdc.201402224.

[32] Nazeem S, Azmi AS, Hanif S, Ahmad A, Mohammad RM, Hadi SM, Kumar KS. Plumbagin induces cell death through a copper-redox cycle mechanism in human cancer cells. Mutagenesis. 2009; 24:413–418. DOI: 10.1093/mutage/gep023.

[33] Monteiro JP, Martins AF, Nunes C, Morais CM, Lúcio M, Reis S, Pinheiro TJT, Geraldes CFGC, Oliveira PJ, Jurado AS. A biophysical approach to menadione membrane interactions: relevance for menadione-induced mitochondria dysfunction and related deleterious/therapeutic effects. Biochim Biophys Acta (BBA)-Biomembranes. 2013; 1828:1899–1908. DOI: 10.1016/j.bbamem.2013.04.006.

[34] Kandioller W, Balsano E, Meier SM, Jungwirth U, Göschl S, Roller A, Jakupec MA, Berger W, Keppler BK, Hartinger CG. Organometallic anticancer complexes of lapachol: Metal centre-dependent formation of reactive oxygen species and correlation with cytotoxicity. Chem Commun. 2013; 49:3348–3350. DOI: 10.1039/c3cc40432c.

[35] Zu X, Xie X, Zhang Y, Liu K, Bode AM, Dong Z, Kim DJ. Lapachol is a novel ribosomal protein S6 kinase 2 inhibitor that suppresses growth and induces intrinsic apoptosis in esophageal squamous cell carcinoma cells. Phyther Res. 2019; 33:2337–2346. DOI: 10.1002/ptr.6415.

[36] Jang SY, Hong D, Jeong SY, Kim J-H. Shikonin causes apoptosis by up-regulating p73 and down-regulating ICBP90 in human cancer cells. Biochem Biophys Res Commun. 2015; 465:71–76. DOI: 10.1016/j.bbrc.2015.07.131.

[37] Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, Minty A, Chalon P, Lelias J-M, Dumont X. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell. 1997; 90:809–819. DOI: 10.1016/s0092-8674(00)80540-1.

[38] Hopfner R, Mousli M, Jeltsch J-M, Voulgaris A, Lutz Y, Marin C, Bellocq J-P, Oudet P, Bronner C. ICBP90, a novel human CCAAT binding protein, involved in the regulation of topoisomerase IIα expression. Cancer Res. 2000; 60:121–128.

[39] Ishteyaque S, Mishra A, Mohapatra S, Singh A, Bhatta RS, Tadigoppula N, Mugale MN. In vitro: cytotoxicity, apoptosis and

ameliorative potential of Lawsonia inermis extract in human lung, Colon and Liver Cancer cell line. Cancer Invest. 2020; 38:476–485. DOI: 10.1080/07357907.2020.1811300.

[40] Li P, Xin H, Liu W. Lawsone inhibits cell growth and improves the efficacy of cisplatin in SKOV-3 ovarian cancer cell lines. African J Tradit Complement Altern Med. 2017; 14:8–17. DOI: 10.21010/ajtcam.v14i5.2.

[41] Pan S-T, Qin Y, Zhou Z-W, He Z-X, Zhang X, Yang T, Yang Y-X, Wang D, Qiu J-X, Zhou S-F. Plumbagin induces G2/M arrest, apoptosis, and autophagy via p38 MAPK-and PI3K/Akt/mTOR-mediated pathways in human tongue squamous cell carcinoma cells. Drug Des Devel Ther. 2015; 9:1601. DOI: 10.2147/DDDT.S76057.

[42] Wang P, Gao C, Wang W, Yao L-P, Zhang J, Zhang S-D, Li J, Fang S-H, Fu Y-J. Juglone induces apoptosis and autophagy via modulation of mitogen-activated protein kinase pathways in human hepatocellular carcinoma cells. Food Chem Toxicol. 2018; 116:40–50. DOI: 10.1016/j.fct.2018.04.004.

[43] Wu Z, Wu L, Li L, Tashiro S, Onodera S, Ikejima T. p53-mediated cell cycle arrest and apoptosis induced by shikonin via a caspase-9-dependent mechanism in human malignant melanoma A375-S2 cells. J Pharmacol Sci. 2004; 94:166–176. DOI: 10.1254/jphs.94.166.

[44] Khaw AK, Sameni S, Venkatesan S, Kalthur G, Hande MP. Plumbagin alters telomere dynamics, induces DNA damage and cell death in human brain tumour cells. Mutat Res Toxicol

Environ Mutagen. 2015; 793:86–95. DOI: 10.1016/j.mrgentox.2015.06.004.

[45] Hwang GH, Ryu JM, Jeon YJ, Choi J, Han HJ, Lee Y-M, Lee S, Bae J-S, Jung J-W, Chang W. The role of thioredoxin reductase and glutathione reductase in plumbagin-induced, reactive oxygen species-mediated apoptosis in cancer cell lines. Eur J Pharmacol. 2015; 765:384–393. DOI: 10.1016/j.ejphar.2015.08.058.

[46] Sun W, Bao J, Lin W, Gao H, Zhao W, Zhang Q, Leung C-H, Ma D-L, Lu J, Chen X. 2-Methoxy-6-acetyl-7-methyljuglone (MAM), a natural naphthoquinone, induces NO-dependent apoptosis and necroptosis by H2O2-dependent JNK activation in cancer cells. Free Radic Biol Med. 2016; 92:61–77. DOI: 10.1016/j.freeradbiomed.2016.01.014.

[47] Nitiss JL, Soans E, Rogojina A, Seth A, Mishina M. Topoisomerase assays. Curr Protoc Pharmacol. 2012; 57:3. DOI: 10.1002/0471141755.ph0303s57.

[48] Silva MN da, Ferreira VF, de Souza MCB V. An overview of the chemistry and pharmacology of naphthoquinones with emphasis on beta-lapachone and derivatives. Quim Nova. 2003; 26:407–416. DOI: 10.1590/S0100-40422003000300019.

[49] Krishnan P, Bastow KF. Novel mechanisms of DNA topoisomerase II inhibition by pyranonaphthoquinone derivatives—eleutherin, α lapachone, and β lapachone∗. Biochem Pharmacol. 2000; 60:1367–1379. DOI: 10.1016/s0006-2952(00)00437-8.

[50] Zhang F-L, Wang P, Liu Y-H, Liu L, Liu X-B, Li Z, Xue Y-X. Topoisomerase I inhibitors, shikonin and topotecan, inhibit growth and induce apoptosis of glioma cells and glioma stem cells. PLoS One. 2013; 8:e81815. DOI: 10.1371/journal.pone.0081815.

[51] Papageorgiou VP, Assimopoulou AN, Samanidou VF, Papadoyannis IN. Recent advances in chemistry, biology and biotechnology of alkannins and shikonins. Curr Org Chem. 2006; 10:2123–2142. DOI: 10.2174/138527206778742704.

[52] Zheng Q, Li Q, Zhao G, Zhang J, Yuan H, Gong D, Guo Y, Liu X, Li K, Lin P. Alkannin induces cytotoxic autophagy and apoptosis by promoting ROS-mediated mitochondrial dysfunction and activation of JNK pathway. Biochem Pharmacol. 2020; 180:114167. DOI: 10.18632/aging.203488.

[53] Plyta ZF, Li T, Papageorgiou VP, Mellidis AS, Assimopoulou AN, Pitsinos EN, Couladouros EA. Inhibition of topoisomerase I by naphthoquinone derivatives. Bioorg Med Chem Lett. 1998; 8:3385–3390. DOI: 10.1016/s0960-894x(98)00600-3.

[54] Bánhegyi G, Baumeister P, Benedetti A, Dong D, Fu Y, Lee AS, Li J, Mao C, Margittai E, Ni MIN. Endoplasmic reticulum stress. Ann N Y Acad Sci. 2007; 1113:58–71. DOI: 10.1196/annals.1391.007.

[55] Parmar VM, Schröder M. Sensing endoplasmic reticulum stress. Self Nonself. 2012:153–168. DOI: 10.1007/978-1-4614-1680-7_10.

[56] Gara RK, Srivastava VK, Duggal S, Bagga JK, Bhatt MLB, Sanyal S, Mishra DP. Shikonin selectively induces apoptosis in human prostate cancer cells through the endoplasmic reticulum stress and mitochondrial apoptotic pathway. J Biomed Sci. 2015; 22:1–12. DOI: 10.1186/s12929-015-0127-1.

[57] Yang H, Zhou P, Huang H, Chen D, Ma N, Cui QC, Shen S, Dong W, Zhang X, Lian W. Shikonin exerts antitumor activity via proteasome inhibition and cell death induction in vitro and in vivo. Int J Cancer. 2009; 124:2450–2459. DOI: 10.1002/ijc.24195.

[58] Liu J, Wang P, Xue Y, Li Z, Qu C, Liu Y. Enhanced antitumor effect of shikonin by inhibiting Endoplasmic Reticulum Stress via JNK/c-Jun pathway in human glioblastoma stem cells. Biochem Biophys Res Commun. 2015; 466:103–110. DOI: 10.1016/j.bbrc.2015.08.115.

[59] Lien J-C, Huang C-C, Lu T-J, Tseng C-H, Sung P-J, Lee H-Z, Bao B-Y, Kuo Y-H, Lu T-L. Naphthoquinone derivative PPE8 induces endoplasmic reticulum stress in p53 null H1299 cells. Oxid Med Cell Longev. 2015; 2015. DOI: 10.1155/2015/453679.

[60] Qi H, Zhang X, Liu H, Han M, Tang X, Qu S, Wang X, Yang Y. Shikonin induced Apoptosis Mediated by Endoplasmic Reticulum Stress in Colorectal Cancer Cells. J Cancer. 2022; 13:243. DOI: 10.7150/jca.65297.

[61] Chao C, Hou S, Huang CC, Hou C, Chen P, Liu J. Plumbagin induces apoptosis in human osteosarcoma through ROS generation, endoplasmic reticulum stress and mitochondrial

apoptosis pathway. Mol Med Rep. 2017; 16:5480–5488. DOI: 10.3892/mmr.2017.7222.

[62] Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009; 9:798–809. DOI: 10.1038/nrc2734.

[63] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454:436–444. DOI: 10.1038/nature07205.

[64] Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, Pilon-Thomas S, Niu G, Kay H, Mulé J, Kerr WG. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med. 2005; 11:1314–1321. DOI: 10.1038/nm1325.

[65] Song H, Wang R, Wang S, Lin J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. Proc Natl Acad Sci. 2005; 102:4700–4705. DOI: 10.1073/pnas.0409894102.

[66] Bhasin D, Etter JP, Chettiar SN, Mok M, Li P-K. Antiproliferative activities and SAR studies of substituted anthraquinones and 1, 4-naphthoquinones. Bioorg Med Chem Lett. 2013; 23:6864–6867. DOI: 10.1016/j.bmcl.2013.09.098.

[67] Joo MK, Park J, Kim SH, Yoo HS, Lee BJ, Chun HJ, Lee SW, Bak Y. Antitumorigenic effect of plumbagin by induction of SH2-containing protein tyrosine phosphatase 1 in human gastric cancer cells. Int J Oncol. 2015; 46:2380–2388. DOI: 10.3892/ijo.2015.2935.

[68] Tian R, Li Y, Gao M. Shikonin causes cell-cycle arrest and induces apoptosis by regulating the EGFR–NF-κB signalling pathway in human epidermoid carcinoma A431 cells. Biosci Rep. 2015; 35. DOI: 10.1042/BSR20150002.

[69] Wang S-B, Tao Z, Li P. Lawsone suppresses azoxymethane mediated colon cancer in rats and reduces proliferation of DLD-1 cells via NF-κB pathway. Biomed Pharmacother. 2017; 89:152–161. DOI: 10.1016/j.biopha.2017.01.169.

[70] Lim SM, Jeong Y, Lee S, Im H, Tae HS, Kim BG, Park HD, Park J, Hong S. Identification of β-lapachone analogs as novel MALT1 inhibitors to treat an aggressive subtype of diffuse large B-cell lymphoma. J Med Chem. 2015; 58:8491–8502. DOI: 10.1021/acs.jmedchem.5b01415.

[71] Cao Y-Y, Yu J, Liu T-T, Yang K-X, Yang L-Y, Chen Q, Shi F, Hao J-J, Cai Y, Wang M-R. Plumbagin inhibits the proliferation and survival of esophageal cancer cells by blocking STAT3-PLK1-AKT signaling. Cell Death Dis. 2018; 9:1–13. DOI: 10.1038/s41419-017-0068-6.

[72] Yu T, Xu Y-Y, Zhang Y-Y, Li K-Y, Shao Y, Liu G. Plumbagin suppresses the human large cell lung cancer cell lines by inhibiting IL-6/STAT3 signaling in vitro. Int Immunopharmacol. 2018; 55:290–296. DOI: 10.1016/j.intimp.2017.12.021.

CHAPTER 5

Recent Studies in Yeast-Based Whole-Cell Biosensors

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Recent Studies in Yeast-Based Whole-Cell Biosensors

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Abstract

Biosensors are sensitive scanning methods used to detect and monitor a target analyte. In recent years, there has been a considerable increase in the need for sensors that can provide rapid and accurate measurements in fields including food, environment, and health. Whole-cell biosensors are cells that both detect and facilitate the creation of signals from the target analyte of interest. Cells obtained by genetic engineering techniques are used as biosensors in application areas such as drug discovery, clinical diagnosis and biotherapy, environmental assessment, mineral exploration, landmine removal and food safety. The last thirty years have seen a rise in interest in whole-cell biosensors as alternatives to electronic or chemical sensors because of their low cost, renewable nature, and environmental friendliness. Yeast-based whole-cell biosensors are biosensors obtained by developing yeast cells through genetic engineering techniques. Despite the problems that need to be overcome such as the determination of the appropriate immobilization method and the necessity of some conditions for the survival of the cells, they

have very important features such as their capabilities to express complex eukaryotic proteins and biosafety compared to other whole-cell based biosensors.

Keywords: whole-cell based biosensors, yeast, detection, sensitivity, specificity, reporters, genetic engineering.

1. Introduction

Biosensors are cost-effective, convenient, and analytical techniques that show high specificity and rapid response. Recent advances in nanotechnology have made it possible to create efficient and smart biosensors that contain nanoparticles or nanocomposites and have superior analytical capabilities [1]. In recent years, there has been a significant increase in the need for sensors that can provide rapid and accurate measurements. Nowadays, biosensors are used in different application areas such as disease diagnosis, drug discovery, environmental pollution (commonly determining heavy metals), and food safety control [2].

One of the sensitive scanning techniques used to identify and monitor environmental toxins, pollutants, and organic or chemical markers that endanger human health is the employment of biosensors. The transducer, signal generator, and molecular recognition layer are the three basic components of a biosensor.

A biosensor is immobilized on the surface of the transducer to create the molecular recognition layer [3, 4]. For specificity, the molecular recognition layer is crucial. In these devices, antibodies, aptamers, cell receptors, enzymes, organelles, tissues, peptides, microorganisms, or whole cells serve as biological recognition elements [5-8]. One of the major challenges in increasing the sensitivity of biosensors is the specific binding of a target analyte [9]. While protein-based biosensors consist of specific enzymes, metalloproteins, and antibodies, cell-based biosensors consist of genetically engineered microorganisms [10-12]. After the target molecule binds to the biosensor, a measurable signal is produced by the transducer, causing a change in the molecular structure by thickness, weight, refractive index, or chemical reaction. In complex samples like blood, cerebrospinal fluid, and physiological fluids like urine and saliva, thousands of molecules can be selectively recognized by biosensors, allowing one or more analytes to be identified, and a quantitative response can be achieved [13].

As one of the most common biological methods to detect hazardous substances in the environment, biosensors were initially developed using fish, mice, algae, daphnia, and bee larvae. However, these organisms are not suitable for rapid detection because they have long growth cycles. The systems have been made more useful by using the cell-based system in toxicity assessment studies. For example, organisms such as

Saccharomyces cerevisiae, Vibrio fischer, Escherichia coli have been used to detect harmful substances and heavy metals in wastewater [14-16]. When comparing the use of animals and microorganisms for toxicity, although the microbiological method is fast and low-cost, this method has some disadvantages such as optical signal insufficiency and detection error, due to the insufficient number of cells [17].

A wide variety of toxins and pollutants such as biocides or heavy metals cause many health problems and loss of ecological diversity. As the pressure on nature increases due to the lifestyle of human beings worldwide and their propensity for consumption, the environment has been polluted with highly toxic pollutants such as pesticides, toxins, chemicals, heavy metals, and endocrine-disrupting molecules [1]. Accurate and rapid detection methods must be developed to deal with such threats. Compared to other fields, there are more studies on the development of biosensors in the field of environmental monitoring [17-20].

The whole cell-based biosensor was developed in 1990 by Sanseverino and colleagues to detect naphthalene [21]. Due to their benefits, extensive application areas, and fast and easy implementation, microbial biosensors are getting more and more attention. Microbial biosensors are widely used for the detection of both organic and inorganic toxic substances (heavy metal detection in wastewater), evaluation of the nutritional quality of

food products, monitoring of the fermentation process (ethanol production), detection of food contaminants, and diagnosis patients (hormones, pathogens, DNA). Most cellular biosensors detect toxic substances, autoinducers and metabolites electrochemically and optically. Electrochemical biosensors are mostly preferred due to their ease of operation, high sensitivity and fast detection [22, 23]. Biochemical signals received by the sensing elements of microbial biosensors are transmitted to the transducers by immobilized microorganisms which are obtained by chemical (e.g., covalent bonding and crosslinking) or physical techniques (e.g., adsorption and trapping) [24, 25].

There are studies on the development of more portable microbial biosensors that can be incorporated into everyday technologies (smartphones) used by society [1, 25-27]. In the not too distant future, microbial biosensors will take place in our lives more and it will be much easier to access these microbial biosensors that can be used in various fields.

2. Biosensors

Biosensors are devices or systems that measure biological components by using them as recognition elements. These biological components can be macromolecules including enzymes and antibodies, lipid bilayer, liposomes and whole cells [28]. The first biosensor was developed by Clark and Lyon in

1962 for glucose detection. In a variety of fields from environmental monitoring to public health to food safety, biosensors have been studied and developed since 1962 [21].

Physiological or biochemical changes in analytes can be detected, transmitted, and recorded by a biosensor that is composed of a bioreceptor, transducer, and signal processor. Transducers convert biological sensing elements into electrical or electrochemical signals which can be measured mechanically, optically, electronically, or electrochemically in response to a target component [21, 29, 30]. It is very important to develop a low background biosensor with high sensitivity and specificity [31]. In recent years, biosensors have been replacing traditional analytical techniques such as immunological tests and biochemical tests, due to their specific, sensitive, reusable, rapid, and multiple analyses capabilities. [32, 33].

Applications of biosensors include medicine and health, pharmaceuticals, environmental monitoring, biological defense, and the food industry. In medicine, gases, ions, and metabolites in the blood can be quickly measured through biosensors to determine the metabolic status of a person. In pharmacology, biosensors can play a role in evaluating the biological activity of a new compound. Biosensors can also be used to determine food quality and safety. In addition, heavy metals, pesticides, fertilizers, industrial wastes, toxic components, and biological

agents such as bacteria and viruses can be determined by biosensors [25, 34-36].

The main principle of biosensors is that the biological recognition element (bioreceptor) is combined with a transducer to produce a measurable signal proportional to the concentration of the analytes. Recently, whole-cell and cell-free transcription-translation biosensors have emerged as efficient, cheap, and easy alternatives to traditional detection methods. Biosensors convert biological responses into physicochemical signals for detection, and various genetic reporters have been used to accomplish this. Biosensor performance and application success depend on reporter gene selection [37].

Reporter genes act as signal transducers to generate a detectable physicochemical signal and they determine the sensitivity and detection limit of a biosensor. Biosensor specificity is determined by the sensing component, which response to analytes. Among the most widely used reporter genes are bacterial luciferase (lux), firefly luciferase (luc), β-galactosidase (lacZ), green fluorescent protein (gfp), and calreticulin (crtA). In addition to these reporters, β-lactamase (bla), cyan fluorescent protein (cfp), yellow fluorescent protein (yfp) and red fluorescent protein (rfp) are also used as reporters [25]. Bioreporters are often considered valuable tools for microbiological, toxicological, and environmental research.

Bioreporters determine the bioavailability of chemicals rather than their quantity. The utilization of bioreporter technology can provide fast screening for stress or general toxicity. They can be used in smaller-budget laboratories or as alternative analytical technology for niche applications [38].

Upon production of the biological response to the analyte, the transducer interprets the response and generates a detectable signal. The signal can be correlated with the analyte concentration. Optical techniques have replaced electrochemical techniques with the adaptation of genetic engineering methods. Measurements made using the optical sensing technique are based on fluorescent, bioluminescent, colorimetric, or other optical signals generated during the interaction of the analyte and the microorganisms. For this purpose, colorimetric, fluorescent, and bioluminescent reporters are used. The inducible gene and a reporter gene are fused in this approach. This inducible gene is either activated or inactivated in the presence of a certain analyte. The presence of the analyte activates the reporter gene, inducing the production of the reporter protein, and an optical signal in the form of light is formed. Colorimetric reporters enable converting the chromogen substrate to a colored compound. Colored compounds could be observed directly with the human eye or a spectrophotometer. The first enzyme used to produce colorimetric output in biosensors was the enzyme β-galactosidase (LacZ) produced from the E. coli lac operon [39]. The enzyme β-

galactosidase is the most preferred enzyme for both whole-cell and cell-free biosensors [21, 37].

An output actuating module, a sensing module, and a computing module are the three components common to both whole-cell and cell-free biosensors. Fluorescent reporters have been used in many biosensors, such as gene expression studies. Characterization has become simpler with fluorescent radiation in laboratory settings. Fluorescent proteins can be easily quantified using a fluorimeter under specific light stimulation because they are relatively stable, mature quickly, and emit light readily. The fluorescent optical intensity is directly proportional to the target analyte concentration. They can also be used to examine sensor cells at the single-cell level by fluorescence microscopy [21].

The strength of bioluminescence is dependent on enzymatic activity rather than protein quantification. Therefore, bioluminescence is a more rapid and sensitive method of detection. Bioluminescence is the emission of light from living organisms and is observed in numerous species, including bacteria, insects, molluscs and coelenterates. Bioluminescence is a valuable bioanalytical tool used in molecular biology due to its high sensitivity and independence from external stimuli. Based on biological processes, bioluminescence reporters generate light without the use of an excitation light source. The majority of whole-cell or cell-free biosensors utilise the bioluminescent

molecules bacterial luciferases (LuxCDABE or LuxAB) [40] and firefly luciferases (LucFF) [41].

The recently developed NanoLuc luciferase has become a favored bioluminescence reporter for whole-cell biosensors because of its small size (19 kDa) and high luminescence activity. For on-site diagnostics or field testing, several of these bioluminescent biosensors are combined with portable devices. One of the technical problems of bioluminescent biosensor applications is their low stability under conditions such as temperature changes and light exposure. No solution has yet been found for this problem. Despite this problem, hybrid devices with immobile or microcells such as bacteria, yeast or mammalian cells have been developed using new biocompatible nanomaterials, and they have been used for drug discovery, environmental monitoring, food control and antidoping screening [37, 42-45].

Enzymes are widely preferred in biosensor construction due to their high specific activity and analyte sensitivity. Some enzyme-based biosensors such as alcohol oxidase-peroxidase coupled systems and alcohol dehydrogenase have been developed. However, enzymes need to be purified in the production of enzyme-based biosensors and the purification process is a very costly application. In addition, to form detectable products, many enzymes or cofactors, or coenzymes are often needed.

Microorganisms have been suggested as an alternative to these bottlenecks. All microbial sensors can be easily and affordably produced since enzyme purification is not required, and enzymes are usually more stable in their native environment in the cell. [46].

Enzymes, antibodies, microorganisms, plant and animal cells or tissues are biological components used in bioreceptors, and the importance of these components depends on their interaction with analytes, meaning these components must be highly specific to minimize their interaction with other substances. Despite the fact that enzymes are the most commonly utilized due to their specificity, safety, and sensitivity, they are very expensive, time-consuming, and inactivate after a short period of time, and they are not appropriate for an in vitro operating environment. Therefore, interest in microorganisms (e.g. bacteria, microalgae, fungi, yeast, and virus) as bioreceptors has increased due to their advantages such as low cost, rapid response, portability, and the ability to detect various chemical substances due to their large number of enzymes [24]. In addition, microorganisms have wide pH and temperature ranges, they can be easily grown in cell culture, modified genetically, and have better vitality and stability in vitro which can enhance and make simpler the performance of the biosensor. As a biosensor, microorganisms have great advantages with their short life cycles and capacity to adapt to their environment, and ability to become better at degrading novel

compounds over time (i.e., evolvability, robustness). On the other hand, cellular biosensors have disadvantages such as low selectivity and longer response time compared to enzyme-based biosensors. In addition, to maintain living cells in their metabolically active form, a suitable immobilization approach should be applied. [25, 46].

3. Whole-Cell Biosensors

"Whole-Cell Biosensors" is a term that has come from the late 1980s to the present day [47]. Because of their high sensitivity and specificity, enzymes have become the most preferred biomolecules for biosensor development. However, they have some negative aspects such as high purification cost, sensitivity to temperature and pH changes, and loss of activity during immobilization. With the development of cell-based biosensors, which are more durable biosensors, these negative features have disappeared. Cell-based biosensors can detect a range of biochemical changes, and they use a transducer to convert this biological response into a visible signal. Whole-cell biosensors can detect and quickly analyze a broad spectrum analyte. In addition, whole-cell biosensors are more cost-effective biosensors compared to conventional biosensors. The whole-cell biosensors have the advantages of high selectivity, enhanced sensitivity, and in situ detection. Therefore, they could be utilized

efficiently in pharmacology and drug screening, environmental monitoring, and food analysis [21].

The naphthalene detection study by King and colleagues in 1990 was one of the earliest examples of how genetic engineering may be used to convert bacteria into living sensors [48]. In their study, they fused the luxCDABE gene from Vibrio harveyi, which is a bacterial bioluminescence gene, with an expression promoter induced by naphthalene, and they inserted these fusion genes into Pseudomonas fluorescens. They used immobilized bacteria to produce a fiber optic biosensor that detects naphthalene and salicylate [49]. Using the approach of fusing an inducible promoter and a promoterless lux gene, biosensors used in the detection of many metals such as mercury, aluminum, arsenic, cadmium, copper, and zinc were developed in the 90s [28]. Since the first whole-cell biosensor was developed nearly 30 years ago, there are many studies on obtaining genetically modified microorganisms for detection and reporting.

Whole-cell biosensors consist of a unit in which cells are immobilized and placed and a biological response transducer. These cells might have a genetic modification or a reporter gene that naturally replies to the target analyte. The other unit is the detection unit, and detection is performed using optical and electrochemical techniques [21]. All cells, eukaryotic or prokaryotic, can be used as reporters in which both biological

receptor and transforming elements are combined [50]. For the purpose of making it more sensitive or involving different reporters and transducers, it is possible to genetically modify cells or microorganisms used as whole-cell biosensors. [51].

In the classical technique, a promoterless gene expressing an easily detectable protein is fused with natural regulatory sequences (transcription regulator promoter/operator) in a microorganism. When the promoter interacts with an analyte, it is activated by the transcription regulator and leads to the expression of the reporter gene, which gives a detectable, interpretable output signal [38]. In whole-cell biosensors, the selection of the reporter gene and the sensitivity and selectivity of the molecular recognition system is crucial in the binding of the target component to the reporter gene.

The analyte causes the target gene to be activated, thereby expressing the reporter protein. This response is transformed into a detectable signal via the transducer [21]. The whole-cell biosensor consists of three modules, which are a signal input module, a regulatory module, and a signal output module. In the signal input module, the target analyte is detected as a stimulus. The regulatory module consists of a promoter connected to the signal input module and generates the response. The signal output module enables the resulting response to be detected, recorded, and quantified as a signal (Figure 1) [52, 53].

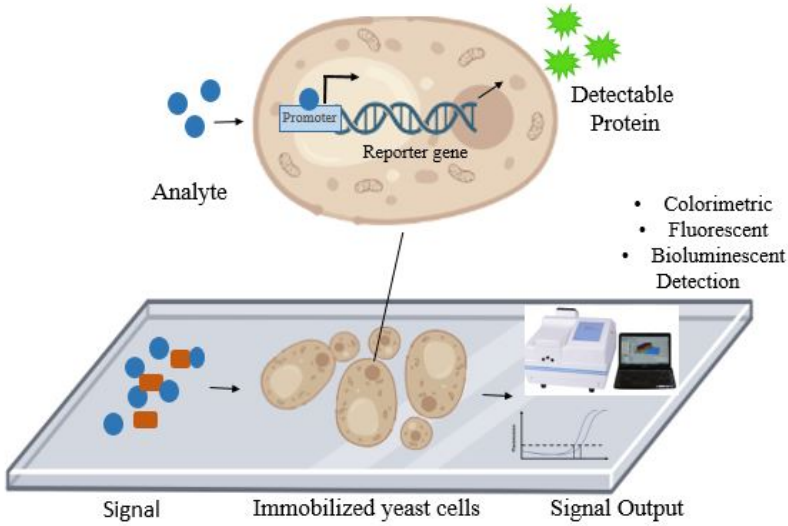

Figure 1. Illustration of genetically engineered yeast-based whole-cell biosensors [21].

Whole-cell biosensors have recently received a lot of attention due to their properties. Whole-cell biosensors can be highly customizable by genetically modifying the sensing element. Cells are more stable than enzymes with their small size and ability to survive in different environmental conditions, being miniaturized, and long-term use that allows portability. However, cell viability which depends on the availability of nutrients and environmental conditions (pH, temperature, ionic strength) are significant limitations [54].

There are many different types of electrochemical biosensors, but enzyme-based biosensors are the most popular [55]. However,

the high purification cost and poor stability of enzymes lead to disadvantages to producing on the large scale and implementing practically these enzyme-based biosensors. Therefore, the development of electrochemical whole-cell biosensors based on surface display technology has been the focus of many studies, as they can overcome the limitations associated with enzyme-based biosensors [56].

Target proteins can be expressed on cell membranes with the surface display and this technique can be applied to virtually any biological entity, from viruses to mammals. Drug screening, library screening, biosensors, biocatalysts, and quantitative testing are just a few applications of this technique in biotechnical and biomedical fields A major role in cell signaling is played by membrane proteins. Signaling molecules are also recognized by biosensors in the same way as molecules are recognized by membrane receptors. In the surface display, proteins that are designed by using membrane protein and protein engineering techniques could be placed on the cell surface. As different from the general protein expression mechanism, membrane protein-bound proteins are located on the surface of the cell. The main target of surface display is affinity proteins, and these proteins could potentially be used as molecular recognition components [13].

Cell immobilization is crucial to successfully functionalize whole-cell based biosensors. Long-term stabilization of cellular

activity can be ensured by immobilization. The sort of cell, its features, and the biosensor development type are important factors in the selection of immobilization type. When producing a biosensor, the immobilization of the microorganism must be stable and effective, not affect cellular activity, and be easy to employ. Various chemical and flocculant agents can be used in immobilization. Most microorganisms naturally have the ability to attach to the surface and then reproduce on the surface [57]. There are numerous methods for immobilization, including covalent bonding, encapsulation, cross-linking, adsorption, and capture. Researchers are developing various immobilization methods for the increased stability and performance of the microorganism. For example, using a cellulose nitrate membrane and a microencapsulation method for heavy metal detection, *Aliivibrio fischeri* was immobilized [21, 58].

3.1. Types of whole-cell biosensors

Whole-cell biosensors may be based on bacteria and eukaryotic microorganisms such as animal tissues, yeast and microalgae. The detection of environmental pollutants, food quality, disease-related compounds, and pathogenic bacteria are some of the major applications of biosensors. Each kind of microorganism used in biosensors has advantages and disadvantages to detect analytes of interest. Moreover, there is an increasing trend to apply genetic modifications in the

development of whole-cell biosensors owing to the potential for portability and high-throughput analysis [1, 25]. Host cell selection is the most important step for biosensor development. The cell type used to detect analytes determines the sensitivity, specificity, and response time of a biosensor. There are also studies in which the presence of heavy metals and toxic substances is determined by using different mammalian cells [21, 59-61]. However, bacteria, microalgae, and yeast-based biosensors are frequently preferred whole-cell biosensors because they are more durable.

3.1.1. Bacterial whole-cell biosensors

Bacteria have advantages such as low cost, rapid replication ability, susceptibility to genetic manipulation, and fast replication time and allow the development of biosensors that can respond to different analytes.

In general, whole-cell bacterial biosensors are based on bacteria genetically engineered to produce measurable signals (eg, light) and to respond in a particular way to a particular analyte [37]. Bacterial whole-cell biosensors show reproducibility and high stability. Bacterial whole-cell-based detection systems are suitable for inclusion in portable devices with their replication, high throughput, and miniaturization features. However, despite being relatively robust, bacterial whole-cell biosensors can be

affected by environmental changes as the expression of the reporter protein relies on cell growth [19, 62].

It is possible to display affinity proteins and active enzymes on the surface of bacterial cells. The target protein's size is unrestricted in the bacterial surface display. For these reasons, peptide or antibody libraries displayed on bacterial surfaces can be useful in biosensor applications [13].

Based on the physiological response of bacterial immobilization in a membrane or gel matrix delivered to a sensing device, many bacterial biosensors have been developed. To perform a faster, more accurate, and selective analysis, bacterial biosensors have recently been developed by integrating a reporter gene with an analyte-detecting component. Generally, the mechanism by which a bacterial biosensor detects analytes is provided by the binding domain of the DNA promoter region, or transcription factors, which directly suppress or induce reporter gene expression. Then the output of detection events is converted into a reporter protein, which can be assessed in the assay (coloration, luminescence, fluorescence) [25, 63].

There are many studies with bacterial whole-cell biosensors. Wen et al. developed a biosensor for methanol detection. They immobilized *Methylobacterium organophilium* on the eggshell membrane with gold nanoparticles (AuNP) and detected the

presence of methanol by the respiration of bacterial cells. This biosensor provides a reliable and simple method with high selectivity to determine the methanol content in samples [46].

Heavy metals are direct or potential hazards that threaten the health of all living organisms, including humans, through bioaccumulation. There are many studies on bacteria-based biosensors developed for heavy metal and toxicity determination. The increase in pollutants discharged into the sea has led to serious marine pollution [64]. Based on exposure studies with marine species such amphipods, fish, copepods, and shrimp, numerous techniques have been developed to determine the toxicity of marine pollutants [65, 66]. These techniques, though, are time-consuming, expensive, and difficult. Additionally, they are unable to respond quickly to emergencies, particularly when there has been accidental marine pollution. Therefore, there is a need for a faster, more reliable, highly efficient, and sensitive method for the assessment of the overall toxicity of pollutants in the marine environment. According to this need, a salinity-tolerant bacterial whole-cell biosensor (*Acinetobacter baylyi* Tox2) has been developed to rapidly detect cytotoxicity in heavy metal-contaminated seawater. *Acinetobacter baylyi* ADP1 bacterium was used, which shows metabolic versatility, easy cloning, and resistance to environmental changes. *Acinetobacter baylyi* Tox2 biosensor works on the principle of bioluminescence. *A. baylyi* cells contain the plasmid pSB417 (pWH1274_lux)

carrying the LuxCDABE gene cassette and this gene is controlled by promoter Ptet expressed constitutively. The biosensor's bioluminescence intensity reduces proportionally according to the concentration of toxic chemicals. To detect genotoxins in soil and groundwater, various biosensors have been also developed by using *A. baylyi* ADP1 [18, 67, 68].

Wong and colleagues developed a biosensor for the detection of Lead-Pb(II), Cadmium-Cd(II) and Copper-Cu(II) by immobilization of *Anabaena torulosa* on a cellulose membrane. The toxicity of the heavy metal inhibits the photosynthetic activity of these cyanobacteria, causing quenching of the fluorescence [69].

Arsenic is one of the most toxic metals in the form of arsenite (AsIII). Water used for food preparation, irrigation, and drinking is assumed to be contaminated. If it is found more than the level determined by the World Health Organization (WHO) (10 μg/L), it becomes dangerous for human health [54, 102]. Also, mercury is a dangerous analyte. The divalent inorganic form of mercury (Hg(II)) is poured into the water as industrial waste and accumulates above the WHO threshold of 1 μg/L, becoming toxic and causing severe neurological disorders, kidney damage, and skin rashes. Sciuto and colleagues developed a whole-cell-based miniature electrochemical biosensor for the detection of heavy metal ions (As(III) and Hg(II)) in water. The system comprises a

whole-cell based on *E. coli* and two advanced sensing modules, which they composed of three microelectrodes, an electrochemical miniature silicon device, and a portable reading system [54].

Furthermore, Jia and colleagues developed likewise a bacterial whole-cell biosensor with a positive feedback amplifier for sensitive, highly specific, and rapid determination of arsenic concentration in drinking water. The biosensor is sensitive enough to detect arsenic concentration below the WHO limit level (0.01 mg/L or 0.13M), such that the biosensor can detect arsenic concentrations up to 0.1 μM. In this study, genetic engineering techniques, especially positive feedback amplifiers, were used in the design of whole-cell biosensors. The arsenic resistance operon of *E. coli* includes arsR (transcriptional regulator), arsB (arsenide permease) and arsC (arsenate reductase). The transcription regulator ArsR binds to the ArsR binding site (ABS) within the ars promoter in the absence of arsenic and prevents transcription. In the presence of arsenic, arsenic binds to ArsR and alters the promoter's structure to activate the transcription of ars genes and clear arsenic from the cell [70]. The positive feedback amplifier amplifies significantly the output signal from the reporter mCherry. The arsR regulator and promoter of this operon have also been utilized to generate whole-cell biosensors capable of detecting arsenic in various microorganism hosts [19, 29, 71].

In the study of Riangrungroj and colleagues, *E. coli* whole-cell biosensors were developed to detect 3-phenoxybenzoic acid (3-PBA), a biomarker for screening synthetic pyrethroid insecticides that are harmful to human health but widely utilized worldwide for household applications and insect control in agriculture. Since pyrethroid insecticides degrade fast and have short half-lives, it is challenging to quantify their amounts directly in human samples (2.5-12 hours in plasma and ~6 hours in urine). Therefore 3-PBA which is a common primary metabolite in the degradation of insecticides is measured. The biosensor in this study works on the ELISA principle and uses the whole-cell surface exhibiting an anti-3-PBA as the sensing part. Cross-linking occurs when biosensor cells are mixed with 3-PBA-protein conjugate. Free 3-PBA in the samples leads to a color change through the expression of the detectable purple-blue amylCP chromoprotein in the output by competing with these crosslinks. Riangrungroj et al. reported that the biosensor cells used in this study could be stored without function loss for at least 90 days by lyophilization, and this would make it possible to produce the whole-cell biosensor in advance in a central location, store it, and then distribute when it needed. They also stated that it exhibits stable function in complex samples such as synthetic urine and plasma [72].

The development of a fluorescent biosensor in genetically modified Pseudomonas aeruginosa allowed for the detection of perfluorinated substances (PFOA and PFOS), also known as

pollutants with endocrine disruptors [73]. According to the findings from the previous studies, bacteria-based biosensors that use genetically modified *E. coli* can detect 2,4-dinitrotoluene (DNT) vapors and 2,4,6-trinitrotoluene (TNT)-included landmines at the micromolar detection threshold [74, 75].

Siedler and colleagues developed a p-coumaric acid sensitive biosensor based on a co-culture of *E. coli* and *Bacillus subtilis* to monitor the production of p-coumaric acid, known as a phenolic acid with a wide range of biological activities such as anti-cancer, antimutagenic, antioxidant, antiplatelet, anti-inflammatory, and anti-ulcer [76]. In another study, a bioluminescent *E. coli* biosensor was developed for the detection of gastrointestinal biomolecules related to gastrointestinal health problems [77, 78].

Bacterial whole-cell biosensors, which are mentioned above and used in different fields such as food, environment, and health, are only a few examples of many studies carried out to date. Much more bacterial whole-cell biosensor studies will be carried out in the future with the ease of use and suitability for genetic manipulation and the development of the technology used in signal conversion.

3.1.2. Microalgal whole-cell biosensors

Compared to other microorganisms, microalgae adapt to various environmental factors, which reduces their susceptibility to physicochemical changes. Microalgae are also widely distributed in nature and are highly sensitive to toxins [79, 80]. Microalgal-based biosensors include benefits including ease of genetic alteration, simplicity, high sensitivity, cheap cost, sustainability, and adaptability [81, 82]. Numerous parameters including fluorescence induction, photosynthetic activity, growth rate, and metabolism related to the inhibitory effects of analytes, are assessed electrochemically or optically [83]. The electrochemical method enables more complicated matrices, whereas the optical method has a higher sensitivity level [84]. Microalgae biosensors are becoming increasingly popular to monitor volatile organic compounds, heavy metals, and other pollutants in marine.

Nowadays microalgal nanoparticles are also used to remove heavy metals [85]. Due to their robustness and adaptability to extreme conditions, *Chlorella*, *Chlamydomonas, Arthrospira*, *Nannochloropsis*, and *Scenedesmus sp*. are among the various microalgae used as biosensors [86, 87]. Wong and colleagues used microalgae to detect heavy metals in water [88]. In another study, *C. vulgaris* was utilized in a bioelectrochemical platform to detect oil spills in marine and aquatic habitats [89]. Microalgae,

as photosynthetic microorganisms, have been used to detect toxic chemicals in water because of their sensitivity and rapid response (2-4 hours) compared to a growth rate-based detection (48-96 hours) [83]. The detection of lead toxicity in the marine ecosystem has been accomplished by using a *Nitzschia closterium* biosensor based on chlorophyll fluorescence parameters [90].

3.1.3. Yeast-based whole-cell biosensors

Yeasts are single-celled eukaryotes, 10-20 μm in size. They are easily cultured and have the ability to multiply rapidly. Thanks to their metabolic activities, yeast can identify molecules that are difficult to detect by converting them to simpler ones. Fungal biosensors have several advantages over bacteria-based or other microbial biosensors [91].

Yeast, a eukaryotic organism, has similar metabolic activities as higher eukaryotes. Most eukaryotes use particular receptors and signaling pathways (MAPKs) [92]. Yeasts are highly sensitive and reproducible organisms. Yeasts can be rapidly grown in an inexpensive medium. A wide range of temperatures can be tolerated by these organisms. In addition, they could be frozen or dried for storage and transportation. The cellular properties of yeasts and their transport and protection possibility make them biological models for the development of biosensors [93].

The selection of yeast cells allows the use of non-animal models or the screening of therapeutic molecules to detect the potential toxicity of a wide variety of compounds. Because of culturing easily and being susceptible to genetic change, yeasts have been widely used as biological components of biosensors since the 1970s [1]. Yeast-based biosensors have attracted attention due to the fact that yeasts are eukaryotic cellular models, they are long-term in a relationship with humans and they can withstand harsh conditions. Additionally, it is a significant advantage that we have deep genetic and technological knowledge about them. In biosensors, yeast cells can not only be used with their metabolic transformation abilities and growth rate but also can be used to monitor target analytes by genetic changes.

Yeast reporters mostly are based on reporter gene expression controlled by inducible promoters. Specific transcription factors activate these inducible promoters under certain conditions. Growth rate variation, fluorescence, luminescence, and colorimetry are among the detection methods of yeast biosensors [91]. In yeast-based whole-cell biosensors, genetically modified yeast cells detect a particular molecule or compound and produce a fluorescent or bioluminescent response in response. Generally, the heterologous gene is transferred in yeast cells, giving the cell the ability to recognize target molecules. The exogenous sensor protein directly or indirectly activates the reporter gene generating a metabolic, colorimetric, luminescent, or fluorescent

signal. These sensor proteins may be membrane-associated receptors or channels, direct transcription factors or intracellular signaling pathway members [1, 94, 95]. Yeast cells have been used to detect a wide variety of analytes, including glucose, copper ions, hormones, toxicants, and antibiotics. Detection of large numbers of substances in their environment is usually by binding transcription factors to responsive elements. Marker proteins, e.g. fluorescent proteins are produced in the presence of the analyte, resulting in an easy-to-follow read signal [96].

Miller et al. stated that yeast-based whole-cell biosensors are portable and inexpensive analytical devices, but readouts of the output signal by yeast biosensors require expensive equipment. They aimed to develop a reporting system that does not need any additional chemicals or equipments. They designed a yeast biosensor that converts the input signal to a unique odor as the output signal, very different from other detection methods. They identified an odorant detection system sensitive to the hormone estradiol (E2). This study demonstrates that odor-based reporters in yeast-based whole-cell biosensors have the potential to meet analytical needs in technology-limited environments [97].

Multiple proteins could be presented on the surface of microorganisms like bacteria and yeast owing to the surface display, which is a powerful technology [98]. Yeast surface display is a whole-cell platform to express immobilized

heterologous proteins on the yeast cell surface. Yeasts are advantageous in that they have eukaryotic mechanisms such as post-translational modifications, protein folding and glycosylation of proteins. In addition to these features, it allows protein immobilization and recovery in the production of biosensors with the ease of cell culture and genetic manipulation. Proteins displayed on the surface of yeast cells could exhibit high stability to pH, proteases, temperature, and organic solvents. Yeast surface display can be utilized for protein-protein interactions, antibody design, and recombinant protein studies [99]. Yeast surface display is advantageous for imaging complex proteins of eukaryotes that had high molecular weight or required post-translational modification [100].

Boder and Wittrup pioneered yeast surface display and they used the Aga1 and Aga2 subunits of α-agglutinin enzymes to anchor the target enzyme to the cell wall in Saccharomyces cerevisiae [101]. When enzymes bind to the yeast cell surface, they can be used directly to enzymatically react with substrates, without the need for their purification. Thus, the cost of preparing enzyme-based biosensors is significantly reduced. Also, the stability of enzymes can be maintained by the surface of yeast cells, which provides a biocompatible microenvironment. In another study, it was stated that the presence of anchored cholesterol oxidase and glucose dehydrogenase on yeast cell wall

had the potential to develop electrochemical biosensing platforms which can detect cholesterol and blood sugar levels [56].

In terms of genetic engineering and safety, yeast cell surface display is more advantageous than bacterial surface display. A yeast cell can display a complex eukaryotic protein on its surface and also, yeast cells are widely used in the pharmaceutical and food industries due to their safety [13]. *S. cerevisiae* is the most preferred host for whole-cell biosensor development. Some other yeast species such as *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus*, *Pichia pastoris* and *Arxula adenivorans* are also used as hosts for biosensor development [1].

Bioassay and biosensors based on yeast cells are used in various application areas such as environment, food and health [1, 25]. Examples of yeast whole-cell biosensor studies used in these areas are given below.

3.1.3.1. Studies in environment and heavy metal detection

As a result of industrial developments and human activities, heavy metal pollution is among the causes of cancer, neurodegenerative and metabolic diseases, and it is one of the problems that should be prevented in the environmental field. There is a need for inexpensive and portable technologies that can be used to identify threats to the environment and human health.

To specifically and sensitively detect toxic heavy metal ions, whole-cell biosensors have recently been developed [19]. Yeasts are excellent cellular models for detecting harmful chemicals and organic pollutants.

In biosensors produced against heavy metal pollution, regulatory elements from a heavy metal resistance operon bind to a reporter gene that can produce detectable responses such as fluorescent, luminescent, or enzyme, and the detected signal strength from the reporter is proportional to the concentration of the heavy metal [19].

Lehmann et al. have developed the *Saccharomyces cerevisiae* biosensor that uses the copper-sensitive promoter PCUP1, which activates the lacZ reporter, to detect Cu(II), but its sensitivity has remained very low [103]. In order to detect Cu(II) ion, Δade2 yeasts were developed that produce red pigment by expression of *ADE5* and *ADE7* under the control of PCUP1 [104]. Yuan and colleagues developed a yeast-based cell sensor with higher sensitivity than other methods and detectable in situ for Cu(II) ion detection using the galactose-inducible (GAL) system [105]. Silver nanoparticles (AgNPs) are increasingly applied in many fields, and Sun and Wang developed yeast-based biosensors to measure Ag+ ions using silver nanoparticles in their work [106]. In another study, they developed a biosensor that can detect Zn(II) ions fluorescently using adenine mutant yeasts [12].

Yeast cells as biosensors have great potential in detecting the cytotoxicity of wastewater. Yeast-based bioassays are used to investigate the presence of pesticides and their endocrine activities in environmental samples such as wastewater effluents [107, 108]. Gong et al. developed a whole-cell biosensor by using *Saccharomyces cerevisiae* to detect heavy metals and the toxicity of chlorothalonil. This biosensor is based on the effect of inhibition of the metabolism of yeast cells by toxic substances [17].

3.1.3.2. Studies in food safety

Both producers and consumers give a high priority to the safety and quality of food produced in the food industry, since it may cause health problems. A Saccharomyces cerevisiae-based electrochemical-impedimetric biosensor was developed by Štukovnik and colleagues for the detection of caffeine. Conventional methods used to detect caffeine (immunoassay, thin-layer chromatography (TLC), and high-performance liquid chromatography-mass spectrometry (HPLC-MS)) are expensive and complex, as well as disadvantageous due to the laborious sample preparation step. The developed biosensor provides a suitable alternative to detect caffeine in foods, drinks, and drugs with its robustness, low cost, low detection limit, and time-saving properties [2].

3.1.3.3. Studies in health

Yeast has been used as a model for a variety of diseases because it shows many of the essential processes found in nearly all higher eukaryotes, including humans. The use of yeast as a biosensor has been beneficial to a wide range of research, from understanding the pathogenesis of disease to studying new drugs [1, 91, 95].

Miller et al. developed the first whole-cell yeast biosensor immobilized on a portable paper substrate for pharmaceutical detection. In order to use in environments where technology is limited, they have produced a fluorescent-based whole-cell yeast biosensor and an inexpensive and portable device that can read the fluorescent signal [109]. In another study, they used the engineered *S. cerevisiae* to develop a cost-effective and simple paper test strip biosensor for the detection of the doxycycline physiological concentration in both bovine and human serum [97]. Cottier et al. developed a cellular assay using *S. cerevisiae* that detects a typical protease activity of human cytomegalovirus (HCMV), a sample of yeast-based biosensor directly related to human health [110].

Yeasts are highly flexible microorganisms for designing whole-cell biosensor systems, not only because their genome is amenable to modifications, but also because it has some

characteristic features, such as the ability to survive with defective mitochondria [95]. Yeast biosensors can be used as biosensors to study chemicals that are unique to yeast and that may alter mitochondrial health status which cannot be accomplished in any other system. In a study by Dhakal and Macreadie, they found that tyramine impairs respiratory growth in yeast cells in the presence of amyloid-β. They utilized the growth properties of yeast for the identification of compounds that could alter mitochondrial health [95, 111].

Yeasts have been used to detect many chemicals such as various carcinogens, polycyclic hydrocarbons, and mycotoxins. Bui and colleagues developed a yeast-based biosensor that detects carcinogens in environmental samples [112]. Becker et al. used yeast cells to detect the toxin ricin, which is used as a deadly poison and biological warfare agent by terrorists [113].

Defects in human matrix metalloproteinases cause diseases such as cardiovascular problems and cancer. Specific matrix metalloproteinase inhibitors are potential targets for treatment development. Diehl et al. developed Pichia pastoris yeast strains expressing human matrix metalloproteinases for the identification of inhibitors of matrix metalloproteinases [114].

Various signals, including chemical compounds and light, are sensed by eukaryotic cells using G-protein coupled receptors. To

build yeast-based biosensors, it is used the G-protein coupled receptors' sensing capabilities. Using the various signal perception ability of G-protein coupled receptors, this technology can be extended to detect a large number of compounds. In yeast-based whole-cell biosensors, Miettinen et al demonstrated the utilization of GPCRs as detection components. It is possible for mammalian cell systems to be affected by the off-target effects of drug precursors, but a yeast-based system can help direct the drug's action to the receptor directly. Due to the yeast-based nature of the biosensor, it is less likely to be affected by toxic compounds than animal cells [31].

The biosensors based on yeast are also effective at detecting a wide range of microbial pathogens, including fungal pathogens. To detect pathogen-derived peptides, a highly specific colorimetric assay using *S. cerevisiae* was developed by Ostrov and colleagues. By integrating G protein-coupled receptors (GPCRs) into a visible lycopene readout, they developed yeast strains that could detect plant, food, and human fungal pathogens at nanomolar levels. The first biosensor developed by the researchers was designed to detect the human fungal pathogen *Candida albicans*. Then, they used this detection system for human, agricultural, and food pathogens *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Magnaporthe*, *Botrytis cinerea*, *Fusarium graminearum*, *Zygosaccharomyces derouxii*,

Lodderomyces elongisporus, *Zygosaccharomyces bailii*, and *Candida glabrata* [115].

Zhao and colleagues created two whole-cell biosensors for the electrochemical detection of glucose and cholesterol by immobilization of yeast cells on electrodes. Both biosensors performed well at room temperature and exhibited good stability over a three-week storage period. In their study, they obtained these enzymes on the surface of *S. cerevisiae* by using the glucose dehydrogenase gene from *Aspergillus oryzae* TI and a cholesterol oxidase gene from *Chromobacterium sp.*. They immobilized these cells on the electrodes to develop electrochemical biosensors, that can be used to detect cholesterol and glucose, and then they measured the catalytic activity of enzymes on the cell surface for detection. Enzymes that are surface-displayed do not require expensive protein purification steps and have a high degree of stability [56].

Lobsiger and colleagues developed a field portable fluorescent biosensor using *S. cerevisiae* for estradiol detection [116]. Cevenini and colleagues have proposed a bioluminescent biosensor that can evaluate estrogenic activity using *S. cerevisiae* [117]. These studies show that yeast-based biosensors have great potential to be used in different applications in the field of health, and S. cerevisiae is generally the preferred yeast species in yeast-based biosensor development.

Conclusion

Detection methods such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry are high-priced, time-consuming, and unportable methods for in situ applications. There is a rising requirement for low-cost, portable technologies to detect risks to both human health and the environment. Yeast-based whole-cell biosensors are easy-to-manipulate, well-characterized, and low-cost platforms for the detection of specific analytes. They can be preferred as potential diagnostic tools to detect various compounds in clinical and environmental samples by producing an easily visible output such as fluorescent or luminescence signals. Nowadays, with technological developments, it has become possible to monitor the output signals obtained through biosensors and to record the data on a smartphone that can be easily accessed by everyone. Considering the advantages of using yeast cells as biosensors, yeast-based whole-cell biosensors will reach wider usage areas in the coming years.

Conflict of Interest

The author declares no conflict of interest.

References

[1] Martin-Yken H. Yeast-based biosensors: current applications and new developments. Biosensors. 2020; 10(5):51. DOI:10.3390/bios10050051.

[2] Štukovnik Z, Godec RF, Bren U. The Use of Yeast Saccharomyces Cerevisiae as a Biorecognition element in the Development of a Model Impedimetric Biosensor for Caffeine Detection. Acta Chimica Slovenica. 2022; 69(2):378-384. DOI: 10.17344/acsi.2021.7301.

[3] Turner A, Karube I, Wilson GS. Biosensors: Fundamentals and Applications. Oxford University Press: Hong Kong, China, 1987.

[4] Afzal A, Iqbal N, Mujahid A, Schirhagl R. Advanced vapor recognition materials for selective and fast responsive surface acoustic wave sensors: A review. Analytica Chimica Acta. 2013; 787:36–49. DOI: 10.1016/j.aca.2013.05.005.

[5] Wang W, Chen C, Qian M, Zhao XS. Aptamer biosensor for protein detection using gold nanoparticles. Analytical Biochemistry. 2008; 373:213–219. DOI: 10.1016/j.ab.2007.11.013.

[6] Liu Q, Wang J, Boyd BJ. Peptide-based biosensors. Talanta. 2015; 136:114–127. DOI: 10.1016/j.talanta.2014.12.020.

[7] Felix FS, Angnes L. Electrochemical immunosensors—A powerful tool for analytical applications. Biosensors and

Bioelectronics. 2018; 102:470–478. DOI: 10.1016/j.bios.2017.11.029.

[8] Yousefi M, Dehghani S, Nosrati R, Zare H, Evazalipour M, Mosafer J, Tehrani BS, Pasdar A, Mokhtarzadeh A, Ramezani M. Aptasensors as a new sensing technology developed for the detection of MUC1 mucin: A review. Biosensors and Bioelectronics. 2019; 130:1–19. DOI: 10.1016/j.bios.2019.01.015.

[9] Sung JH, Koo J, Shuler ML. Mimicking the Human Physiology with Microphysiological Systems (MPS). BioChip Journal. 2019; 13:115–126. DOI: 10.1007/s13206-019-3201-z.

[10] Chouteau C, Dzyadevych S, Durrieu C, Chovelon JM. A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosensors and Bioelectronics. 2005; 21:273–281. DOI: 10.1016/j.bios.2004.09.032.

[11] Ren W, Huang PJJ, de Rochambeau D, Moon WJ, Zhang J, Lyu M, Wang S, Sleiman H, Liu J. Selection of a metal ligand modified DNAzyme for detecting Ni2+. Biosensors and Bioelectronics. 2020; 165:112285. DOI: 10.1016/j.bios.2020.112285.

[12] Sun A, Wang WX. Adenine deficient yeast: A fluorescent biosensor for the detection of Labile Zn (II) in aqueous solution. Biosensors and Bioelectronics. 2021; 179:113075. DOI: 10.1016/j.bios.2021.113075.

[13] Park M. Surface display technology for biosensor applications: a review. Sensors. 2020; 20(10):2775. DOI: 10.3390/s20102775.

[14] Scariot FJ, Jahn L, Delamare APL, Echeverrigaray S. Necrotic cell death induced by dithianon on, Saccharomyces cerevisiae. Pesticide Biochemistry and Physiology. 2018; 149:137-142. DOI: 10.1016/j.pestbp.2018.06.006.

[15] Kusumahastutia KAD, Sihtmäe M, Kapitanov IV, Karpichev Y, Gathergood N, Kahru A. Toxicity profiling of 24 L~phenylalanine derived ionic liquids based on pyridinium, imidazolium and cholinium cations and varying alkyl chains using rapid screening Vibrio fischeri bioassay. Ecotoxicology and Environmental Safety. 2019; 172:556-565. DOI: 10.1016/j.ecoenv.2018.12.076.

[16] Zhang Y, Ren T, He J, Tian H, Jin B. Acute heavy metal toxicity test based on bacteriahydrogel. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2019; 563:318-323. DOI: 10.1016/j.colsurfa.2018.12.016.

[17] Gong L, Yang G, Yang B, Gu J. Development of the yeast Saccharomyces cerevisiae as a biosensor for the toxicity detection of toxic substances. bioRxiv. 2020. DOI: 10.1101/2020.01.07.898106.

[18] Cui Z, Luan X, Jiang H, Li Q, Xu G, Sun C, Zheng L, Song Y, Davison PA, Huang WE. Application of a bacterial whole cell biosensor for the rapid detection of cytotoxicity in heavy metal

contaminated seawater. Chemosphere. 2018; 200:322-329. DOI: 10.1016/j.chemosphere.2018.02.097.

[19] Jia X, Bu R, Zhao T, Wu K. Sensitive and specific whole-cell biosensor for arsenic detection. Applied and Environmental Microbiology. 2019; 85(11):e00694-19. DOI: 10.1128/AEM.00694-19.

[20] Fan C, Zhang D, Mo Q, Yuan J. Engineering Saccharomyces cerevisiae-based biosensors for copper detection. Microbial Biotechnology. 2022. DOI: 10.1111/1751-7915.14105.

[21] Saini R, Hegde K, Brar SK, Verma M. Advances in whole cell-based biosensors in environmental monitoring. In Tools, Techniques and Protocols for Monitoring Environmental Contaminants. 2019; 263-284. DOI: 10.1016/B978-0-12-814679-8.00013-3.

[22] Low SS, Loh HS, Boey JS, Khiew PS, Chiu WS, Tan MTT. Sensitivity enhancement of graphene/zinc oxide nanocomposite-based electrochemical impedance genosensor for single stranded RNA detection. Biosensors and Bioelectronics. 2017; 94:365–373. DOI: 10.1016/j.bios.2017.02.038.

[23] Low SS, Ji D, Chai WS, Liu J, Khoo KS, Salmanpour S, Karimi F, Deepanraj B, Show PL. Recent progress in nanomaterials modified electrochemical biosensors for the detection of MicroRNA. Micromachines. 2021; 12(11):1409. DOI: 10.3390/mi12111409.

[24] Dai C, Choi S, Technology and applications of microbial biosensor. Open Journal of Applied Biosensor. 2013; 2:83–93. DOI:10.4236/ojab.2013.23011.

[25] Ma Z, Meliana C, Munawaroh HSH, Karaman C, Karimi-Maleh H, Low SS, Show PL. Recent advances in the analytical strategies of microbial biosensor for detection of pollutants. Chemosphere. 2022; 135515. DOI: 10.1016/j.chemosphere.2022.135515.

[26] Li S, Zhang D, Liu J, Cheng C, Zhu L, Li C, Lu Y, Low SS, Su B, Liu Q. Electrochemiluminescence on smartphone with silica nanopores membrane modified electrodes for nitroaromatic explosives detection. Biosensors and Bioelectronics. 2019; 129:284–291. DOI: 10.1016/j.bios.2018.09.055.

[27] Low SS, Pan Y, Ji D, Li Y, Lu Y, He Y, Chen Q, Liu Q. Smartphone-based portable electrochemical biosensing system for detection of circulating microRNA-21 in saliva as a proof-of-concept. Sensors and Actuators B: Chemical. 2020; 308:127718.

[28] Bousse L. Whole cell biosensors. Sensors and Actuators B: Chemical. 1996; 34(1-3):270-275. DOI: 10.1016/S0925-4005(96)01906-5.

[29] Su L, Jia W, Hou C, Lei Y. Microbial biosensors: a review, Biosensors and Bioelectronics. 2011; 26(5):1788–1799. DOI: 10.1016/j.bios.2010.09.005.

[30] Chu H, Wei X, Wu M, Yan J, Tu Y. An electrochemiluminescent biosensor based on polypyrrole immobilized uricase for ultrasensitive uric acid detection. Sensors

and Actuators B: Chemical. 2012; 163(1):247–252. DOI: 10.1016/j.snb.2012.01.047.

[31] Miettinen K, Leelahakorn N, Almeida A, Zhao Y, Hansen LR, Nikolajsen IE, Andersen JB, Givskov M, Staerk D, Bak S, Kampranis SC. A GPCR-based yeast biosensor for biomedical, biotechnological, and point-of-use cannabinoid determination. Nature communications. 2022; 13(1):1-16. DOI: 10.1038/s41467-022-31357-6.

[32] Justino CIL, Duarte AC, Rocha-Santos TAP. Recent progress in biosensors for environmental monitoring: a review. Sensors. 2017; 17:2918. DOI: 10.3390/s17122918.

[33] Low SS, Chen Z, Li Y, Lu Y, Liu Q. Design principle in biosensing: critical analysis based on graphitic carbon nitride (G-C3N4) photoelectrochemical biosensor. TrAC Trends in Analytical Chemistry. 2021; 145:116454. DOI: 10.1016/j.trac.2021.116454.

[34] Amaro F, Turkewitz AP, Martín-Gonz´alez A, Guti´errez JC. Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from Tetrahymena thermophila. Microbial Biotechnology. 2011; 4(4):513–522. DOI: 10.1111/j.1751-7915.2011.00252.x.

[35] Scognamiglio V, Arduini F, Palleschi G, Rea G. Biosensing technology for sustainable food safety. TrAC Trends in Analytical Chemistry. 2014; 62:1–10. DOI: 10.1016/j.trac.2014.07.007.

[36] Inda ME, Lu TK. Microbes as biosensors. Annual Review of Microbiology. 2020; 74:337–359. DOI: 10.1146/annurev-micro-022620-081059.

[37] Lopreside A, Calabretta MM, Montali L, Zangheri M, Guardigli M, Mirasoli M, Michelini E. Bioluminescence goes portable: recent advances in whole-cell and cell-free bioluminescence biosensors. Luminescence. 2021, 36(2):278-293. DOI: 10.1002/bio.3948.

[38] Harms H, Wells MC, Van der Meer JR. Whole-cell living biosensors—are they ready for environmental application?. Applied Microbiology and Biotechnology. 2006; 70(3):273-280. DOI: 10.1007/s00253-006-0319-4.

[39] Turdean GL. Design and development of biosensors for the detection of heavy metal toxicity. International Journal of Electrochemical Science. 2011; 2011. DOI: 10.4061/2011/343125.

[40] Mitani Y, Yasuno R, Futahashi R, Oakley TH, Ohmiya Y. Luciferase gene of a Caribbean fireworm (Syllidae) from Puerto Rico. Scientific Reports. 2019, 9(1), 1-6. DOI: 10.1038/s41598-019-49538-7.

[41] Lagarde F, Jaffrezic-Renault N. Cell-based electrochemical biosensors for water quality assessment. Analytical and Bioanalytical Chemistry. 2011; 400(4):947-964. DOI: 10.1007/s00216-011-4816-7.

[42] Alloush HM, Anderson E, Martin AD, Ruddock MW, Angell JE, Hill PJ, Mehta P, Smith MA, Smith JG, Salisbury VC.

A bioluminescent microbial biosensor for in vitro pretreatment assessment of cytarabine efficacy in leukemia. Clinical Chemistry. 2010; 56(12):1862-1870.

[43] Zhang D, He Y, Wang Y, Wang H, Wu L, Aries E, Huang WE. Whole-cell bacterial bioreporter for actively searching and sensing of alkanes and oil spills. Journal of Microbial Biotechnology. 2012; 5(1):87-97. DOI: 10.1111/j.1751-7915.2011.00301.x.

[44] Jouanneau S, Durand MJ, Thouand G. Online detection of metals in environmental samples: comparing two concepts of bioluminescent bacterial biosensors. Environmental Science & Technology. 2012; 46(21):11979-11987. DOI: 10.1021/es3024918.

[45] Cevenini L, Michelini E, D'Elia M, Guardigli M, Roda A. Dual-color bioluminescent bioreporter for forensic analysis: evidence of androgenic and anti-androgenic activity of illicit drugs. Analytical and Bioanalytical Chemistry. 2013; 405(2):1035-1045. DOI: 10.1007/s00216-012-6416-6.

[46] Wen G, Wen X, Shuang S, Choi MM. Whole-cell biosensor for determination of methanol. Sensors and Actuators B: Chemical. 2014; 201:586-591. DOI: 10.1016/j.snb.2014.04.107.

[47] Rawson DM, Willmer AJ, Turner AP. Whole-cell biosensors for environmental monitoring. Biosensors. 1989; *4*(5):299-311. DOI: 10.1016/0265-928X(89)80011-2.

[48] King JMH, DiGrazia PM, Applegate B, Burlage R, Sanseverino J, Dunbar P, Larimer F, Sayler GA. Rapid, sensitive

bioluminescent reporter technology for naphthalene exposure and biodegradation. Science. 1990; 249(4970):778-781. DOI: 10.1126/science.249.4970.778.

[49] Heitzer A, Malachowsky K, Thonnard JE, Bienkowski PR, White DC, Sayler GS. Optical biosensor for environmental on-line monitoring of naphthalene and salicylate bioavailability with an immobilized bioluminescent catabolic reporter bacterium. Applied and Environmental Microbiology. 1994; 60(5):1487-1494. DOI: 10.1128/aem.60.5.1487-1494.1994.

[50] Belkin S. Microbial whole-cell sensing systems of environmental pollutants. Current opinion in microbiology. 2003; 6(3):206–212. DOI: 10.1016/S1369-5274(03)00059-6.

[51] Han L, Zhao Y, Cui S, Liang B. Redesigning of microbial cell surface and its application to whole-cell biocatalysis and biosensors. Applied Biochemistry and Biotechnology. 2018; 185:396–418. DOI: 10.1007/s12010-017-2662-6.

[52] De Paepe B, Peters G, Coussement P, Maertens J, De Mey M. Tailor-made transcriptional biosensors for optimizing microbial cell factories. Journal of Industrial Microbiology and Biotechnology. 2017; 44:623–645. DOI: 10.1007/s10295-016-1862-3.

[53] Rebets Y, Schmelz S, Gromyko O, Tistechok S, Petzke L, Scrima A, Luzhetskyy A. Design, development and application of whole-cell based antibiotic-specific biosensor. Metabolic Engineering. 2018; 47:263-270. DOI: 10.1016/j.ymben.2018.03.019.

[54] Sciuto EL, Petralia S, van der Meer JR, Conoci S. Miniaturized electrochemical biosensor based on whole-cell for heavy metal ions detection in water. Biotechnology and Bioengineering. 2021; 118(4):1456-1465. DOI: 10.1002/bit.27646.

[55] Kucherenko I, Soldatkin O, Dzyadevych S, Soldatkin A. Electrochemical biosensors based on multienzyme systems: Main groups, advantages and limitations—A review. Analytica Chimica Acta. 2020; 1111:114–131. DOI: 10.1016/j.aca.2020.03.034.

[56] Zhao S, Guo D, Zhu Q, Dou W, Guan W. Display of microbial glucose dehydrogenase and cholesterol oxidase on the yeast cell surface for the detection of blood biochemical parameters. Biosensors. 2020; 11(1):13. DOI: 10.3390/bios11010013.

[57] Xu X, Ying Y. Microbial biosensors for environmental monitoring and food analysis. Food Reviews International. 2011; 27(3):300–329. DOI: 10.1080/87559129.2011.563393.

[58] Futra D, Heng L, Surif S, Ahmad A, Ling T. Microencapsulated Aliivibrio fischeri in alginate microspheres for monitoring heavy metal toxicity in environmental waters. Sensors. 2014; 14(12):23248. DOI: 10.3390/s141223248.

[59] Curtis TM, Widder MW, Brennan LM, Schwager SJ, van der Schalie WH, Fey J, Salazar N. A portable cell-based impedance sensor for toxicity testing of drinking water. Lab on a Chip. 2009; 9(15):2176-2183. DOI: 10.1039/B901314H.

[60] Rakers S, Imse F, Gebert M. Real-time cell analysis: sensitivity of different vertebrate cell cultures to copper sulfate measured by xCELLigence®. Ecotoxicology. 2014; 23(8):1582–1591. DOI: 10.1007/s10646-014-1279-6.

[61] Pan T, Li H, Khare S, Huang B, Huang DY, Zhang W, Gabos S. High-throughput screening assay for the environmental water samples using cellular response profiles. Ecotoxicology and Environmental Safety. 2015; 114:134-142. DOI: 10.1016/j.ecoenv.2015.01.020.

[62] Bereza-Malcolm L, Aracic S, Kannan R, Mann G, Franks AE. Functional characterization of gram-negative bacteria from different genera as multiplex cadmium biosensors. Biosensors and Bioelectronics. 2017; 94:380-387. DOI: 10.1016/j.bios.2017.03.029.

[63] Tecon R, Van Der Meer JR. Bacterial biosensors for measuring availability of environmental pollutants. Sensors. 2008; 8, 4062–4080. DOI: 0.3390/s8074062.

[64] Henry R, Schang C, Coutts S, Kolotelo P, Prosser T, Crosbie N, Grant T, Cottam D, O'Brien P, Deletic A. Into the deep: evaluation of Source-Tracker for assessment of faecal contamination of coastal waters. Water Research. 2016; 93:242-253. DOI: 10.1016/j.watres.2016.02.029.

[65] Campbell, A.L., Mangan, S., Ellis, R.P., Lewis, C., Ocean acidification increases copper toxicity to the early life history stages of the polychaete Arenicola marina in artificial seawater.

Environmental Science & Technology. 2014; 48(16):9745-9753. DOI: 10.1021/es502739m.

[66] Simpson SL, Spadaro DA. Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. Environmental Science & Technology. 2016; 50(7):4061-4070. DOI: 10.1021/acs.est.6b00203.

[67] Song Y, Li G, Thornton SF, Thompson IP, Banwart SA, Lerner DN, Huang WE. Optimization of bacterial whole cell bioreporters for toxicity assay of environmental samples. Environmental Science & Technology. 2009; 43(20):7931-7938. DOI: 10.1021/es901349r.

[68] Jiang B, Zhu D, Song Y, Zhang D, Liu Z, Zhang X, Huang WE, Li G. Use of a whole-cell bioreporter, Acinetobacter baylyi, to estimate the genotoxicity and bioavailability of chromium(VI)-contaminated soils. Biotechnology Letters. 2015; 37 (2):343-348. DOI: 10.1007/s10529-014-1674-3.

[69] Wong LS, Lee YH, Surif S. Whole cell biosensor using Anabaena torulosa with optical transduction for environmental toxicity evaluation. Journal of Sensors. 2013; 2013:8. DOI: 10.1155/2013/567272.

[70] Páez-Espino D, Tamames J, de Lorenzo V, Cánovas D. Microbial responses to environmental arsenic. Biometals 2009; 22:117–130. DOI: 10.1007/s10534-008-9195-y.

[71] Van Der Meer JR, Belkin S. Where microbiology meets microengineering: design and applications of reporter bacteria.

Nature Reviews Microbiology. 2010; 8:511–522. DOI: 10.1038/nrmicro2392.

[72] Riangrungroj P, Bever CS, Hammock BD, Polizzi KM. A label-free optical whole-cell Escherichia coli biosensor for the detection of pyrethroid insecticide exposure. Scientific Reports. 2019; 9(1):1-9. DOI: 10.1038/s41598-019-48907-6.

[73] Sunantha G, Vasudevan N. A method for detecting perfluorooctanoic acid and perfluorooctane sulfonate in water samples using genetically engineered bacterial biosensor. Science of The Total Environment. 2021; 759:143544. DOI: 10.1016/j.scitotenv.2020.143544.

[74] Kabessa Y, Eyal O, Bar-On O, Korouma V, Yagur-Kroll S, Belkin S, Agranat AJ. Standoff detection of explosives and buried landmines using fluorescent bacterial sensor cells. Biosensors and Bioelectronics. 2016; 79:784–788. DOI: 1 0.1016/j.bios.2016.01.011.

[75] Shemer B, Shpigel E, Hazan C, Kabessa Y, Agranat AJ, Belkin S. Detection of buried explosives with immobilized bacterial bioreporters. Microbial biotechnology. 2021; 14:251–261. DOI: 10.1111/1751-7915.13683.

[76] Siedler S, Khatri NK, Zsoh´ar A, Kjærbølling I, Vogt M, Hammar P, Nielsen CF, Marienhagen J, Sommer MOA, Joensson HN. Development of a bacterial biosensor for rapid screening of yeast p-Coumaric acid production. ACS Synthetic Biology. 2017; 6:1860–1869. DOI: 10.1021/acssynbio.7b00009.

[77] Mimee M, Nadeau P, Hayward A, Carim S, Flanagan S, Jerger L, Collins J, McDonnell S, Swartwout R, Citorik RJ, Bulovi´c V, Langer R, Traverso G, Chandrakasan AP, Lu TK. An ingestible bacterial-electronic system to monitor gastrointestinal health. Science. 2018; 360:915–918. DOI: 10.1126/science.aas9315.

[78] Woo SG, Moon SJ, Kim SK, Kim TH, Lim HS, Yeon GH, Sung BH, Lee CH, Lee SG, Hwang JH, Lee DH. A designed whole-cell biosensor for live diagnosis of gut inflammation through nitrate sensing. Biosensors and Bioelectronics. 2020; 168:112523. DOI: 10.1016/j.bios.2020.112523.

[79] Antonacci A, Scognamiglio V. Biotechnological advances in the design of algae-based biosensors. Trends in Biotechnology. 2020; 38:334–347. DOI: 10.1016/j.tibtech.2019.10.005..

[80] Eom H, Park M, Jang A, Kim S, Oh SE. A simple and rapid algal assay kit to assess toxicity of heavy metal-contaminated water. Environmental Pollution. 2021; 269:116135. DOI: 10.1016/j.envpol.2020.116135.

[81] Brayner R, Cout´e A, Livage J, Perrette C, Sicard C. Micro-algal biosensors. Analytical and bioanalytical chemistry. 2011; 401:581–597. DOI: 10.1007/s00216-011-5107-z.

[82] Jacob JM, Ravindran R, Narayanan M, Samuel SM, Pugazhendhi A, Kumar G. Microalgae: a prospective low cost green alternative for nanoparticle synthesis. Current Opinion in Environmental Science & Health. 2021; 20:100163. DOI: 10.1016/j.coesh.2019.12.005.

[83] Han S, Zhang Q, Zhang X, Liu X, Lu L, Wei J, Li Y, Wang Y, Zheng G. A digital microfluidic diluter-based microalgal motion biosensor for marine pollution monitoring. Biosensors and Bioelectronics. 2019; 143:111597. DOI: 10.1016/j.bios.2019.111597.

[84] Boron I, Ju´arez A, Battaglini F. Portable microalgal biosensor for herbicide monitoring. ChemElectroChem. 2020; 7:1623–1630. DOI: 10.1002/celc.202000210.

[85] Chai WS, Tan WG, Halimatul Munawaroh HS, Gupta VK, Ho SH, Show PL. Multifaceted roles of microalgae in the application of wastewater biotreatment: a review. Environmental Pollution. 2021; 269:116236. DOI: 10.1016/j.envpol.2020.116236.

[86] Wollmann F, Dietze S, Ackermann JU, Bley T, Walther T, Steingroewer J, Krujatz F. Microalgae wastewater treatment: biological and technological approaches. Engineering in Life Sciences. 2019; 19:860–871. DOI: 10.1002/elsc.201900071.

[87] Maryjoseph S, Ketheesan B. Microalgae based wastewater treatment for the removal of emerging contaminants: a review of challenges and opportunities. Case Studies in Chemical and Environmental Engineering. 2020; 2:100046. DOI: 10.1016/j.cscee.2020.100046.

[88] Wong LS, Judge SK, Voon BWN, Tee LJ, Tan KY, Murti M, Chai MK. Bioluminescent microalgae-based biosensor for metal detection in water. IEEE Sensors Journal. 2018; 18:2091–2096. DOI: 10.1109/JSEN.2017.2787786.

[89] Nandimandalam H, Gude VG. Indigenous biosensors for in situ hydrocarbon detection in aquatic environments. Marine Pollution Bulletin. 2019; 149:110643. DOI: 10.1016/j.marpolbul.2019.110643.

[90] Gan T, Zhao N, Yin G, Chen M, Wang X, Liu J, Liu W. Optimal chlorophyll fluorescence parameter selection for rapid and sensitive detection of lead toxicity to marine microalgae Nitzschia closterium based on chlorophyll fluorescence technology. Journal of Photochemistry and Photobiology B: Biology. 2019; 197:111551. DOI: 10.1016/j.jphotobiol.2019.111551.

[91] Adeniran A, Sherer M, Tyo KEJ. Yeast-based biosensors: design and applications. FEMS Yeast Research. 2015; 15:1-15. DOI: DOI:10.1111/1567-1364.12203.

[92] Chamas A, Pham HTM, Baronian K, Kunze G. Biosensors based on yeast/ fungal cells. Biotechnology of Yeasts and Filamentous Fungi. 2017; 2017:351–371. DOI: 10.1007/978-3-319-58829-2_12.

[93] Walmsley RM, Keenan P. The eukaryote alternative: Advantages of using yeasts in place of bacteria in microbial biosensor development. Biotechnology and Bioprocess Engineering. 2000; 5(6):387–394. DOI: 10.1007/BF02931936.

[94] Tobehn-Steinhäuser I, Günther M, Görlandt S, Herbs S, Wünscher H, Ortlepp T, Gerlach G. Multisensors for whole-cell analytics. Informatyka, Automatyka, Pomiary w Gospodarce i

Ochronie Środowiska. 2018; 2018,8. DOI: 10.5604/01.3001.0012.0702.

[95] Dhakal S, Macreadie I. The Use of Yeast in Biosensing. Microorganisms. 2022; 10(9):1772. DOI: 10.3390/microorganisms10091772.

[96] Hahne K, Rödel G, Ostermann K. A fluorescence-based yeast sensor for monitoring acetic acid. Engineering in Life Sciences. 2021; 21(5):303-313. DOI: 10.1002/elsc.202000006.

[97] Miller RA, Lee S, Fridmanski EJ, Barron E, Pence J, Lieberman M, Goodson HV. "Scentsor": A Whole-Cell Yeast Biosensor with an Olfactory Reporter for Low-Cost and Equipment-Free Detection of Pharmaceuticals. ACS sensors. 2020; 5(10):3025-3030. DOI: 10.1021/acssensors.0c01344.

[98] Pham ML, Polakovič M. Microbial cell surface display of oxidoreductases: Concepts and applications. International Journal of Biological Macromolecules. 2020; 165:835–841. DOI: 10.1016/j.ijbiomac.2020.09.237.

[99] Teymennet-Ramírez KV, Martínez-Morales F, Trejo-Hernández MR. Yeast surface display system: strategies for improvement and biotechnological applications. Frontiers in Bioengineering and Biotechnology. 2021; 9. DOI: 10.3389/fbioe.2021.794742.

[100] Ueda M. Yeast Cell Surface Engineering: Biological Mechanisms and Practical Applications; Springer: Singapore, 2019.

[101] Boder ET, Wittrup KD. Yeast surface display for screening combinatorial polypeptide libraries. Nature biotechnology. 1997; 15:553–557.

[102] World Health Organization (2018, February 15). Arsenic. Retrieved from https://www.who.int/news-room/fact sheets/detail/arsenic.

[103] Lehmann M, Riedel K, Adler K, Kunze G. Amperometric measurement of copper ions with a deputy substrate using a novel Saccharomyces cerevisiae sensor. Biosensors and bioelectronics. 2000; 15:211-219. DOI: 10.1016/S0956-5663(00)00060-9.

[104] Vopálenská I, Váchová L, Palková Z. New biosensor for detection of copper ions in water based on immobilized genetically modified yeast cells. Biosensors and bioelectronics. 2015; 72:160-167. DOI: 10.1016/j.bios.2015.05.006.

[105] Yuan J, Fan C, Zhang D, Mo Q. High-performance Saccharomyces cerevisiae-based biosensor for heavy metal detection. bioRxiv. 2021 DOI: 10.1101/2021.12.13.472527.

[106] Sun A, Wang WX. A yeast-based biosensor for silver nanoparticle accumulation and cellular dissolution. Biosensors and Bioelectronics. 2022; 205:114082. DOI: 10.1016/j.bios.2022.114082.

[107] Wang J, Eldridge M, Menn F, Dykes T, Sayler G. Standardized application of yeast bioluminescent reporters as endocrine disruptor screen for comparative analysis of wastewater effluents from membrane bioreactor and traditional

activated sludge. Ecotoxicology. 2015; 24:2088–2099. DOI: 10.1007/s10646-015-1556-z.

[108] Westlund P, Yargeau V. Investigation of the presence and endocrine activities of pesticides found in wastewater effluent using yeast-based bioassays. Science of the Total Environment. 2017; 607–608:744–751. DOI: 10.1016/j.scitotenv.2017.07.032.

[109] Miller RA, Brown G, Barron E, Luther JL, Lieberman M, Goodson HV. Development of a paper-immobilized yeast biosensor for the detection of physiological concentrations of doxycycline in technology-limited settings. Analytical Methods. 2020; 12(16):2123-2132. DOI: 10.1039/D0AY00001A.

[110] Cottier V, Barberis A, Lüthi U. Novel yeast cell-based assay to screen for inhibitors of human cytomegalovirus protease in a high-throughput format. Antimicrobial Agents and Chemotherapy. 2006; 50:565–571. DOI: 10.1128/AAC.50.2.565-571.2006.

[111] Dhakal S, Macreadie I. Tyramine and Amyloid Beta 42: A Toxic Synergy. Biomedicines. 2020; 8(6):145. DOI: 10.3390/biomedicines8060145.

[112] Bui VN, Nguyen TTH, Mai CT, Bettarel Y, Hoang TY, Trinh TTL, Truong NH, Chu HH, Nguyen VTT, Nguyen, H.D, et al. Procarcinogens—Determination and Evaluation by Yeast-Based Biosensor Transformed with Plasmids Incorporating RAD54 Reporter Construct and Cytochrome P450 Genes. PLoS ONE. 2016; 11: e0168721. DOI: 10.1371/journal.pone.0168721.

[113] Becker B, Schnöder T, Schmitt MJ. Yeast Reporter Assay to Identify Cellular Components of Ricin Toxin a Chain Trafficking. Toxins. 2016; 8(12):366. DOI: 10.3390/toxins8120366.

[114] Diehl B, Hoffmann TM, Mueller NC, Burkhart JL, Kazmaier U, Schmitt MJ. Novel yeast bioassay for high-throughput screening of matrix metalloproteinase inhibitors. Applied and Environmental Microbiology. 2011; 77(24):8573–8577. DOI: 10.1128/AEM.06111-11.

[115] Ostrov N, Jimenez M, Billerbeck S, Brisbois J, Matragrano J, Ager A, Cornish VW. A modular yeast biosensor for low-cost point-of-care pathogen detection. Science Advances. 2017; 3:e1603221. DOI: 10.1126/sciadv.1603221.

[116] Lobsiger N, Venetz JE, Gregorini M, Christen M, Christen B, Stark WJ. YestroSens, a field-portable S. cerevisiae biosensor device for the detection of endocrine-disrupting chemicals: reliability and stability. Biosensors and Bioelectronics. 2019; 146:111710. DOI: 10.1016/j.bios.2019.111710.

[117] Cevenini L, Lopreside A, Calabretta MM, D'Elia M, Simoni P, Michelini E, Roda A, A novel bioluminescent NanoLuc yeast-estrogen screen biosensor (nanoYES) with a compact wireless camera for effect-based detection of endocrine-disrupting chemicals. Analytical and bioanalytical chemistry. 2018; 410(4):1237–1246. DOI: 10.1007/s00216-017-0661-7.

CHAPTER 6

Aptamers and Biotechnological Applications

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Aptamers and Biotechnological Applications

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Abstract

Aptamers are short, single- stranded DNA or RNA molecules capable of interacting with various targets with high affinity and specifity. SELEX method is used for aptamer selection. Various modifications to the SELEX method have been made over the years in order to make the procedure more efficient and less time-consuming, as well as to increase the affinity of the aptamers that are selected and automate the process. The use of aptamer molecules has been widespread in faciliating discoveries in basic research, ensuring food safety and monitoring environmental condition. Moreover, aptamers have promising therapeutic and diagnostic potential. This chapter provides general information about aptamers, selection process and recent advances in aptamer researches.

Keywords: Aptamer, DNA SELEX, RNA SELEX, aptasensors, therapeuticals

1. Introduction

Natural selection was one of Darwin's key discoveries over 150 years ago, which contributed to the theory of evolution. In-vitro selection, on the other hand, was not recognized until much later [1].

Nucleic acids have the intrinsic ability to fold into explicit three-dimensional structures which allow them to interact with multiple target molecules [2]. To put it simply, it is possible to selectively generate nucleic acids with the highest specificity and affinity for the target molecules [3]. These molecules are called "aptamers" and are oligonucleotides with single-stranded DNA or RNA with 6-30 kDa molecular weight (generally 20 to 80 nucleotides) [4]. The term "aptamer" originated from the Latin *aptus* (to fit) and Greek *meros* (particle) [5,6].

Aptamers have similar conformational recognition with antibody-antigen recognition that bind to their conjugate targets using van der Waals forces, electrostatic interactions, hydrogen bonding, shape complementarity [7], and dissociation constansts (Kd) generally range from picomolar to nanomolar [8]. For all these reasons, aptamers are called as "chemical antibodies" and functionally used as targeting ligands, antagonists or agonists [7,8].

Compared with antigens, aptamers have been shown to be more advantages due to their nucleic acid characteristics and smaller size that can be used for clinical and industrial purposes [9]. In many ways, aptamers are more useful than antibodies in clinical applications [table 1]. Despite being small, they can vigorously penetrate tissue barriers and easily enter target cells; they are nontoxic in vivo and nonimmunogenic; they could be developed to attack many different targets including small inorganic ions, organic peptides, drugs, proteins and also tissues [12]; they have also thermal stability, so they could be easily stored and transported [7]; they could be produced and modified in a short period of time (hours) in large scale due to well-established modification and chemical synthesis technologies [7].

Table 1. Comparision features of aptamer and antibody

Features	**Antibody**	**Aptamer**
Specifity	Low	High
Cost	High	Low
Stability	Unstable	Stable
Synthesis	Up to 6 months	2-8 weeks
Size	Relatively large	Small
Affinity	Low	High
Modification	Limited	Easily modified
Potential targets	Immunological targets	Wide range

2. Generation of Aptamers

2.1. SELEX technology

SELEX is a method used to select aptamers in vitro, which is a process developed independently by two different research groups in 1990 [5,6]. Since then, numerous improvements and modifications have been succeeded. SELEX is the gold-standard methodology for improving specific aptamers.

Traditionally, aptamers are generated by SELEX in three steps: selection, partitioning and amplification (figure 1) [13, 17]. An oligonucleotides library containing up to 10^{15} unique sequences is synthesized before the selection step. It consists of random 20 to 50 nt bases between two conserved primer binding sites, and these primers are used for PCR amplification. As part of the selection process, random sequences into an initial oligonucleotide library are folded into secondary and tertiary structures and then incubated with target molecules under optimal conditions in order to generate aptamer-target complexes. The partitioning step involves separating unbound sequences from the area by various methods, including membrane filtration, affinity columns, magnetic beads or capillary electrophoresis. Following partitioning, aptamer-target complexes are amplified by PCR for DNA aptamers (DNA SELEX) or RT-PCR for RNA aptamers (RNA SELEX) [14]. A new sub pool of PCR products is created

and used for the next round of selection. Approximately 8-20 rounds of selection were required to obtain specific aptamers. The entire procedure could take weeks to months. As a result, several modified SELEX procedures were developed to shorten the selection time and improve specifity [15,16].

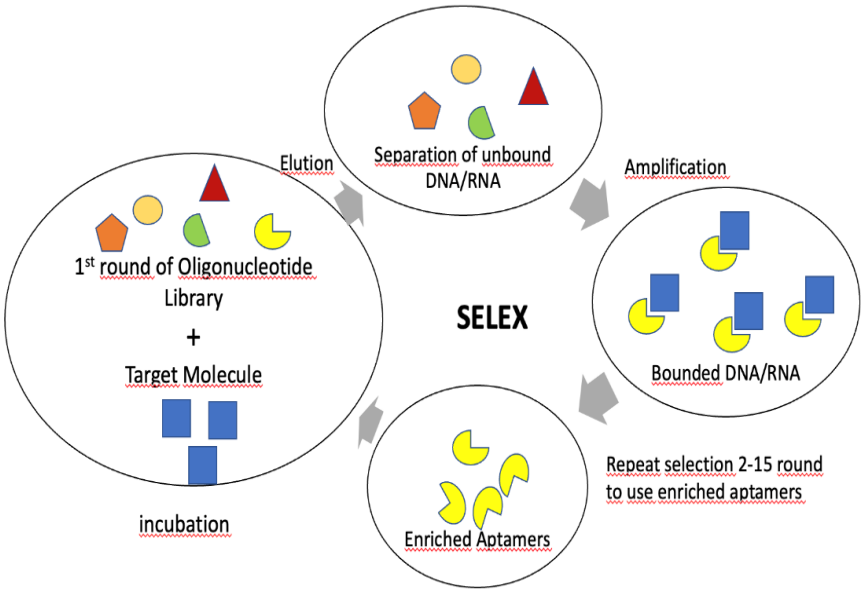

Figure 1. Schematic representation of SELEX [17].

Table 2. Frequently used SELEX methods and explanations

Method	Description	Reference
Negative SELEX	The primitive type of SELEX	[3,6]
Capture SELEX	Aptamers are identified by immobilizing oligonucleotide libraries on a support instead of targets	[64]
Cell SELEX	Aptamers can be generated from whole live cells	[65]
Genomic SELEX	An organism's genomic DNA is used to constuct the SELEX library	[66]
IP-SELEX	Includes immunoprecipitation	[67]
Counter or Subtractive SELEX	An aptamer is incubated with a closely related molecule or cell	[68]
In vivo SELEX	A method for in vivo aptamer evolution.	[69]
Indirect SELEX	SELEX uses a metal ion-dependent oligonucleotide approach	[70]
Animal SELEX	Aptamers selected among live animal model	[71]
Capillar electrophoresis SELEX	Electrophoresis is used to separate ions based their mobility	[72]

2.1.1. Production of random DNA/RNA oligonucleotide library

An initial step in the SELEX procedure is the chemical synthesis of randomly selected DNA oligonucleotides. Several ssDNA fragments are included in this library (~10 15 molecules). The fragments range in length from 22 nucleotides to 220 nucleotides, with an average length of 30 to 80 nucleotides [14]. These fragments are flanked by 18-21 nt specific sequences named as primer binding sequences (PBS) for PCR reaction. As a first step in a DNA SELEX, this library could be used for selecting DNA aptamers. In each SELEX round, sense and antisense primers derived from specific sequences at the 5' and 3' ends allow the amplification of selected oligonucleotides [14, 18].

It is necessary to transform the random DNA library into an RNA library before starting the RNA SELEX procedure in order to select RNA aptamers [19]. A specific sense primer with T7 promoter sequences at the 5' end and antisense primer are required to transform the ssDNA library to dsDNA library via PCR. These dsDNA is transcribed by the T7 RNA polymerase and become a randomized RNA library. This RNA library is used to start RNA SELEX and must be revers transcribed in every SELEX cycle [14, 18].

An aptamer's length can be a major factor in whether SELEX works or fails, so it should be carefully considered. Oligonucleotide library length depends on amount of starting material [20]. For example, libraries with short random sequences are more suitable the small or low complexity targets; if the more complex molecules are targeted, the longer aptamer sequences could be an advantage. In contrast, a meta-review on aptamer selection concluded that individual sequence and structure were more important in aptamer affinity than library length [21]. Because the equal distribution between A- T/U and G-C is expected, optimization of the molar ratio is required. It has been stated that because DNA libraries consist of more G and C bases than A and T bases, majority of G and C bases in aptamer could increase the structural stability and diversity of library [22-24].

In some SELEX experiments, chemically modified oligonucleotide libraries were used to enhance the complexity of the library. There are a number of different ways to modify aptamers, including adding new functional groups that can modulate their ability to interact with target molecules, or increasing their stability, or increasing their resistance to nucleases, which are important for many aptamer applications [25-27]. Another method is to modify nucleic acids' phosphate backbones to select in vitro aptamers. A common type is the replacement of the non-binding oxygen by sulfur [14]. The aim of this process is increasing to resistance of oligonucleotides against

nuclease digestion. other modifications could enable quantification of selected oligonucleotides during the SELEX process. Commonly used techniques are incorporating of radioactive labeled nucleotides or attaching to the 5' end of oligonucleotides with fluorescent molecules [14].

2.1.2. Selection

Selection involves three general principles: binding the target molecule with the oligonucleotide library, removing unbound oligonucleotides, and eluting the bindin oligonucleotides. The purpose of selection step is to find out the oligonucleotides with highest specifity and affinity to the target molecule. Thus, target molecule and oligonucleotide library is incubated for a period of time in the same test tube. The direct interaction binds target molecule and oligonucleotide library.

The separation of binding and nonbinding oligonucleotides is one of the most important steps in the SELEX process [22, 25]. Immobilization of target molecule on a particular matrix material is an effective methodology for separation process. Affinity chromotography is frequently used method in partitioning step [28]. The biggest disadvantage of this technique is substantial amount of target are needed to bind to the column [29]. Another immobilization technique is use of magnetic beads [30-33]. This method is easy to use and very small amount of target is sufficient

for immobilization. On the other hand, ultrafiltration method by use of nitrocellulose filtrers separate unbounds without immobilization [34]. However, it could be observed unspecific interactions or losing of target binding molecules in both methods. Therefore, in recent years, several authors develop possible separation methods for SELEX process [35-42].

2.1.3. Amplification

In the selection step only few functional oligonucleotides bind the target. Therefore, an additional amplification step is required for enrichment of selected aptamer pool. On the other hand, it is also possible to add additional functional groups or properties to the special primers for detection to DNA aptamers for different applications [44-46]. Amplification step for RNA oligonucleotides differs from DNA oligonucleotides in point of reverse transcription PCR (RT-PCR) stage. First, cDNA is obtained from RNA and then amplified in a subsequent PCR.

2.1.4. Single-stranded DNA (ssDNA) or RNA production

After amplification step, double-stranded DNAs (dsDNA) are obtained. Aptamer strands hybridized with their complementary sequences. ssDNA or RNA aptamers are needed to regenerate to continue to the next selection step. For RNA aptamers, a transcription with T7 RNA polymerase is sufficient [46]. In case

of ssDNA aptamers, DNA strands must be separated. For this purpose, there are various methods in literature. The most common method is streptavidin/biotin system that could be used two different ways [47, 48]. In the first way, biotin molecule is added to the unwanted strand by researchers, and they distinguish unwanted strand in the gel electrophoresis. In the second way, biotinylated strand binds to streptavidin beads or plates and is separated from unwanted strand after DNA denaturation. Another method is generating of ssDNA with asymmetric PCR amplification that produces ssDNA by using vastly different amount (eg. 100:1forward/reverse) of sense and antisense primers in the PCR reaction [49]. Thereby, the PCR reaction is splited into two phases. The first phase is like the regular PCR and dsDNA is produced at an exponential rate. The second phase starts after reverse primer used up. The final PCR product contains devastating amounts of ssDNA and little amount of dsDNA. After PCR step, native gel electrophoresis is carried on and this give the possibility to distinguish the dsDNA bands from the ssDNA bands according to the size [50]. Another method for production of ssDNA is enzymatic digestion [51]. In this technique, 5' phosphate labelled reverse primer is used in the PCR and antisense strands are labelled. Then lambda-exonuclease is added and enzyme degrades the antisense strand but the sense strands are intact. The enzyme must be removed in time due to some of ssDNA material could be lost [51].

2.1.5. Sequencing

After 6-20 cycles of SELEX, aptamers binded with target should be abundant in the oligonucleotide pool[14]. Identifying individual aptamers requires sequencing the DNA pool and evaluating the data. Traditional method is cloning of the pool into a bacterial vector and after that selecting individual aptamer colonies and sequencing with Sanger Sequencing method [52]. Begining to using of Next Generation Sequencing (NGS) methods into SELEX makes easier to reading of more than a hundred million sequence from a single oligonucleotide pool [53-55]. Lastly, nanopore sequencing method has been indicated that could be promising for future applications. Briefly, this technique depends on passage of oligonucleotides through a nanopore and detection of nucleotides according to the voltage changes [56,57]. Theoretically, this technique offers to sequence different types of modified DNA, RNA and proteins.

2.2. Biosensor Design Methods with Aptamers

When aptamer sequence is determined, an assay could be designed for detection of target molecule. Aptamers are known that using similar way with antibody-antigen binding. But beyond that, varius assay configurations with various signaling methods has been designed unique to aptamers.

2.2.1. Optical sensors

Optical assays are common methods for detecting molecules of interest. Aptamer based optical assays are constituted with recognition and transducer parts [1]. Aptamers are used as recognition element while signals produced by biological, chemical or physical phenomenon are used as transducer. Transducers turned these signals into visible, infrared (IR) or ultraviolet (UV) radiations [58]. There are five different optical techniques that are colorimetric, chemiluminescence, fluorescence, surface plasmon resonance and surface-enhanced Raman scattering [59, 60].

One of the most common optical methods for detecting molecules is ELISA (enzyme-linked immunosorbent assay) [1]. Because aptamers show similar affinity with antibodies, they could replace antibodies in ELISA. The “sandwich” ELISA is one of the most used techniques in aptamer detection. In this method, the target molecule is sandwiched between two aptamers, one capture probe and one reporter probe. While reporter probes are typically conjugated to signaling components such as fluorophores, enzymes, or nanoparticles, capture probes are generally immobilized to solid support surfaces such as glass chips or nanoparticles [60].

2.2.2. Electrochemical sensors

Another detection method is electrochemical aptasensor technique. This technique depends on electric change produced by oxidation-reduction reactions occurring on the surface of electrode [60]. Electrochemical aptasensors are produced by immobilizing the aptamer to the carbon or gold-based electrode substrates [61]. When aptamer and substrate interact, a signal is produced. This signal has to be transformed and measured by different methods such as amperometric, potentiometric, voltammetric and conductometric [62]. Compared to the optical techniques, these assays are cost effective and need fewer reaction reagent and specific instrument [63].

2.3. Aptamer Applications

Aptamers show promise due to the fact that they are analogue with antibodies. They could specifically recognize and bind to their target [73]. Therefore, aptamers could be used several therapeutic and diagnostic applications. Additionally, they have been used in different areas such as biotechnology, biomedicine and molecular biology due to easy modification, stability and simple preparation.

2.3.1. Aptamers for diagnostic purposes

There are several areas such as biomedical diagnostic, enviromental contamination detection and monitoring food safety to use the aptamers successfully.

2.3.1.1. Aptamers used as biomedical diagnostic agent

Because aptamers show high specifity and affinity, they become ideal diagnostic tools. Additionally, beacuse aptamers can easily be conjugated and labelled, they combined with other high throughput techniques such as flow cytometry, microfluidic cell separation or nanoparticle based sensing techniques [13]. The first diagnostic DNA aptamer was developed for detecting anthrax spores in 1999 [74]. To date, researchers has develeoped aptamers for diagnosis of cardiovascular diseases, cancer diseases and ophtalmology [75-77].

Additionally, many researchers are utilized SELEX based approaches in the design of biosensors to detect infectious agents in infectious diseases. Vaccinia virus [78], herpes simplex virus [79], hepatitis C virus [80], hepatitis B virus [80], human immunodeficiency virus (HIV) [81], influenza virus [82] and severe acute respiratory syndrome (SARS) [83] coronavirus are a few examples of aptasensors. Furthermore, *Lactobacillus acidophilus* [84], *E. Coli* [84], *Staphylococcus aureus* [85],

Mycobacterium tuberculosis [86] are examples of the whole cell based SELEX procedure for pathogen detection.

2.3.1.2. Aptamers used as environmental and food monitoring detection

Nowadays, monitoring of environmental contaminants are an important issue since human and animals can expose to pollutants via water, soil or foods [87]. Therefore, sensitive, rapid and robust, user-friendly detection systems are needed to detect pollutants in the environment. Although traditional techniques such as mass spectrometry or HPLC have high specifity, there could be some challenges in practice such as degradation of contaminants during analysis, wrong analysis result due to low concentration of contaminants [87]. To minimize that analysis errors, researchers try to develop different detection methods. Aptamer-based biosensors, aptasensors, are quite convenient for detection of harmful agents in the environment.

Aptasensors for monitoring environmental contamination detect bacteria, toxins, toxic chemicals, pesticides, antibiotics and pharmaceuticals in the water, soil or foods. Aptasensors are developed by researchers for detecting pathogenic bacteria caused to widespread illness and death such as *Salmonella* [88], *Staphylococcus aureus* [89, 90], *Listeria monocytogenes* [91, 92], *Escherichia coli* [93], *Pseudomonas aeruginosa* [94] and *Vibrio*

[95] in food and water sources. Furthermore, there are several developed aptasensors for multiplex detection of bacteria [96-100].

Another type of aptasensors is developed against to various toxins and toxic chemicals could be found in foods or water. Aptasensors against Ochratoxin A (OTA), bacterial endotoxins are a few examples of designed biosensors for water and food monitoring [101, 102]. Aptamers against heavy metals such as arsenic, copper and mercury have also been produced to detect contamination [103-105]. Furthermore, there are several developed aptasensors for multiplex detection of heavy metals in the same sample [106-108].

In addition, aptamers have been generated against some antibiotics and pesticides which may cause several damage in humans. Aptamers against tetracycline antibiotic, acetamiprid and atrazine pesticides are a few important examples of developed aptasensors [109-111].

2.3.2. Aptamers used for drug delivery and therapeutics

Biosensors are designed for drug delivery to the target region by utilizing the ability of aptamers to bind to a specific region and send them to the intended site, easily modified chemical structure and high affinity. Additionally, aptamers have a unique position

that therapeutics could be developed for intracellular, extracellular or cell surface targets with antisense nucleotides (siRNAs, miRNAs). The first siRNA therapeutic was developed in 2006. [112]. In this study, researchers linked an RNA aptamer against human prostate-specific membrane antigen (PSMA) to therapeutic siRNA and was targeted overexpressed PLK1 and BCL2 survival genes to inhibit overexpression and tumor growth in prostate cancer. After this study, targeted siRNA delivery using with aptamer is actively studied as promising therapeutic [113-117]. miRNAs are other promising therapeutics. Dysregulation of expression of miRNAs are related many diseases. Therefore, the principle of developed aptamer-miRNA chimeras is restoration of miRNA levels with specific delivery [118, 119].

Traditional drugs used in cancer treatment generally lack selectivity, resulting in severe side effects in patient. Using aptamers with drugs in the cancer treatment is very good option to reduce the side effects and increase the success of treatment. Doxorubicin, used in curing various cancers, is intercalated into aptamer and targeted to prostate specific membrane antigen (PSMA) which is expressed on the surface of prostatic adenocarcinoma cells [120]. Doxorubicin is also used in lymphoblastic leukemia by linking another aptamer [121]. Nanomaterial linked aptamers are also used for smart drug carrier for targeted delivery of drugs [122, 123]. Principle of that drug and drug carrier complex is enhancing of anti-tumor activity and

diminishing of toxicity. Another promising candidate for drug delivery are Liposomes. Liposomes could encapsulate both hydrophilic or hydrophobic drugs; attach with different ligands and enhance its accumulation at the target sites. Thus, they are suitable drug carrier to targeted site. There are developed aptamers with liposomes specific for different cancer cells [124, 125].

A number of aptamers have been developed and entered clinical trials for various conditions such as ocular diseases, cancer, haematologic diseases, coagulation and inflammation [126, 127]. Vascular endothelial growth factor (VEGF) specific aptamer named as Pegaptanib is the first approved aptamer that is used for treatment of Age Related Macular Degeneration (AMD) [128]. In addition, several other aptamers are evaluated in clinical trials for many diseases. The current list of therapeutic aptamers could be reached at the Clinical Trials website of the National Institute of Health (NIH) [https://clinicaltrials.gov/].

Conclusion

After many years of development, aptamer researches has made great progress. An aptamer is more suitable than an antibody as detecting molecule by its short preparation time, stability, lower cost, easy labeling, wide range of target and high specifity and affinity.

The success of SELEX depends on different factors such as target type, oligonucleotide library, selection method, cycle number of amplification. Therefore, researchers developed different SELEX variants that fit the target molecule.

As described in this chapter, aptamers have great number of medically relevant and biotechnological applications. Using aptamers in many different fields as biosensors, named as aptasensors, for detection of toxic chemicals, pathogen bacteria, toxins and environmental pollutants has rapidly increased. Another promising application is using aptamers for their therapeutic potential in several diseases including infectious diseases, oncology, inflammatory disorders and vascular disease. Aptamers also have been used as site specific drug delivery agents to deliver the drug directly to the target site. Aptamer technology is potential research area developing new classes of pharmaceuticals and next generation screening tools. This chapter gives summary of developed aptamers for diagnosis and therapeutical.

References

[1] Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. TrAC Trends in Analytical Chemistry. 2008; 27(2), 108-117. DOI: 10.1016/j.trac.2007.12.004.

[2] Marimuthu C, Tang TH, Tominaga J, Tan SC, Gopinath SC. Single-stranded DNA (ssDNA) production in DNA aptamer generation, Analyst, 2012; 137(6), 1307-1315. DOI: 10.1039/c2an15905h.

[3] Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science,1990; 249(4968), 505-510. DOI: 10.1126/science.2200121.

[4] Sun H, Zu Y. A Highlight of Recent Advances in Aptamer Technology and Its Application, Molecules, 2015; 30;20(7):11959-80. DOI: 10.3390/molecules200711959.

[5] Jayasena SD. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clinical chemistry, 1999; 45(9), 1628-1650.

[6] Ellington AD, Szostak JW. Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. Nature, 1992; 355(6363), 850-852.

[7] Sun H, Zu Y. A highlight of recent advances in aptamer technology and its application. Molecules, 2015; 20(7), 11959-11980. DOI: 10.3390/molecules200711959.

[8] Almasi F, Mousavi Gargari SL, Bitaraf F, Rasoulinejad S. Development of a single stranded DNA aptamer as a molecular probe for lncap cells using cell-selex, Avicenna J. Med. Biotechnol. 2016, 8, 104–111.

[8] Lao YH, Phua KK, Leong KW. Aptamer nanomedicine for cancer therapeutics: Barriers and potential for translation, ACS Nano 2015, 9, 2235–2254.

[9] Xiang D, Shigdar S, Qiao G, Wang T, Kouzani AZ, Zhou SF, Kong L, Li Y, Pu C, Duan W. Nucleic acid aptamer-guided cancer therapeutics and diagnostics: The next generation of cancer medicine. Theranostics 2015; 5, 23–42. DOI: 10.7150/thno.10202

[10] Ireson CR, Kelland LR. Discovery and development of anticancer aptamers. Mol. Cancer Ther. 2006; 5, 2957–2962. DOI: 1158/1535-7163.mct-06-0172.

[11] Xiang D, Shigdar S, Qiao G, Wang T, Kouzani AZ, Zhou SF, Kong L, Li Y, Pu C, Duan W. Nucleic acid aptamer-guided cancer therapeutics and diagnostics: The next generation of cancer medicine. Theranostics 2015; 5, 23–42. DOI: 10.7150%2Fthno.10202.

[12] Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, Mallikaratchy P, Sefah K, Yang CJ, Tan W. Aptamers evolved from live cells as effective molecular probes for cancer study. Proc. Natl. Acad. Sci. USA 2006; 103, 11838–11843. DOI: 10.1073/pnas.0602615103.

[13] Zhuo Z, Yu Y, Wang M, Li J, Zhang Z, Liu J, ... & Zhang, B. Recent advances in SELEX technology and aptamer applications in biomedicine. International journal of molecular sciences, 2017; 18(10), 2142. DOI: 10.3390/ijms18102142.

[14] Stoltenburg R, Reinemann C, Strehlitz, B. SELEX—a (r) evolutionary method to generate high-affinity nucleic acid ligands. Biomolecular engineering, 2007; 24(4), 381-403.

[15] Sinha A, Gopinathan P, Chung YD, Lin HY, Li KH, Ma HP, Huang PC, Shiesh SC, Lee GB. An Integrated Microfluidic Platform to Perform Uninterrupted SELEX Cycles to Screen Affinity Reagents Specific to Cardiovascular Biomarkers. Biosens. Bioelectron. 2018; 122, 104−112.

[16] Weng CH, Hsieh IS, Hung LY, Lin HI, Shiesh SC, Chen YL, Lee GB. An Automatic Microfluidic System for Rapid Screening of Cancer Stem-Like Cell-Specific Aptamers. Microfluid. Nanofluid. 2013; 14, 753−765.

[17] Zhang Y, Lai BS, Juhas M. Recent advances in aptamer discovery and applications. Molecules, 2019; 24(5), 941.

[18] Marshall KA, Ellington AD. In vitro selection of RNA aptamers, Methods Enzymol. 2000; 318, 193–214. [19] Darmostuk M, Rimpelova S, Gbelcova H, Ruml T. Current approaches in SELEX: An update to aptamer selection technology. Biotechnology Advances, 2015; 33, 1141-1161.

[20] Klussmann S, The Aptamer Handbook. Functional Oligonucleotides and Their Applications. WILEY-VCH Verlag GmbH & Co., 2006; KGaA, Wein- heim.

[21] Wang T, Chen C, Larcher LM, Barrero RA, Veedu RN. Three decades of nucleic acid aptamer technologies: Lessons learned, progress and opportunities on aptamer development.

Biotechnol Adv, 2019; 37, 28-50. DOI: 1016/j.biotechadv.2018.11.001.

[22] Kohlberger, M., & Gadermaier, G. SELEX: Critical factors and optimization strategies for successful aptamer selection. Biotechnology and Applied Biochemistry, 2021; 69 (5): 1771-1792. DOI:10.1002/bab.2244.

[23] Gevertz J, Gan HH, Schilick T. In vitro RNA random pools are not structurally diverse: A computational analysis, Rna, 2005;, 853-863. DOI: 10.1261%2Frna.727140511.

[24] Asensio A, Kobko N, Dannenberg JJ. Cooperative hydrogen-bonding in adenine- thymine and guanine-cytosine base pairs. Density functional theory and Moller-Plesset molecular orbital study. J Phys Chem, 2003; A107, 6441-6443. DOI: 10.1021/jp0344646.

[25] Ni S, Yao H, Wang L, Lu J, Jiang F, Lu A, Zhang G. Chemical Modifications of Nucleic Acid Aptamers for Therapeutic Purposes. Int J Mol Sci, 2017; 18. DOI:10.3390/ijms18081683.

[26] Kubik MF, Bell C, Fitzwater T, Watson SR,Tasset DM. Isolation and characterization of 2'-fluoro-, 2'-amino-and 2'-fluoro/amino-modified RNA ligands to human IFN- gamma that inhibit receptor binding. J Immunol, 1997; 159, 259-267.

[27] Rothlisberger P, Hollenstein M. Aptamer chemistry. Adv Drug Deliver Rev, 2018; 134, 3-21. DOI: 10.1016/j.addr.2018.04.007.

[28] Liu JJ, Stormo GD, Combining SELEX with quantitative assays to rapidly obtain accurate models of protein–DNA interactions. Nucleic Acids Res. 2005; 33, e141. DOI: 10.1093%2Fnar%2Fgni139.

[29] Tombelli S, Minunni A, Mascini A, Analytical applications of aptamers. Biosens. Bioelectron, 2005; 20, 2424–2434. DOI: 10.1016/j.bios.2004.11.006.

[30] Stoltenburg R, Reinemann C, Strehlitz B, FluMag-SELEX as an advantageous method for DNA aptamer selection. Anal. Bioanal. Chem. 2005; 383, 83–91. DOI: 10.1007/s00216-005-3388-9.

[31] Murphy MB, Fuller ST, Richardson PM, Doyle, S.A., 2003. An improved method for the in vitro evolution of aptamers and applications in protein detection and purification. Nucleic Acids Res, 2003; 31, e110. DOI: 10.1093/nar/gng110.

[32] Lupold SE, Hicke BJ, Lin Y, Coffey DS. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. Cancer Res. 2002; 62, 4029–4033.

[33] Kikuchi K, Umehara T, Fukuda K, Hwang J, Kuno A, Hasegawa T, Nishikawa S. RNA aptamers targeted to domain II of hepatitis C virus IRES that bind to its apical loop region. J. Biochem, 2003; 133, 263– 270. DOI: 10.1093/jb/mvg036.

[34] Bianchini M, Radrizzani M, Brocardo MG, Reyes GB, Gonzalez SC, Santa-Coloma TA. Specific oligobodies against ERK-2 that recog- nize both the native and the denatured state of

the protein. J. Immunol. Methods, 2001; 252, 191–197. DOI: 10.1016/s0022-1759(01)00350-7.

[35] Mendonsa SD, Bowser MT. In vitro selection of high-affinity DNA ligands for human IgE using capillary electrophoresis. Anal. Chem, 2004; 76, 5387–5392. DOI: 10.1021/ac049857v.

[36] Mosing RK, Mendonsa SD, Bowser MT. Capillary electrophoresis- SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. Anal. Chem, 2005; 77, 6107–6112. DOI: 10.1021/ac050836q.

[37] Misono TS, Kumar PKR. Selection of RNA aptamers against human influenza virus hemagglutinin using surface plasmon resonance. Anal. Biochem.2005; 342, 312–317. DOI: 10.1016/j.ab.2005.04.013.

[38] Tang JJ, Xie JW, Shao NS, Yan Y. The DNA aptamers that specifically recognize ricin toxin are selected by two in vitro selection methods. Electrophoresis,2006; 27, 1303–1311. DOI: 10.1002/elps.200500489.

[39] Tsai RYL, Reed RR. Identification of DNA recognition sequences and protein interaction domains of the multiple-Zn-finger protein Roaz. Mol. Cell. Biol. 1998; 18, 6447–6456.

[40] Rhie A, Kirby L, Sayer N, Wellesley R, Disterer P, Sylvester I, Gill A, Hope J, James W, Tahiri-Alaoui A, Characterization of 2^0-fluoro- RNA aptamers that bind preferentially to disease-associated conformations of prion

protein and inhibit conversion. J. Biol. Chem. 2003; 278, 39697–39705. DOI: 10.1074/jbc.m305297200.

[41] Yang XB, Li X, Prow TW, Reece LM, Bassett SE, Luxon BA, Herzog NK, Aronson J, Shope RE, Leary JF, Gorenstein DG. Immunofluorescence assay and flow-cytometry selection of bead-bound aptamers. Nucleic Acids Res. 2003; 31, e54.

[42] Davis JH, Szostak JW. Isolation of high-affinity GTP aptamers from partially structured RNA libraries. Proc. Natl. Acad. Sci. U. S. A. 2002; 99, 11616–11621. DOI: 10.1073/pnas.182095699.

[43] Komarova N, Kuznetsov A. Inside the Black Box: What Makes SELEX Better? Molecules, 2019; 24. DOI: 10.3390/molecules24193598.

[44] Shao K, Ding W, Wang F, Li H, Ma D, Wang H. Emulsion PCR: a high efficient way of PCR amplification of random DNA libraries in aptamer selection. PLoS One, 2011; 6, e24910. DOI: 10.1371/journal.pone.0024910.

[45] Yang DK, Chou CF, Chen LC. Selection of aptamers for AMACR detection from DNA libraries with different primers. Rsc Adv, 2018; 8, 19067-19074. DOI: 10.1039/C8RA01808A

[46] Tsuji S, Hirabayashi N, Kato S, Akitomi J, Egashira H, Tanaka T, Waga I, Ohtsu T. Effective isolation of RNA aptamer through suppression of PCR bias. Biochemical and Biophysical Research Communications, 2009; 386, 223-226. DOI: 10.1016/j.bbrc.2009.06.013.

[47] Sharma TK, Bruno JG, Dhiman A. ABCs of DNA aptamer and related assay development. Biotechnology Advances, 2017; 35, 275-301. DOI: 10.1016/j.biotechadv.2017.01.003.

[48] Pagratis NC. Rapid preparation of single stranded DNA from PCR products by streptavidin induced electrophoretic mobility shift. Nucleic Acids Research, 1996; 24, 3645-3646. DOI: 10.1093/nar/24.18.3645.

[49] Nehdi A, Samman N, Aguilar-Sanchez V, Farah A, Yurdusev E, Boudjelal M, Perreault J. Novel Strategies to Optimize the Amplification of Single-Stranded DNA. Front Bioeng Biotech, 2020; 8. DOI: 10.3389/fbioe.2020.00401.

[50] Zhang YZ, Xu H, Zhou HY, Wu F, Su YQ, Liang YL, Zhou D. Indirect purification method provides high yield and quality ssDNA sublibrary for potential aptamer selection. Anal Biochem, 2015; 476, 84-90. DOI: 10.1016/j.ab.2015.02.027.

[51] Avci-Adali M, Paul A, Wilhelm N, Ziemer G, Wendel HP. Upgrading SELEX Technology by Using Lambda Exonuclease Digestion for Single-Stranded DNA Generation. Molecules, 2010; 15, 1-11. DOI: 10.3390/molecules15010001.

[52] Ozer A, Pagano JM, Lis JT. New Technologies Provide Quantum Changes in the Scale, Speed, and Success of SELEX Methods and Aptamer Characterization. Molecular Therapy-Nucleic Acids, 2014; 3. DOI: 10.1038%2Fmtna.2014.34.

[53] Schutze T, Wilhelm B, Greiner N, Braun H, Peter F, Morl M, Erdmann VA, Lehrach H, Konthur Z, Menger M, Arndt PF, Glokler J. Probing the SELEX Process with Next- Generation

Sequencing. Plos One, 2011; 6. DOI: 10.1371/journal.pone.0029604.

[54] Alam KK, Chang JL, Burke DH. FASTAptamer: A Bioinformatic Toolkit for High- throughput Sequence Analysis of Combinatorial Selections. Molecular Therapy-Nucleic Acids, 2015; 4, e230. DOI: 10.1038%2Fmtna.2015.4.

[55] Luo ZF, He L, Wang JJ, Fang XN, Zhang LY. Developing a combined strategy for monitoring the progress of aptamer selection. Analyst, 2017; 142, 3136-3139. DOI: 10.1039/c7an01131h.

[56] Deamer D, Akeson M, Branton D. Three decades of nanopore sequencing. Nat Biotechnol, 2016; 34, 518-524. DOI: 10.1038%2Fnbt.3423.

[57] Reynaud L, Bouchet-Spinelli A, Raillon C, Buhot, A. Sensing with Nanopores and Aptamers: A Way Forward. Sensors-Basel, 2020; 20, 4495. DOI: 10.3390/s20164495.

[58] Hermann T, Patel, DJ. Adaptive recognition by nucleic acid aptamers. Science, 2000; 287(5454), 820-825. DOI: 10.1126/science.287.5454.820.

[59] Feng C, Dai S, Wang L. Optical aptasensors for quantitative detection of small biomolecules: A review, Biosensors and Bioelectronics, 2014; 59, 64-74. DOI: 10.1016/j.bios.2014.03.014.

[60] Majdinasab M, Hayat A, Marty JL. Aptamer-based assays and aptasensors for detection of pathogenic bacteria in food

samples. TrAC Trends in Analytical Chemistry, 2018; 107, 60-77. DOI: 10.1016/j.trac.2018.07.016.

[61] Hayat A, Marty JL. Aptamer based electrochemical sensors for emerging environmental pollutants, Frontiers in chemistry, 2014; 2, 41. DOI: 10.3389/fchem.2014.00041.

[62] Majdinasab M, Yaqub M, Rahim A, Catanante G, Hayat A, Marty JL. An Overview on Recent Progress in electrochemical biosensors for antimicrobial drug residues in animal-derived food, Sensors, 2017; 17, 1947. DOI: 10.3390/s17091947.

[63] Radi AE. Electrochemical aptamer-based biosensors: recent advances and perspectives, International Journal of Electrochemistry, 2011; 1-17. DOI: 10.4061/2011/863196.

[64] Stoltenburg R, Nikolaus N, Strehlitz B. Capture-SELEX: selection of DNA aptamers for aminoglycoside antibiotics, J. Anal. Methods Chem, 2012; 415697. DOI: 10.1155/2012/415697

[65] Homann M, Göringer HU. Combinatorial selection of high affinity RNA ligands to live African trypanosomes. Nucl. Acids Res. 1999; 27. DOI: 10.1093%2Fnar%2F27.9.2006.

[66] Singer BS, Shtatland T, Brown D, Gold L. Libraries for genomic SELEX. Nucleic Acids Res. 1997; 25 (4), 781–786. DOI: 10.1093%2Fnar%2F25.4.781.

[67] Mayer,G, Ahmed MS, Dolf A, Endl E, Knolle PA, Famulok M. Fluorescence-activated cell sorting for aptamer SELEX with cell mixtures. Nat. Protoc. 2010; 5, 1993–2004. DOI: 10.1038/nprot.2010.163.

[68] Jenison RD, Gill SC, Pardi A, Polisky B. High-resolution molecular discrimina- tion by RNA. Science, 1994; 263 (5152), 1425–1429. DOI: 10.1126/science.7510417.

[69] Coulter LR, Landree MA, Cooper TA. Identification of a new class of exonic splic- ing enhancers by in vivo selection. Mol. Cell. Biol, 1997; 17 (4), 2143–2150. DOI: 10.1128%2Fmcb.17.4.2143.

[70] Kawakami J, Imanaka H, Yokota Y, Sugimoto N. In vitro selection of aptamers that act with Zn2+. J. Inorg. Biochem. 2000; 82 (1–4), 197–206. DOI: 10.1016/s0162-0134(00)00158-6.

[71] Cheng C, Chen YH, Lennox KA, Behlke MA, Davidson BL. In vivo SELEX for Identification of Brain-penetrating Aptamers. Mol. Ther. Nucleic Acids,2013; 2, e67. DOI: 1038/mtna.2012.59.

[72] Mendonsa SD, Bowser MT. In vitro selection of aptamers with affinity for neuropeptide Y using capillary electrophoresis. J. Am. Chem. Soc. 2005; 127, 9382–9383. DOI: 10.1021/ja052406n.

[73] Ospina-Villa JD, López-Camarillo C, Castañón-Sánchez CA, Soto-Sánchez J, Ramírez-Moreno E, Marchat LA. Advances on Aptamers against Protozoan Parasites. Genes 2018, 9, 584. DOI: 10.3390/genes9120584.

[74] Bruno JG, Kiel JL. In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. Biosens. Bioelectron. 1999, 14, 457–464. DOI: 10.1016/s0956-5663(99)00028-7.

[75] Wan Y, Kim YT, Li N, Cho SK, Bachoo R, Ellington AD, Iqbal SM. Surface-immobilized aptamers for cancer cell isolation and microscopic cytology. Cancer Res. 2010; 70, 9371–9380. DOI: 10.1158/0008-5472.can-10-0568.

[76] Komarova N, Panova O, Titov A, Kuznetsov A. Aptamers Targeting Cardiac Biomarkers as an Analytical Tool for the Diagnostics of Cardiovascular Diseases: A Review. Biomedicines, 2022; 10(5), 1085. DOI: 10.3390/biomedicines10051085.

[77] Drolet DW, Green LS, Gold L, Janjic N. Fit for the eye: aptamers in ocular disorders. nucleic acid therapeutics, 2026; 26(3), 127-146. DOI: 10.1089/nat.2015.0573.

[78] Labib M, Zamay AS, Muharemagic D, Chechik AV, Bell JC, Berezovski MV. Aptamer-based viability impedimetric sensor for viruses. Anal. Chem. 2012; 84, 1813–1816. DOI: 10.1021/ac203412m.

[79] Gopinath SC, Hayashi K, Kumar PK. Aptamer that binds to the gD protein of herpes simplex virus-1 and
efficiently inhibits viral entry. J. Virol. 2012; 86, 6732–6744. DOI: 10.1128%2FJVI.00377-12.

[80] Fukuda K, Vishinuvardhan D, Sekiya S, Kakiuchi N, Shimotohno K, Kumar PK, Nishikawa S. Specific RNA aptamers to NS3 protease domain of hepatitis C virus. Nucleic Acids Symp. Ser. 1997; 37, 237–238.

[81] Boiziau C, Dausse E, Yurchenko L, Toulmé JJ. DNA aptamers selected against the HIV-1 trans-activation-responsive

RNA element form RNA-DNA kissing complexes. J. Biol. Chem. 1999; 274, 12730–12737 DOI: 10.1074/jbc.274.18.12730.

[82] Gopinath SC, Kawasaki K, Kumar PK. Selection of RNA-aptamer against human influenza B virus. Nucleic Acids Symp. Ser. 2005; 49, 85–86. DOI: 10.1093/nass/49.1.85.

[83] Jang KJ, Lee NR, Yeo WS, Jeong YJ, Kim DE. Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. Biochem. Biophys. Res. Commun. 2008; 366, 738–744. DOI: 10.1016/j.bbrc.2007.12.020.

[84] Hamula CL, Zhang H, Guan LL, Li XF, Le XC. Selection of aptamers against live bacterial cells. Anal. Chem. 2008; 80, 7812–7819. DOI: 10.1021/ac801272s.

[85] Cao X, Li S, Chen L, Ding H, Xu H, Huang Y, Li J, Liu N, Cao W, Zhu Y, et al. Combining use of a panel of ssDNA aptamers in the detection of Staphylococcus aureus. Nucleic Acids Res. 2009; 37, 4621–4628. DOI: 10.1093/nar/gkp489

[86] Chen F, Zhou J, Luo F, Mohammed AB, Zhang XL. Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent Mycobacterium tuberculosis. Biochem. Biophys. Res. Commun. 2007; 357, 743–748. DOI: 10.1016/j.bbrc.2007.04.007

[87] McConnell EM, Nguyen J, Li Y. Aptamer-based biosensors for environmental monitoring. Frontiers in chemistry, 2020; 8, 434.

[88] Zhang Z, Liu D, Bai Y, Cui Y, Wang D, and Shi X. Identification and characterization of two high affinity aptamers specific for Salmonella Enteritidis. Food Control; 2019; 106:106719. Doi:10.1016/j.foodcont.2019.106719.

[89] Díaz-Amaya S, Lin LK, Deering AJ, and Stanciu LA. Aptamer-based SERS biosensor for whole cell analytical detection of E. coli O157:H7. Anal. Chim. Acta, 2019; 1081, 146–156. doi: 10.1016/j.aca.2019.07.028.

[90] Chang YC, Yang CY, Sun RL, Cheng YF, Kao WC, and Yang PC. Rapid single cell detection of Staphylococcus aureus by aptamer- conjugated gold nanoparticles. Sci. Rep.2013; 3:1863. doi: 10.1038/srep01863.

[91] Shrivastava S, Lee W. II, and Lee NE. Culture-free, highly sensitive, quantitative detection of bacteria from minimally processed samples using fluorescence imaging by smartphone. Biosens. Bioelectron. 2019; 109, 90–97. doi: 10.1016/j.bios.2018.03.006.

[92] Suh SH, Choi SJ, Dwivedi HP, Moore MD, Escudero-Abarca BI, and Jaykus LA. Use of DNA aptamer for sandwich type detection of Listeria monocytogenes. Anal. Biochem. 2018; 557, 27–33. doi: 10.1016/j.ab.2018.04.009.

[93] Suh SH, Dwivedi HP, Choi SJ, and Jaykus LA. Selection and characterization of DNA aptamers specific for Listeria species. Anal. Biochem. 2014; 459, 39–45. doi: 10.1016/j.ab.2014.05.006.

[94] Shi X, Zhang J, and He F. A new aptamer/polyadenylated DNA interdigitated gold electrode piezoelectric sensor for rapid detection of Pseudomonas aeruginosa. Biosens. Bioelectron. 2019; 132, 224–229. doi: 10.1016/j.bios.2019.02.053.

[95] Osunla C, and Okoh A. Vibrio pathogens: a public health concern in rural water resources in sub-saharan Africa. Int. J. Environ. Res. Public Health, 2017; 14:1188. doi: 10.3390/ijerph14101188.

[96] Wei X, Zhou W, Sanjay ST, Zhang J, Jin Q, Xu F, et al. Multiplexed instrument-free bar-chart spinchip integrated with nanoparticle- mediated magnetic aptasensors for visual quantitative detection of multiple pathogens. Anal. Chem. 2018; 90, 9888–9896. doi: 10.1021/acs.analchem.8b02055.

[97] Somvanshi SB, Ulloa AM, Zhao M, Liang Q, Barui AK, Lucas A, ... & Stanciu LA. Microfluidic paper-based aptasensor devices for multiplexed detection of pathogenic bacteria. Biosensors and Bioelectronics, 2022; 207, 114214. DOI:10.1016/j.bios.2020.112649.

[98] Zhang K, Li H, Wang W, Cao J, Gan N, Han H. Application of multiplexed aptasensors in food contaminants detection. ACS sensors, 2020; 5(12), 3721-3738. DOI: 10.1021/acssensors.0c01740.

[99] Yu J, Wu H, He L, Tan L, Jia Z, Gan N. The universal dual-mode aptasensor for simultaneous determination of different bacteria based on naked eyes and microfluidic-chip together with

magnetic DNA encoded probes. Talanta, 2020; 225, 122062. DOI: 10.1016/j.talanta.2020.122062.

[100] Yan M, Li H, Li M, Cao X, She Y, Chen Z. Advances in Surface-Enhanced Raman Scattering-Based Aptasensors for Food Safety Detection. Journal of Agricultural and Food Chemistry, 2021; 69(47), 14049-14064.

[101] Cruz-Aguado JA, Penner G.Determination of ochratoxin a with a DNA aptamer. J.Agric.Food Chem, 2008; 56, 10456–10461. DOI: 10.1021/acs.jafc.1c05274.

[102] Kim SE, Su W, Cho M, Lee Y, Choe WS. Harnessing aptamers for electrochemical detection of endotoxin. Anal. Biochem. 2012; 424, 12–20. DOI: 10.1016/j.ab.2012.02.016.

[103] Zeng G, Zhang C, Huang D, Lai C, Tang L, Zhou Y, Xu P, Wang H Qin L. Cheng,M.Practical and regenerable electrochemical aptasensor based on nanoporous gold and thymine-Hg. Biosens. Bioelectron, 2017; 90, 542–548. DOI: 10.1016/j.bios.2016.10.018.

[104] Cui L, Wu J, Ju H. Label-free signal-on aptasensor for sensitive electrochemical detection of arsenite. Biosens. Bioelectron, 2016; 79, 861–865. DOI: 10.1016/j.bios.2016.01.010.

[105] Chen Z, Li L, Mu X, Zhao H, Guo L. Electrochemical aptasensor for detection of copper based on a reagentless signal-on architecture and amplification by gold nanoparticles. Talanta, 2011, 85, 730–735. DOI: 10.1016/j.talanta.2011.04.056.

[106] Abu-Ali H, Nabok A, Smith TJ. Development of novel and highly specific ssDNA-aptamer-based electrochemical biosensor for rapid detection of mercury (II) and lead (II) ions in water. Chemosensors, 2019; 7:27. DOI: 10.3390/chemosensors7020027.

[107] Berlina AN, Zherdev AV, Pridvorova SM, Gaur MS, Dzantiev BB. Rapid visual detection of lead and mercury via enhanced crosslinking aggregation of aptamer-labeled gold nanoparticles. J. Nanosci. Nanotechnol. 2019; 19, 5489–5495. DOI: 10.1166/jnn.2019.16575.

[108] Zhang J, Huang Y, Yan J, Zhu C, Zhang C, Chen A. Dulplex analysis of mercury and silver ions using a label-free fluorescent aptasensor. Int. J. Environ. Anal. Chem. 2018; 98, 349–359. DOI: 10.1080/03067319.2018.1466879.

[109] Kim YJ, Kim YS, Niazi JH, Gu MB. Electrochemicalaptasensorfortetracyclinedetection.Bioprocess Biosyst. Eng. 2010; 33, 31–37. DOI: 10.3390%2Fmd8041323.

[110] Hu W, Chen Q, Li H, Ouyang Q, Zhao J. Fabricating a novel label-free aptasensor for acetamiprid by fluorescence resonance energy transfer between NH2-NaYF4: Yb, Ho@SiO2 and Au nanoparticles. Biosens. Bioelectron. 2016; 80, 398–404. DOI: 10.1016/j.bios.2016.02.001.

[111] Madianos L, Tsekenis G, Skotadis E, Patsiouras L, Tsoukalas D. A highly sensitive impedimetric aptasensor for the selective detection of acetamiprid and atrazine based on microwires formed by platinum nanoparticles. Biosens.

Bioelectron. 2018; 101, 268–274. DOI: 10.1016/j.bios.2017.10.034.

[112] McNamara JO, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, Sullenger BA, Giangrande PH. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat. Biotechnol. 2006; 24, 1005–1015. DOI: 10.1038/nbt1223.

[113] Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, Meyerholz DK, McCaffrey AP, McNamara JO, Giangrande PH. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of psma-expressing tumors. Nat. Biotechnol. 2009; 27, 839–849. DOI: 10.1038/nbt.1560.

[114] Liang C, Guo B, Wu H, Shao N, Li D, Liu J, Dang L, Wang C, Li H, Li S, et al. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel rna interference-based bone anabolic strategy. Nat. Med. 2015; 21, 288–294. DOI: 10.1038/nm.3791.

[115] Jeong H, Lee SH, Hwang Y, Yoo H, Jung H, Kim SH, Mok H. Multivalent aptamer-rna conjugates for simple and efficient delivery of doxorubicin/siRNA into multidrug-resistant cells. Macromol. Biosci. 2016. DOI: 10.1002/mabi.201600343.

[116] Subramanian N, Kanwar JR, Kanwar RK, Krishnakumar S. Targeting cancer cells using lna-modified aptamer-siRNA chimeras. Nucleic Acid Ther. 2015; 25, 317–322. DOI: 10.1089/nat.2015.0550.

[117] Herrmann A, Priceman SJ, Swiderski P, Kujawski M, Xin H, Cherryholmes GA, Zhang W, Zhang C, Lahtz C, Kowolik C,

et al. CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. J. Clin. Investig. 2014; 124, 2977–2987. DOI: 10.1172/jci73174.

[118] Esposito CL, Cerchia L, Catuogno S, De Vita G, Dassie JP, Santamaria G, Swiderski P, Condorelli G, Giangrande PH, de Franciscis V. Multifunctional aptamer-miRNA conjugates for targeted cancer therapy. Mol. Ther. 2014; 22, 1151–1163. DOI: 10.1038/mt.2014.5.

[119] Esposito CL, Nuzzo S, Kumar SA, Rienzo A, Lawrence CL, Pallini R, Shaw L, Alder JE, Ricci-Vitiani L, Catuogno S, et al. A combined microRNA-based targeted therapeutic approach to eradicate glioblastoma stem-like cells. J. Control Release 2016; 238, 43–57. DOI: 10.1016/j.jconrel.2016.07.032.

[120] Bagalkot V, Farokhzad OC, Langer R, Jon S. An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. Angew. Chem. Int. Ed. Engl. 2006; 45, 8149–8152. DOI: 10.1002/anie.200602251.

[121] Huang YF, Shangguan D, Liu H, Phillips JA, Zhang X, Chen Y, Tan W. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. ChemBioChem 2009; 10, 862–868. DOI: 10.1002/cbic.200800805.

[122] Luo YL, Shiao YS, Huang YF. Release of photoactivatable drugs from plasmonic nanoparticles for targeted cancer therapy. ACS Nano 2011; 5, 7796–7804. DOI: 10.1021/nn201592s.

[123] Dam DH, Culver KS, Kandela I, Lee RC, Chandra K, Lee H, Mantis C, Ugolkov A, Mazar AP, Odom TW. Biodistribution and in vivo toxicity of aptamer-loaded gold nanostars. Nanomedicine 2015; 11, 671–679. DOI: 10.1016%2Fj.nano.2014.10.005.

[124] Chang HI, Yeh MK. Clinical development of liposome-based drugs: Formulation, characterization, and therapeutic efficacy. Int. J. Nanomed. 2012; 7, 49–60. DOI: 10.2147/ijn.s26766.

[125] Zununi Vahed S, Salehi R, Davaran S, Sharifi S. Liposome-based drug co-delivery systems in cancer cells. Mater. Sci. Eng. C Mater. Biol. Appl. 2017; 71, 1327–1341. DOI: 10.1016/j.msec.2016.11.073.

[126] Gragoudas ES, Adamis AP, Cunningham ET, Feinsod M, Guyer DR. VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N. Engl. J. Med. 2004; 351, 2805–2816. DOI: 10.1056/nejmoa042760.

[127] Rusconi CP, Scardino E Layzer J, Pitoc GA, Ortel TL, Monroe D, Sullenger BA. RNAaptamersas reversible antagonists of coagulation factor IXa. Nature 2002; 419, 90–94. DOI: 10.1038/nature00963.

[128] Bates PJ, Laber DA, Miller DM, Thomas SD, Trent JO. Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. Exp. Mol. Pathol. 2009; 86, 151–164. DOI: 10.1016/j.yexmp.2009.01.004.