Archives of Orofacial Sciences
www.dental.usm.my/aos/ © Penerbit Universiti Sains Malaysia. 2018
print ISSN: 1823-8602
e-ISSN: 2231-7163

Arch Orofac Sci (2018), **13**(1): 1-5.

Original Article

Effects of modified hydrothermal nanotitania on the viability of *Staphylococcus aureus*

Nor Farid Mohd Noor[a]*, Ahmad Mukifza Harun[b], Shahril Yusof[b], Mohamad Ezany Yusoff[a], Suharni Mohamad[a]

[a] School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[b] Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

* Corresponding author: drfarid@usm.my

Submitted: 03/12/2017. Accepted: 04/06/2018. Published online: 04/06/2018.

Abstract *Staphylococcus aureus* (*S. aureus*) is an important bacterium with significant pathological implications in the field of medicine. Attempting to cure bacterial infections at an advanced stage results in considerable waste of time, effort and expenditure. Thus, the prevention of such illnesses is paramount. Besides using chemical drugs to treat infections, several non-organic extracts have been tested in trials and been shown to impede the bacteria's growth. This paper proposes that the modified hydrothermal nanotitania extract has great potential to combat this lethal organism. The viability of *S. aureus* was shown to be markedly reduced following the addition of nanotitania extract with 0.01%, 0.03% and 0.05% silver after 24, 48, and 72 hours. The ability of the nanotitania extract to inhibit the growth of *S. aureus* indicates its antimicrobial characteristics.

Keywords: Bacterial eradication; nanotitania extract; respiratory tract infection; *Staphylococcus aureus*; TiO_2.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most notorious bacteria in the medical field, being commonly associated with a host of skin and soft tissue infections (Nathwani *et al.,* 2016). In addition, it is one of the main organisms responsible for catheter-related infections leading to serious heart infections, such as infective endocarditis as a complication (Lata *et al.,* 2016). In the surgical operating theatre, *S. aureus* is the main cause of surgical site infections and deadly post-operative complications (Inui and Bandyk, 2015).

Septicemia is a potentially fatal consequence of any infection, and it is frequently caused by bacterial infection. The most prevalent factor for hospital-acquired bacteremia is *S. aureus* infection (Morris and Russell, 2016). Other illnesses associated with the *S. aureus* are pneumonia, skin, bone and joint infections. The most serious *S. aureus* related infection is methicillin-resistant staphylococcus aureus (MRSA) which requires costly treatment (VanEperen and Segreti, 2016).

One of the main sources of *S. aureus* infection is from colonization in the nasal cavity and throat (Marzec and Bessesen, 2016; Chipolombwe *et al.,* 2016). In addition to the problem of treating the infection; *S. aureus* results in frequent and subsequently costly admissions to the ward, especially in the case of children with various respiratory problems associated with cystic fibrosis (Mukifza *et al.,* 2016; Harik *et al.,* 2016).

Titanium dioxide (TiO_2) has been extensively studied for its role as an antibacterial agent. For example, increasing the concentration of TiO_2 nanoparticles has been shown to reduce the growth rate of *Escherichia coli* (Ahmad, and Sardar, 2013). In addition, Martinez-Gutierrez *et al.* (2010) have shown that the combination of TiO_2 with other substances such as silver is able to induce antimicrobial activity. Naturally, synthesized TiO_2 nanoparticles obtained via extraction from the *Psidium guajava* aqueous leaf has an antibacterial effect on

S. aureus, Pseudomonas aeruginosa, E. coli and *Proteus mirabilis* (Santhoshkumar *et al.,* 2014). In this study, we report, on one of the key benefits of modified hydrothermal nanotitania extraction, namely the inhibition of *S. aureus* growth.

Materials and methods

Bacteria and material preparation

Bacteria *S. aureus* was obtained from a stock culture from the Craniofacial Science Laboratory, School of Dental Sciences, Universiti Sains Malaysia (USM). The modified hydrothermal nanotitania extract was prepared using TiO_2 in combination with silver at concentrations of 0.01%, 0.03% and 0.05%. To test these materials, a control TiO_2 was also produced devoid of any silver (undoped). It is also important to note that the product was not diluted in water, dimethyl sulfoxide (DMSO) and sulphuric acid.

Preparation of the nanotitania extraction

The nanotitania extraction was done according to the method previously described by Mukifza *et al.* (2016). In brief, the effect of molarity and the mix ratio on the caustic hydrothermal process produced optimum growth conditions for the nanoparticle TiO_2 when situated in ilmenite waste. These TiO2 nanoparticles were then further mixed with Argentum nitrate in a molten salt synthesis process to produce highly crystalline nanoparticles, which gave dopant concentrations in the samples.

Evaluation of antibacterial activity

The antibacterial activity of nanotitania extracts was performed as described Mukifza *et al.* (2016). Suspensions of 250 mg/ml nanotitania extract with 0.01%, 0.03% and 0.05% silver and undoped (positive control containing TiO_2 only) were prepared by mixing 1 g of these material in 4 ml of Mueller Hinton (MH) Broth. Then 1 ml of these extraction were mix with 1 ml of *S. aureus* suspension (equivalent to 0.5 MacFarland). Bacterial suspension, without nanotitania extract was used as a negative control. The mixture was then placed in an incubator shaker at 37°C for 24 hours. Then, 100 µl of the mixture was spread on MH agar and incubated at 37°C for another 24, 48, and 72 hours. The growth of bacteria was observed after each incubation period. Adding and mixing the nanotitania extract into the agar before the addition of bacteria is similar to the method employed by Mukifza *et al.* (2016).

Results

The present study of nanotitania extraction containing TiO_2 in combination with silver (0.01%, 0.03% and 0.05%) and TiO_2 alone (undoped) was shown to inhibit the growth of *S. aureus* after 24, 48 and 72-hour incubation periods at concentration 250 mg/ml (Table 1). Figure 1 shows that bacteria (*S. aureus*) was grown on the plate A for control negative (bacteria + MH broth). It is indicated that no inhibition occurred. While on the plate B, C, D and E (undoped, 0.01%, 0.03% and 0.05%) shows that no bacteria were grown which is indicated that the bacteria were inhibited. The results are summarized in Fig. 1.

Table 1 The effect of nanotitania extracts in combination with different concentrations of silver on the growth of *S. aureus*

Test	Incubation time		
	24 hours	**48 hours**	**72 hours**
Bacteria only (negative control)	Growth	Growth	Growth
Undoped (positive control) +bacteria	No growth	No growth	No growth
Nanotitania extraction TiO_2 with 0.01% silver+ bacteria 250 mg/ml	No growth	No growth	No growth
Nanotitania extraction TiO_2 with 0.03% silver +bacteria 250 mg/ml	No growth	No growth	No growth
Nanotitania extraction TiO_2 with 0.05% silver +bacteria 250 mg/ml	No growth	No growth	No growth

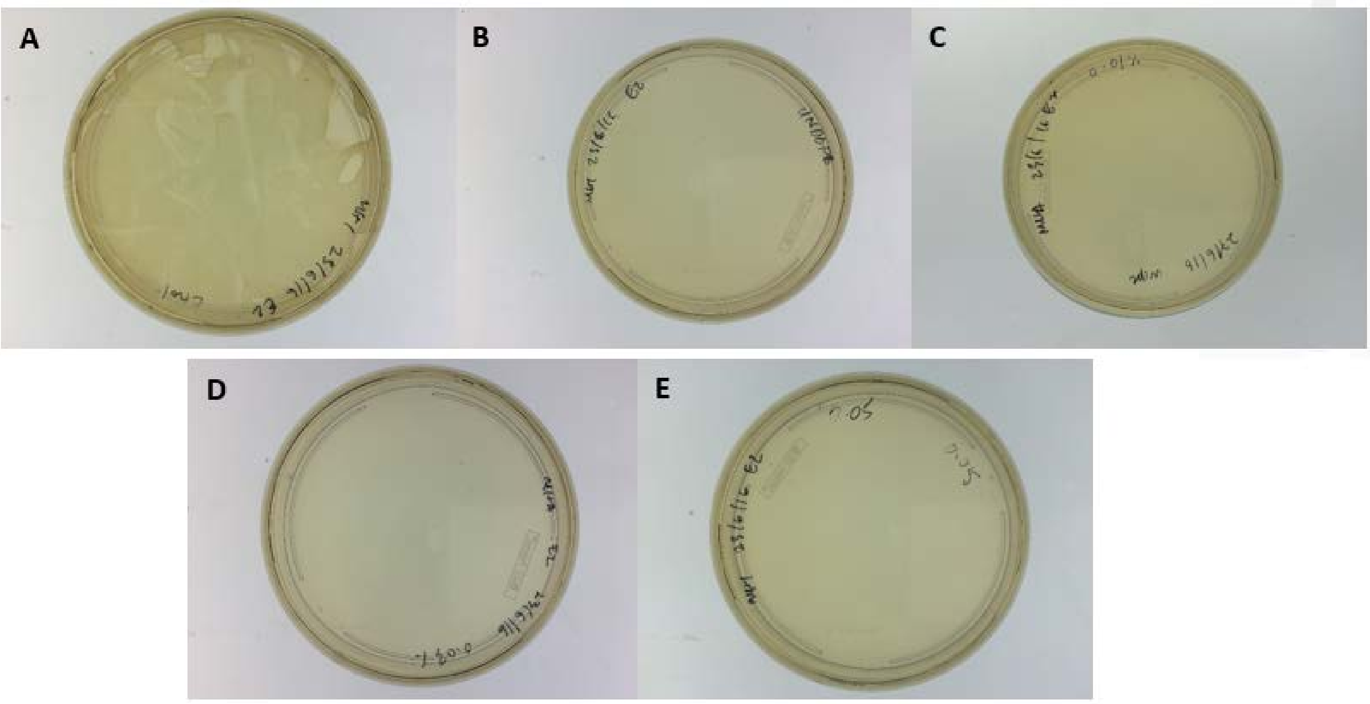

Fig. 1 (A) Negative control. (B) Nanotitania extraction containing TiO_2 only / positive control. (C) Nanotitania extraction: TiO_2 containing 0.01% silver. (D) Nanotitania extraction: TiO_2 containing 0.03% silver. (E) Nanotitania extraction: TiO_2 containing 0.05% silver. It should be noted that there was no bacterial growth observed in agar plates (B), (C), (D) and (E).

Discussion

The resistance of bacteria to antibacterial medications creates anxiety and worry among medical health personnel and patients worldwide. Although considerable efforts have been made to prevent the onset of deadly bacterial infections, the significant increase in bacterial resistance to a range of conditions is alarming. In this study, a novel nanotitania extract has been shown to give the promise of hope to the medical field. The capability of this nanotitania extract to inhibit S*. aureus* has at least partly restored our confidence in the potential of antibacterial medications. In this study, the TiO_2 was synthesized via caustic hydrothermal method as practiced by several other researchers (Ou and Lo, 2007; Zhang *et al.,* 2009; Meor Yusoff *et al.,* 2010; Mahdi *et al.,* 2013). Mukifza *et al.* (2016) reported that after employing the caustic hydrothermal decomposition method, the formed sodium titanate underwent a leaching process which exemplified a moderate molarity and ratio. The acid-washing was performed for the ion exchangeable process (Ou and Lo, 2007). The higher the acid molarity, the greater the amount of titanium extracted, as reported by several researchers (Jia *et al.,* 2014; Sasikumar *et al.,* 2004; Zhang and Nicol, 2010; Li *et al.,* 2007; Mehdilo and Irannajad, 2012; Zhang *et al.,* 2011; Razavi *et al.,* 2014). However, it has been previously reported that titanium waste with high acidity poses a considerable danger to the environment (Lane, 1991). Several studies have shown the effect of titanium on the bacteria and have highlighted the antibiotic qualities of the nanotitania. For example, TiO_2 nanoparticles were shown to increase the effect of beta lactam, cephalosporin and aminoglycosides on methicillin-resistant *S. aureus* (MRSA) (Roy *et al.,* 2010). In addition, the use of nanotitania on medical equipment indicated the possibility of its significantly arresting bacterial activity. For example, the combination of TiO_2 with silver nitrate when used in nanoparticle-coated surgical face masks reduced *E. coli* and *S. aureus* viability within 48 hours (Li *et al.,* 2006). The next step in understanding the nanotitania's antibacterial effect is to discover its mechanism of action. Several studies have shown the possible mechanism of TiO_2 as an antibacterial substance. For example, nanoparticles of TiO_2 caused the mortality of the oral bacteria, *Streptococcus mutans*, through precipitating a reduction in its lactate production over 24 hours (Besinis *et al.,* 2014). In addition, a TiO_2-based nanocomposite was observed as having a photocatalytic action on *P. aeruginosa* PAO1 cells, in which the reduced expression of specific genes and proteins involved in regulatory, signalling and growth functions of the cell was noted (Kubacka *et al.,* 2014). In this study, a novel nanotitania extraction containing TiO_2 mixed with various concentrations of silver (0.01%, 0.03% and 0.05%) together with an undoped version (containing TiO_2 only) were tested against *S. aureus*. The main reason was to determine whether these substances were capable of inhibiting the growth of the bacteria, as well as to study the mechanism and usage of this substance in various ways. As with other antibacterial tests, water dilution is the main objective before testing begins, together with dilution in various other fluids. However, this substance was not dissolved in water, dimethyl sulfoxide (DMSO) and sulphuric acid. From the results, this substance has been shown to successfully inhibit the growth of *S. aureus* up to 72 hours of incubation. These findings explain the potential of these substances to be used due to their prolonged antibacterial effect, and therefore as successful antibacterial agents. With regard to the limitations of the study, the nanotitania extract would not dissolve in water, thus restricting the number of tests permissible for various bacteria.

Conclusion

The novel nanotitania extract was shown to inhibit the growth of S. aureus for up to 72 hours.

Acknowledgements

We would like to thank Mr Zawdy Badruddin (School of Dental Sciences, USM Health Campus) for capturing the photos and to the Craniofacial Sciences Laboratory, School of Dental Sciences, USM for providing the required facilities and technical support in conducting the work.

References

Ahmad R, Sardar M (2013). TiO_2 nanoparticles as an antibacterial agents against *E. coli. Int J Innov Res Sci Eng Technol*, **2**(8): 3569-3574.

Besinis A, De Peralta T, Handy RD (2014). The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on *Streptococcus mutans* using a suite of bioassays. *Nanotoxicology*, **8**(1): 1-16.

Chipolombwe J, Török ME, Mbelle N, Nyasulu P (2016). Methicillin-resistant *Staphylococcus aureus* multiple sites surveillance: a systemic review of the literature. *Infect Drug Resist*, **9**: 34-52.

Harik NS, Com G, Tang X, Melguizo Castro M, Stemper ME, Carroll JL (2016). Clinical characteristics and epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in children with cystic fibrosis from a center with a high MRSA prevalence. *Am J Infect Control*, **44**(4): 409-415.

Inui T, Bandyk DF (2015). Vascular surgical site infection: risk factors and preventive measures. *Semin Vasc Surg*, **28**(3-4): 201-207.

Jia L, Liang B, Lü L, Yuan S, Zheng L, Wang X, Li C *et al.* (2014). Beneficiation of titania by sulfuric acid pressure leaching of Panzhihua ilmenite. *Hydrometallurgy*, **150**: 92-98.

Kubacka A, Diez MS, Rojo D, Bargiela R, Ciordia S, Zapico I *et al.* (2014). Understanding the antimicrobial mechanism of TiO_2-based nanocomposite films in a pathogenic bacterium. *Sci Rep*, **4**: 4134.

Lane DA (1991). Pollution caused by waste from the titanium dioxide industry: Directive 89/428. *Boston Coll Int Comp Law Rev*, **14**(2): 425-434.

Lata C, Girard L, Parkins M, James MT (2016). Catheter-related bloodstream infection in end-stage kidney disease: a Canadian narrative review. *Can J Kidney Health Dis*, **3**: 24.

Li C, Liang B, Guo LH (2007). Dissolution of mechanically activated Panzhihua ilmenites in dilute solutions of sulphuric acid. *Hydrometallurgy,* **89**: 1-10.

Li Y, Leung P, Yao L, Song QW, Newton E (2006). Antimicrobial effect of surgical masks coated with nanoparticles. *J Hosp Infect*, **62**(1): 58-63.

Mahdi EM, Hamdi M, Meor Yusoff MS, Wilfred P (2013). Characterization of titania nanoparticles synthesized by the hydrothermal method with low grade mineral precursors. *J Nano Res*, **21**: 71-76.

Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM *et al.* (2010). Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomedicine*, **6**(5): 681-688.

Marzec NS, Bessesen MT (2016). Risk and outcomes of methicillin-resistant Staphylococcus aureus (MRSA) bacteremia among patients admitted with and without MRSA nares colonization. *Am J Infect Control,* **44**(4): 405-408.

Mehdilo A, Irannajad M (2012). Iron removing from titanium slag for synthetic rutile production. *Physicochem Probl Miner Process*, **48**(2): 425-439.

Meor Yusoff MS, Masliana M, Paulus W, Devi P, Mahmoud ME (2010). Fabrication of titania nanotubes by a modified hydrothermal method. *J Sci Technol* (UTHM), **2**(2): 15-24.

Morris AK, Russell CD (2016). Enhanced surveillance of Staphylococcus aureus bacteraemia to identify targets for infection prevention. *J Hosp Infect*, **93**(2): 169-174.

Mukifza A, Yusof S, Awang HB, Farid EM (2016). Synthesis and characterization of titanium dioxide using a caustic hydrothermal with moderate molarity and ratio from synthetic rutile waste. *Eur J Sci Technol*, **4**(8): 42-45.

Nathwani D, Dryden M, Garau J (2016). Early clinical assessment of response to treatment of skin and soft-tissue infections: how can it help clinicians? Perspectives from Europe. *Int J Antimicrob Agents,* **48**(2): 127-136.

Ou HH, Lo SL (2007). Review of titania nanotubes synthesized via the hydrothermal treatment: fabrication, modification, and application. *Sep Purif Technol*, **58**(1): 179-191.

Razavi R, Hosseini SMA, Ranjbar M (2014). Production of nanosized synthetic rutile from ilmenite concentrate by sonochemical HCl and H_2SO_4 leaching. *Iran J Chem Chem Eng*, **33**(2): 29-36.

Roy AS, Parveen A, Koppalkar AR, Prasad, MA (2010). Effect of nano-titanium dioxide with different antibiotics against methicillin-resistant *Staphylococcus aureus*. *J Biomater Nanobiotechnol,* **1**(1), 37-41.

Santhoshkumar T, Rahuman AA, Jayaseelan C, Rajakumar G, Marimuthu S, Kirthi AV *et al.* (2014). Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. *Asian Pac J Trop Med*, **7**(12): 968-976.

Sasikumar C, Rao DS, Srikanth S, Ravikumar B, Mukhopadhyay NK, Mehrotra SP (2004). Effect of mechanical activation on the kinetics of sulfuric acid leaching of beach sand ilmenite from Orissa, India. *Hydrometallurgy,* **75**: 189-204.

VanEperen AS, Segreti J (2016). Empirical therapy in methicillin-resistant Staphylococcus aureus infections: An up-to-date approach. *J Infect Chemother*, **22**(6): 351-359.

Zhang S, Nicol MJ (2010). Kinetics of the dissolution of ilmenite in sulfuric acid solutions under reducing conditions. *Hydrometallurgy,* **103**: 196-204.

Zhang W, Zhu Z, Cheng CY (2011). A literature review of titanium metallurgical processes. *Hydrometallurgy,* **108**: 177-188.

Zhang Y, Qi T, Zhang Y (2009). A novel preparation of titanium dioxide from titanium slag. *Hydrometallurgy,* **96**: 52-56.