FAuNPs on Bacterial Colonization

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Contributor: Farid Menaa

This work reports the design and development of a green synthesis route for flavonoid-AuNPs (FAuNPs) which were successfully tested in vitro and in vivo against bacterial colonization.

Keywords: FAuNPs ; Bacterial colonization ; Liver ; Kidneys ; E. faecalis ; Nanomedicine

1. Introduction

Functional nanoparticles (NPs) have a wide range of medical applications that encompasses a broad spectrum of fields, including imaging, molecular diagnosis, and targeted drug delivery [1][2]. As drug nanocarriers, they move inside the body to repair damaged tissues, cross the cell barriers, and access those cells and tissues where other drugs/antibodies cannot reach in appropriate concentrations [2]. Their superior features are due to high surface area-to-volume ratio, small size, stability, and biocompatibility [2][3].

The history of the gold nanoparticles (AuNPs) for the delivery of antibacterial agents dates to more than a decade. Various stable complexes of antibiotics with colloidal gold have been developed. These include gold complexes with vancomycin, ciprofloxacin, and fluorouracil [4][5][6]. However, ampicillin, kanamycin, streptomycin, and many other antibiotics form unstable complexes with gold [7][8][9]. Later, gold nanoparticles functionalized with ampicillin [10][11], vancomycin [12], and lysozyme [13] have been reported against various strains, such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes,* and multidrug resistance (MDR) *Staphylococcus aureus*.

The hard bacterium, *Enterococcus faecalis,* is an opportunistic pathogen that can withstand high environmental stresses [14]. Despite being mammalian gut residents, it also causes nosocomial infections such as urinary tract infection (UTI), bacteremia, surgical wound infection, and endocarditis [15]. Its characteristic feature resides in its acquired and intrinsic resistance against main groups of antibiotics such as aminoglycosides, β-lactams, cephalosporins, glycopeptides, tetracyclines, and trimethoprim-sulfamethoxazole [16][17]. This MDR is manifested by mutations or by the horizontal exchange of foreign genetic material through the transfer of plasmids and transposons [18]. Antibiotic development against Enterococci is, therefore, an active and challenging research area.

Various nanocarrier systems were documented to be effective against *E. Faecalis*.These include gold nanorods for phototherapy [1], vancomycin bound biogenic AuNPs [19], calcium hydroxide NPs [20], chitosan NPs [21], Ag–Ca–Si mesoporous NPs [22], silver NPs [23], and polysaccharide-maghemite composite NPs [24]. However, these studies are focused on in-vitro antibacterial activity. NPs stability and activity under physiological conditions are also a matter of interest as many factors can alter the inherent antibacterial activity of NPs, such as pH and temperature [25]. Besides, plasma proteins and plasma components can also interact with the NPs surface, and this can affect their biodistribution [26][27][28], cellular uptake, and bioactivity [29][30][31]. Recently, a few in vivo studies have been carried out that include combined therapy of silver NPs, and visible blue light against *Pseudomonas* [32], quercetin loaded PLGA for *Escherichia coli* [33], and tridecaptin-antibiotic conjugates against *Klebsiella pneumoniae* [34].

AuNPs are not considered as antibacterial agents individually; however, when conjugated with small active substances (e.g., antibiotics and antibodies), they usually exhibit more potent antimicrobial activity [4][5][6]. Plant, bacterial, fungal, yeast and algal extracts were frequently used for the preparation of bioactive AuNPs according to previous studies. [35][36][37]. The bioactive compounds in these extracts not only reduce gold (Au^{+3}) but also impart characteristic bioactive properties to these NPs [38]. Flavonoids are one of the extensively studied bioactive secondary metabolites from plants. The antibacterial activity of Flavonoids is ascribed to intracellular targeting such as inhibition of bacterial enzymes, damage to cytoplasmic membranes and cell wall components, inhibition of the bacterial efflux pump, and disruption of energy metabolism pathways [39]. Flavonoids can be extracted from *Berberis lycium* Royle, a spiny shrub prevalent in

milder climates and subalpine regions.They are commonly used to treat a wide variety of human pathologies in the Pakistan, India, and Bangladesh Indian Himalayan Region. The phytochemical analysis of *B. Lycium* plant parts indicated the presence of important contents, including flavonoids, phenols, alkaloids, terpenoids, tannin, fat, and resin [40].

Moreover, its leaves and fruits contain a high amount of different nutritive components such as vitamin C, calcium, sulfur, protein, fiber, fat, palmitine, and berberine [40]. Importantly, *B. lycium* roots contain berberine, a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids, which is reported for wide medicinal applications [40]. However, the bioactivities of leaves of this plant have not yet been investigated. Therefore, the present research focuses on the green synthesis of flavonoid-coated gold nanoparticles (FAuNPs) from methanol extracts of *B. lyceum,* its physical characterization, and the determination of its potential effect on colonization of *E. faecalis* both in vitro and in vivo.

2. Discussion

It is well assumed that NPs carrying antibiotics are found to be quite effective against resistant bacteria [41]. This is particularly due to the striking features of NPs attributed to their size and unique physiochemical properties at the nanoscale that help them to evade drug efflux pumps. Flavonoids, albeit depending upon their type and structure, often display poor absorption and bioavailability [42]. One way to combat the above constraints is to use drug nanocarriers [2][43]. For their development, flavonoids can act as reducing as well as a capping agent for the synthesis of metallic NPs. Green synthesis is, therefore, a better option than using toxic reducing agents, especially for biomedical applications.

Flavonoids were used for a range of medicinal applications, such as antibacterial, antiviral, antioxidative, anti-inflammatory, anticancer, cardio-protective, skin-protective, and antidiabetic activities [44]. Flavonoids can be obtained from *B. lycium*, which is a known medicinal plant containing a variety of bioactive constituents [40]. Medicinal plants are a good source of potent and safe natural extracts that could act as adjuvants or even be an alternative to costly antibiotics against which microbes are becoming resistant day by day. Moreover, these extracts are cost-effective and work effectively against a variety of microbes, including bacteria, fungus, and viruses [45][46][47].

3. Conclusions and Perspectives

The enhanced antibacterial activity of bioactive compounds in nanoformulations has been extensively studied. However, most of such studies are focused on in-vitro aspects. The present study was focused on a green synthesis and characterizations of FAuNPs, which were further assessed for their in-vitro and in-vivo antibacterial potential against Gram-positive bacterium *E. faecalis*. The FAuNPs were successfully synthesized, and different physical characterizations confirmed their formation. The synthesized FAuNPs were sphere-shaped shaped with a 23 nm diameter average size. The results for optimizations revealed that a 1:3 ratio of Au and flavonoid, a pH of 4, and a temperature of 70 °C are effective conditions for FAuNPs formation. Stability results showed that temperature had negligible effect on stability of optimized FAuNPs. However, FAuNPs cannot remain stable in salt solution. These FAuNPs when tested for antibacterial activity showed enhanced activity against *E. faecalis* with MIC of 25 µg/mL. Similarly, they also showed reduced bacterial colonizing activity of *E. faecalis* in liver and kidneys of the mice. FAuNPs were more biocompatible owing to its reduced hemolytic behavior with varying concentrations. The results of this study conclude that FAuNPs can be very effective antibacterial agents against *E. faecalis* infections. The present study was based on a non-biofilm forming *E. faecalis* strain. In the future, further studies are required for biofilm-forming and vancomycin-resistant *E. faecalis* strains.

References

1. Dykman, L.; Khlebtsov, N. Gold nanoparticles in biomedical applications: Recent advances and perspectives. Chem. Soc. Rev. 2012, 41, 2256–2282.
2. Menaa, F. When Pharma Meets Nano or The Emerging Era of Nano-Pharmaceuticals. Pharm. Anal. Acta 2013, 4, 223.
3. Loomba, L.; Scarabelli, T. Metallic nanoparticles, and their medicinal potential. Part I: Gold and silver colloids. Ther. Deliv. 2013, 4, 859–873.
4. Gu, H.; Ho, P.; Tong, E.; Wang, L.; Xu, B. Presenting Vancomycin on Nanoparticles to Enhance Antimicrobial Activities. Nano Lett. 2003, 3, 1261–1263.
5. Rosemary, M.; MacLaren, I.; Pradeep, T. Investigations of the Antibacterial Properties of Ciprofloxacin@SiO2. Langmuir 2006, 22, 10125–10129.
6. Selvaraj, V.; Alagar, M. Analytical detection and biological assay of antileukemic drug 5-fluorouracil using gold nanoparticles as probe. Int. J. Pharm. 2007, 337, 275–281.

7. Chakraborty, S.; Sahu, S.; Pramanik, P.; Roy, S. In vitro antimicrobial activity of nanoconjugated vancomycin against dr ug resistant Staphylococcus aureus. Int. J. Pharm. 2012, 436, 659–676.
8. Grace, N.; Pandian, K. Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles a brief study. C olloids and Surfaces A: Physicochem. Eng. Asp. 2007, 297, 63–70.
9. Grace, N.; Pandian, K. Quinolone antibiotic-capped gold nanoparticles and their antibacterial efficacy against gram posi tive and gram-negative organisms. J. Bionanosci. 2007, 1, 96–105.
10. Brown, A.; Smith, K.; Samuels, T.; Lu, J.; Obare, S.; Scott, M. Nanoparticles Functionalized with Ampicillin Destroy Multi ple-Antibiotic-Resistant Isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and Methicillin-Resistant St aphylococcus aureus. Appl. Environ. Microbiol. 2012, 78, 2768–2774.
11. Chamundeeswari, M.; Sobhana, S.; Jacob, J.; Kumar, M.; Devi, M.; Sastry, T.; Mandal, A. Preparation, characterization and evaluation of a biopolymeric gold nanocomposite with antimicrobial activity. Biotechnol. Appl. Biochem. 2010, 55, 2 9–35.
12. Varisco, M.; Khanna, N.; Brunetto, P.; Fromm, K. New Antimicrobial and Biocompatible Implant Coating with Synergic S ilver-Vancomycin Conjugate Action. ChemMedChem 2014, 9, 1221–1230.
13. Chen, W.; Lin, J.; Chen, W.; Luo, L.; Wei-Guang Diau, E.; Chen, Y. Functional gold nanoclusters as antimicrobial agent s for antibiotic-resistant bacteria. Nanomedicine 2010, 5, 755–764.
14. Rana, N.; Sauvageot, N.; Laplace, J.; Bao, Y.; Nes, I.; Rincé, A.; Posteraro, B.; Sanguinetti, M.; Hartke, A. Redox Balan ce via Lactate Dehydrogenase Is Important for Multiple Stress Resistance and Virulence in Enterococcus faecalis. Infec t. Immun. 2013, 81, 2662–2668.
15. Arias, C.; Contreras, G.; Murray, B. Management of multidrug-resistant enterococcal infections. Clin. Microbiol. Infect. 2 010, 16, 555–562.
16. Michaux, C.; Sanguinetti, M.; Reffuveille, F.; Auffray, Y.; Posteraro, B.; Gilmore, M.; Hartke, A.; Giard, J. SlyA Is a Trans criptional Regulator Involved in the Virulence of Enterococcus faecalis. Infect. Immun. 2011, 79, 2638–2645.
17. Gentry-Weeks, C.; Estay, M.; Loui, C.; Baker, D. Intravenous Mouse Infection Model for Studying the Pathology of Ente rococcus faecalis Infections. Infect. Immun. 2003, 71, 1434–1441.
18. Blair, M.; Webber, A.; Baylay, J.; Ogbolu, O.; Piddock, J. Molecular mechanisms of antibiotic resistance. Nat. Rev. Micro biol. 2015, 13, 42–51.
19. Mohammed Fayaz, A.; Girilal, M.; Mahdy, S.; Somsundar, S.; Venkatesan, R.; Kalaichelvan, P. Vancomycin bound biog enic gold nanoparticles: A different perspective for development of anti VRSA agents. Process Biochem. 2011, 46, 636 –641.
20. Dianat, O.; Saedi, S.; Kazem. M.; Alam. M. Antimicrobial Activity of Nanoparticle Calcium Hydroxide against Enterococc us Faecalis: An In vitro study. Iran Endod. J. 2014, 10, 39–43.
21. Barreras, U.; Méndez, F.; Martínez, R.; Valencia, C.; Rodríguez, P.; Rodríguez, J. Chitosan nanoparticles enhance the antibacterial activity of chlorhexidine in collagen membranes used for periapical guided tissue regeneration. Mater. Sci. Eng. C 2016, 58, 1182–1187.
22. Fan, B. Substantivity of Ag–Ca–Si mesoporous nanoparticles on dentin and its ability to inhibit Enterococcus faecalis. J. Mater. Sci. Mater. Med. 2015, 27.
23. Wu, D.; Fan, W.; Kishen, A.; Gutmann, J.L.; Fan, B. Evaluation of the Antibacterial Efficacy of Silver Nanoparticles agai nst Enterococcus faecalis Biofilm. J. Endod. 2014, 40, 285–290.
24. Iconaru, S.L.; Prodan, A.M.; Motelica-Heino, M.; Sizaret, S.; Predoi. D. Synthesis and characterization of polysaccharid e-maghemite composite nanoparticles and their antibacterial properties. Nanoscale Res. Lett. 2012, 7, 576.
25. Abdel-Raouf, N.; Al-Enazi, N.M.; Ibraheem, I.B. Green biosynthesis of gold nanoparticles using Galaxaura elongata an d characterization of their antibacterial activity. Arab. J. Chem. 2017, 10, S3029–S3039.
26. Dwivedi, P.; Nayak, V.; Kowshik, M. Role of gold nanoparticles as drug delivery vehicles for chondroitin sulfate in the tre atment of osteoarthritis. Biotechnol. Prog. 2015, 31, 1416–1422.
27. Sahayaraj, K.; Rajesh, S. Bionanoparticles: Synthesis and antimicrobial applications. In Science against microbial path ogens: Communicating current research and technological advances. Badajoz Spain 2011, 23, 228–244.
28. Ehrenberg, M.S.; Friedman, A.E.; Finkelstein, J.N.; Oberdörster, G.; Mcgrath, J.L. The influence of protein adsorption o n nanoparticle association with cultured endothelial cells. Biomaterials 2009, 30, 603–610.
29. Dobrovolskaia, M.A.; Aggarwal, P.; Hall, J.B.; Mcneil, S.E. Preclinical Studies To Understand Nanoparticle Interaction wi th the Immune System and Its Potential Effects on Nanoparticle Biodistribution. Mol. Pharm. 2008, 5, 487–495.

30. Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Rémésy, C. Bioavailability and bioefficacy of polyphenols in hum ans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 2005, 81, 230S–242S.
31. Moghimi, S.M.; Hunter, A.C.; Murray, J.C. Long-circulating and target-specific nanoparticles: Theory to practice. Pharm acol. Rev. 2001, 53, 283–318.
32. . El-Azizi, M.M.; Din, S.N.E.; El-Tayeb, T.; Aisha, K.A. In vitro and in vivo antimicrobial activity of combined therapy of sil ver nanoparticles and visible blue light against Pseudomonas aeruginosa. Int. J. Nanomed. 2016, 11, 1749.
33. Sun, D.; Li, N.; Zhang, W.; Yang, E.; Mou, Z.; Zhao, Z.; Liu, H.; Wang, W. Quercetin-loaded PLGA nanoparticles: A highl y effective antibacterial agent in vitro and anti-infection application in vivo. J. Nanoparticle Res. 2015, 18, 3.
34. Cochrane, S.A.; Li, X.; He, S.; Yu, M.; Wu, M.; Vederas, J.C. Synthesis of Tridecaptin–Antibiotic Conjugates with in Vivo Activity against Gram-Negative Bacteria. J. Med. Chem. 2015, 58, 9779–9785.
35. Kuppusamy, P.; Yusoff, M.M.; Maniam, G.P.; Govindan, N. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications—An updated report. Saudi Pharm. J. 2016, 24, 473–484.
36. Oberdörster, G. Safety assessment for nanotechnology and nanomedicine: Concepts of nanotoxicology. J. Intern. Med. 2010, 267, 89–105.
37. Cushnie, T.T.; Lamb, A.J. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicro b. Agents 2011, 38, 99–107.
38. Ateeq, M.; Shah, M.R.; Ain, N.U.; Bano, S.; Anis, I.; Lubna; Faizi, S.; Bertino, M.F.; Naz, S.S. Green synthesis and mole cular recognition ability of patuletin coated gold nanoparticles. Biosens. Bioelectron. 2015, 63, 499–505.
39. Farhadi, F.; Khameneh, B.; Iranshahi, M.; Iranshahy, M. Antibacterial activity of flavonoids and their structure-activity rel ationship: An update review. Phytother. Res. 2018, 33, 13–40.
40. Ali, H.; Uddin, S.; Jalal, S. Chemistry and Biological Activities of Berberis lyceum Royle. J. Biol. Act. Prod. Nat. 2015, 5, 295–312.
41. Pawlak, A.; Mucha, M. Thermogravimetric and FTIR studies of chitosan blends. Thermochim. Acta 2003, 396, 153–16 6.
42. Cushnie, T.T.; Lamb, A.J. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 2005, 26, 343–356.
43. Jacob, A.E.; Hobbs, S.J. Conjugal Transfer of Plasmid-Borne Multiple Antibiotic Resistance in Streptococcus faecalis v ar. zymogenes. J. Bacteriol. 1974, 117, 360–372.
44. Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines 2018, 5, 93.
45. Gonelimali, F.D.; Lin, J.; Miao, W.; Xuan, J.; Charles, F.; Chen, M.; Hatab, S.R. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Front. Microbiol. 2018, 9, 163 9.
46. Ogbole, O.O.; Akinleye, T.E.; Segun, P.A.; Faleye, T.C.; Adeniji, A.J. In vitro antiviral activity of twenty-seven medicinal p lant extracts from Southwest Nigeria against three serotypes of echoviruses. Virol. J. 2018, 15, 110.
47. Jaime, M.F.V.; Redko, F.; Muschietti, L.V.; Campos, R.H.; Martino, V.S.; Cavallaro, L.V. In vitro antiviral activity of plant extracts from Asteraceae medicinal plants. Virol. J. 2013, 10, 245.