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The Antimicrobial Action of Silver Halides in Calcium Phosphate

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Abstract. Silver halides represent a yet unexplored avenue for imparting antimicrobial activity to calcium phosphates**.** Negtively charged silver halide colloids (AgI, AgBr and AgCl) were added to synthesized amorphous calcium phosphate. Concurrent melting of silver halides and crystallization to carbonated apatite at 700 ^{o}C increased the silver halide surface area available to bacteria and formed a lower solubility apatite. The effect of the matrix solubility on antimicrobial response could then be investigated.

Pseudomonas aeruginosa was more sensitive to silver iodide and silver bromide than *Staphylococcus aureus*. Silver iodide demonstrated greater activity than silver bromide. Silver chloride did not produce an antibacterial response. Both amorphous calcium phosphate and carbonated apatite displayed similar antibacterial activity when accompanied by silver halides. It is thought that amorphous calcium phosphate dissolves more readily and increases the bioavailability of the silver halide particles. Silver iodide displays a greater antibacterial response of all silver halides, with a response that is improved in a more resorbable matrix.

Introduction

The incidence of infection can have devastating effects for the longevity of implants with greater occurrence in revision surgeries. The two different strategies for imparting antimicrobial activity to implants involves adding active agents or including ionic species in the structure of the biomaterial. The easiest approach is to add a secondary phase possessing antimicrobial action with the only requirement to adjust the resorbability of the matrix. The antimicrobial activity of silver is well known and, of all the elements; described as exhibiting the highest levels of toxicity to microorganisms and the lowest levels of toxicity to animal cells [1].

Nanoscale materials have recently appeared as new antimicrobial agents, due to their high surface area to volume ratio, and unique chemical and physical properties [2-4]. Silver nanoparticles (NPS) exhibit very strong bactericidal activity against both Gram-positive and Gram-negative bacteria, including multiresistant strains [2,5].

The antimicrobial properties of silver have been linked to the cation Ag^+, which has the ability to form a strong bond with electron donor groups in biological molecules. Traditionally, $AgNO_3$ solutions were the most widely used. Over time, silver compounds (carbonates in dressings [6], phosphates in hydroxyapatites [7,8] have been recognized for their antibacterial properties. Among different chemical forms of silver AgCl has been found to be the least cyto-toxic [9].

Research and interest on the use of silver as an antibacterial agent has grown tremendously over the past few years. Research results have been described in literature reviews, which describe the positive characteristics of silver, such as its' ability to provide bacterial resistance. Research compiled from earlier research offers practical and theoretical explanations on the antimicrobial properties of silver [10]. However, more attention is now being paid to the associated risks with its use, especially as nanoparticles [11]. Special attention is given to silver microbial resistance, together with questions about the standardization of antimicrobial testing methods [12]. Given the high concentration of chloride ions in biological solutions, attention has been given to the different forms of silver chloride in water and its marked complex formation ability in the presence of a greater concentration of chloride ions.

Research, as far as we know, has not been conducted on the use of silver bromide and silver iodide, primarily due to the low solubility. Previous work on the inclusion of silver iodide in amorphous calcium phosphate [13] will be combined with new studies on silver bromide and silver chloride to provide a comparison between all three types of halides. This work sets out to investigate the incorporation of silver halogens in amorphous calcium phosphate and apatite crystalized by heating. Our research will determine the silver halogen that is most effective in reducing the concentration of common wound pathogens. The AgI, AgCl and AgBr will be negatively charged based on more favourable antimicrobial activity, as found in a previous study [12].

Materials and Methods

Synthesis. Nanoparticles of silver halogens were synthesized using a condensation reaction route from silver nitrate and potassium halogen solutions: $AgNO_3 + KX= AgX + KNO_3$, where X=I, Br or Cl. Silver halogen charge arises from an excess of ionically bonded constituent ions in all cases. A 0.01M solution of $AgNO_3$ was added dropwise to a rapidly stirred 0.01M KX solution. The ξ potential for all silver halides changed from -46 mV for colloidal negatively charged silver iodide, to -67 mV for colloidal negatively charged silver bromide and -51 mV for negatively charged colloidal silver chloride.

Amorphous calcium phosphate (ACP) suspensions were produced by pouring a calcium nitrate solution into a stirred ammonium hydrogen phosphate solution buffered to pH 10 using ammonium hydroxide so as to provide a Ca/P ratio of 1.7. The ACP was stirred for 10 minutes before modification by the silver halogens. Stirring was conducted for 15 minutes and the silver halogen combined with ACP was kept for 20 minutes before vacuum filtration. The filtrates were washed with water by pouring water onto the filter cake during vacuum filtration and then dried by freeze-drying.

Characterization and testing. Absorption spectra of AgX nanoparticles were obtained using a Shimadzu UV spectrometer. Spectra were recorded using a 1 mm path length quartz cell.

Raman spectra measurements were performed on a Renishaw InVia Raman spectrometer, He-Ne laser – 633 nm at 1 mW.

Antimicrobial testing. Antibacterial activity was estimated against two bacterial strains that are typical wound pathogens: *Staphylococcus aureus* MSCL 334 (Gram-positive bacteria) and *Pseudomonas aeruginosa* MSCL 331 (Gram-negative bacteria). Minimum inhibitory concentrations (MIC) against test bacterial strains were determined by a standardized macrodilution method using Muller-Hinton broth medium [14]. Experiments established the antibacterial activity of each powder suspended in water after 24 hours.

Results and Discussion

Solubility of silver halides

Silver halides are more soluble in water than apatite (Table 1). Considering the low water solubilities of silver halides at room temperature, it is possible to release more silver from silver halides sols. Silver halogens were added to amorphous calcium phosphate. Since the halogen molecular weight differs, it is necessary to add a different weight of silver compounds to deliver an equal amount of silver (1mg). The smallest content was 1.33 mg of AgCl and the largest amount was 2.18 mg AgI. Compared to the solubility of hydroxyapatite, the halides are fairly soluble (K_{sp} of hydroxyapatite is 2.34×10^{-59}) [15].

Table 1. Solubility constants for various silver inorganic and equilibrium concentration

	Silver compound	Fw, g/M	Solubility constant $(K_{sp})^{a)}$	Solubility, mol/L	Ag^+, mg/L	$Ag,^{c)}$, mg/L
1.	$AgNO_3$	169.9	Highly soluble$^{b)}$	13.8	1	1.6
2.	AgCl	143.3	1.8×10^{-10}	1.3×10^{-5}	1	1.3
3.	AgBr	187.8	5.4×10^{-13}	7.3×10^{-7}	1	1.7
4.	AgI	234.8	8.3×10^{-17}	9.2×10^{-9}	1	2.2
5.	Ag	107.9	insoluble			
	a) Handbook of Chemistry and Physics, 66th Ed.; CRC Press: Boca Raton, FL., 1985-1986. b) Highly soluble, it is possible to make comparable high concentrations of Ag^+ ionic parts in solutions, but not nanoparticles; c) Amount of silver halides (mg/L for incorporation of 1mg/L Ag^+)					

Characterization of AgI^-, $AgBr^-$ and $AgCl^-$ sols

The spectra obtained immediately after preparation by a condensation reaction route from silver nitrate and potassium halogen solutions in water are shown in Fig 1. The spectra are in agreement with results obtained by other researchers [16,17].

The position and the number of silver halide peaks in the absorption spectra depend on the shape and size of the particles. The amount of halides, in all of the synthesized sols, was greater than the silver to capture all Ag^+ ions.

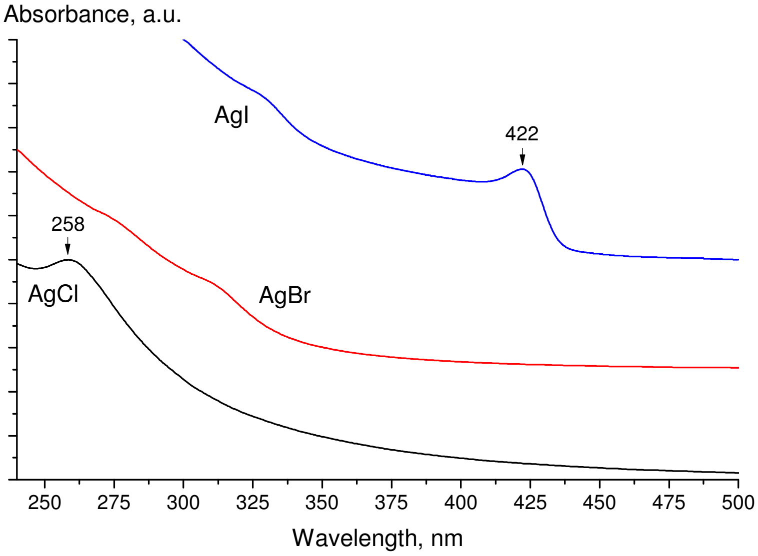

Figure 1. UV–VIS absorption spectra of silver halides recorded immediately after the chemical condensation reaction at room temperature.

Raman spectroscopy

Since silver halides have low vibration frequencies and are highly photo-sensitive, we used Raman spectroscopy [18] as a suitable tool for determining the presence of silver halides in amorphous calcium phosphates. The recorded Raman spectra for the basic synthesis (ACP) are depicted in Figure 2 together with Raman spectra of AgI and AgI with carbonated hydroxyapatite. The bands at 948 cm^{-1} and 958 cm^{-1} are attributed to the symmetric stretching mode ν_1 (PO_4^{3-}) and display the crystalline order: the lower frequency occur for amorphous calcium phosphate and the higher wavenumber occurs for carbonated hydroxyapatite. Silver iodide has characteristic absorption within the spectra range of 63 cm^{-1} – 110 cm^{-1}, depending on the size of the silver iodide particle [19].

Silver halides in ACP and calcium apatite were detected using EDAX, however since the silver halides distribution was not necessarily homogenous the measurement of the concentration was not deemed sufficiently accurate and will not be shown here.

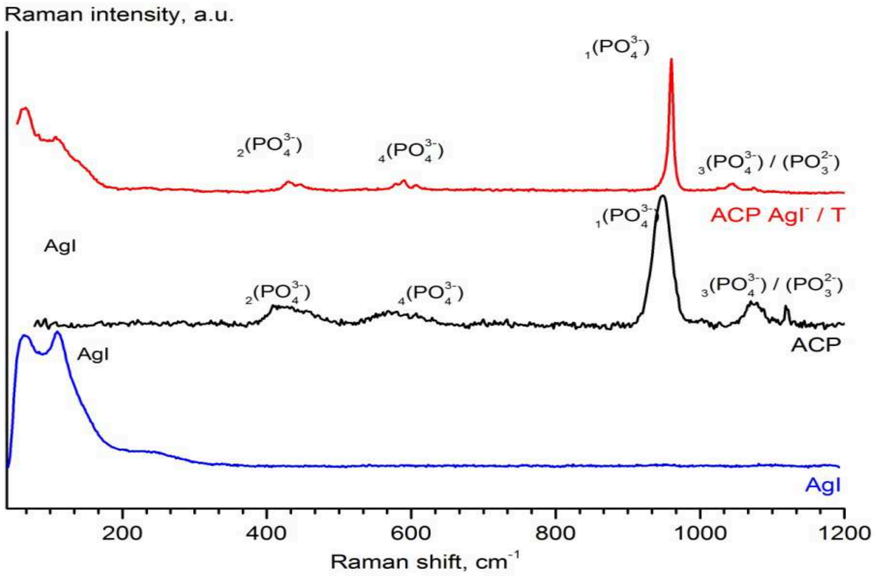

Figure 2. Raman spectrum of AgI, ACP- basic synthesis of amorphous calcium phosphate and ACP AgI$^-$ /T - AgI modified with ACP that has been crystallized to carbonated hydroxyapatite.

Antibacterial properties

The broth macrodilution susceptibility test was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the amorphous apatite (blank - ACP) and apatite loaded with silver halides.

Table 2.The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) in mg/ml for negatively charged AgI, AgBr and AgCl nanoparticles with ACP and crystallized apatite by heating (T) for 20 min at 700 ^{o}C.

Powder	*Pseudomonas aeruginosa* MSCL 331 (Gram–negative)			*Staphylococcus aureus* MSCL 334 (Gram-positive)		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
Blank - ACP	>10	>10		>10	>10	
ACP - 5%AgI$^-$	0.3	2.5	8.3	1.3	10	7.7
ACP - 10%AgI$^-$	0.6	> 10	>16	1.3	>10	>7.7
ACP/T - 5%AgI$^-$	0.6	2.5	4.2	0.6	2.5	4.2
ACP/T - 10%AgI$^-$	2.5	5	2	2.5	10	4
ACP - 5%AgBr$^-$	5	5		>5	>5	
ACP - 10%AgBr$^-$	2.5	2.5		>5	>5	
ACP/T - 5%AgBr$^-$	2.5	2.5		>5	>5	
ACP/T -10%AgBr$^-$	2.5	2.5		1.25	1.25	
ACP - 5%AgCl$^-$	>5	>5		>5	>5	
ACP - 10%AgCl$^-$	>5	>5		>5	>5	
ACP/T - 5%AgCl$^-$	>5	>5		>5	>5	
ACP/T -10%AgCl$^-$	>5	>5		>5	>5	

In exposure to *Pseudomonas aeruginosa,* the greatest antimicrobial activity was found with AgI colloidal particles enriched calcium phosphate. ACP - silver iodide demonstrated greater activity than ACP – silver bromide. The MBC that kills bacteria was the same as MIC, for silver bromide loaded ACP and apatite. MIC for ACP – silver chloride exceeded the detection limit, i.e. 5 mg/ml.

Pseudomonas aeruginosa, the Gram- negative bacterium, was more sensitive to both AgI and AgBr than *Staphylococcus aureus.* The use of a crystalline matrix (ACP/T) loaded with 10% AgBr for comparison to the more soluble amorphous calcium provides the greater effect to *Staphylococcus aureus* than to *Pseudomonas aeruginosa.*

Summary

Silver iodide and silver bromide, synthesized as colloids with a negative charge and incorporated into an amorphous calcium phosphate (ACP) provided an antibacterial action and prevented growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Silver iodide displayed the greatest antibacterial response of all silver halides, with a response that is improved in a more resorbable matrix. *Pseudomonas aeruginosa* was more sensitive to AgI and AgBr containing calcium phosphates.

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References

[1] J.P. Guggenbichler, M. Böswald, S. Lugauer, T. Krall, A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters. *Infection*, 1999; 27(Suppl.) S16—S23.

[2] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez, M.J. Yacaman, The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16 (2005) 2346–2353.

[3] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H.Kim, Y.K. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, M.H. Cho, Antimicrobial effects of silver nanoparticles. *Nanomed.-Nanotechnol. Biol. Med.* 3 (2007) 95-101.

[4] M. Rai , A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv*., 27 (2009) 76-83.

[5] A. Panacek, L. Kvítek, L.R. Prucek, M. Kolar, R. Vecerova , N. Pizúrova ,V.K. Sharma, T. Nevecna, R. Zboril, Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J. Phys. Chem. B*. 110 (2006) 16248–16253.

[6] M.H. Cavanagh, R.E. Burrell, P.L. Nadworny, Evaluating antimicrobial efficacy of new commercially available silver dressings. *Int. Wound J.* 7 (2010) 394–405

[7] J.J. Buckley, A.F. Lee, L. Olivi, K. Wilson, Hydroxyapatite supported antibacterial Ag_3PO_4 nanoparticles. *J. Mater. Chem.* 20 (2010) 8056-63.

[8] C.S. Ciobanu, S.L. Iconaru, P. de Coustumer, L.V. Constantin, D. Predoi, Antibacterial activity of silver-doped hydroxyapatite nanoparticle against gram –positive and gram-negative bacteria. *Nanoscale Res Lett.* 7 (2012) 324.

[9] S. Zhang, C. Du, Z. Wang, X. Han, K. Zhang, I. Liu, Reduced cytotoxicity of silver ions to mammalian cells at high concentration due to the formation of silver chloride. *Toxicol. in Vitro* 27 (2013) 739-744.

[10] S. Eckhardt, P.S. Brunetto, J. Gagnon, M. Priebe, B. Giese, K.M. Forman, Nanobio Silver : Its Interactions with Peptides and Bacteria, and its Uses in Medicine. *Chem. Rev.* 113 (2013) 4708-4754.

[11] C. Marambio-Jones, E. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and environment. *J. Nanopart. Res.* 12 (2010) 1531-1551.

[12] I. Chopra, The increasing use of silver–based products as antimicrobial agents: a useful development or a cause for concern? *J. Antimicrob. Chemoth.*, 59 (2007) 587-590.

[13] K.A. Gross, D. Kalnina, Z. Stankeviciute, V. Nikolajeva, Charge state of silver halide colloids determines the antibacterial activity in amorphous calcium phosphate. *Key Eng. Mat.* 587 (2014) 74-79.

[14] S. Qaiyumi, Macro- and microdilution methods of antimicrobial susceptibility testing. in: R. Schwalbe, L. Steele-Moore, A.C. Goodwin (Eds.), Antimicrobial susceptibility testing protocols. CRC Press, 2007, pp. 75-79.

[15] H. Mc Dowell, T.M. Gregory, W.E. Brown. Solubility of $Ca_5(PO_4)_3OH$ in the system $Ca(OH)_2$-H_3PO_4-H_2O at 5, 15, 25, and 37 °C. *J. Res. Nat. Bur. Stand.* 81 (1977) 273–281.

[16] H. Kanzaki, S. Sakuragi, Optical absorption & luminescence of excitons in silver halides containing isoelectronic impurities. Part II. AgBr:Cl- and AgBr, *J. Phys. Soc. Jpn.* 29 (1970) 924-35.

[17] F. Moser, R.K. Ahrenkiel, and S.L. Lyu, Optical Absorption and Luminescent Emission of the I– Center in AgCl, Phys. Rev. 161, 897 – Published 15 September 1967.

[18] I. Martina, R. Wiesinger, D. Jembrih-Simburger, M. Schreiner, Micro-Raman characterisation of silver corrosion products: instrumental set and reference database. *Preservation Science*, 9 (2012) 1-8.

[19] R.C. Hanson, T.A. Fjeldly, H.C. Hochheomer, Raman Scattering from Five Phases of Silver Iodide. *Phys. Stat. Sol.* 70 (1975) 567.

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DOI References

[3] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, M.H. Cho, Antimicrobial effects of silver nanoparticles. Nanomed. -Nanotechnol. Biol. Med. 3 (2007) 95-101.
http://dx.doi.org/10.1016/j.nano.2006.12.001
[15] H. Mc Dowell, T.M. Gregory, W.E. Brown. Solubility of Ca5(PO4)3OH in the system Ca(OH)2-H3PO4-H2O at 5, 15, 25, and 37 °C. J. Res. Nat. Bur. Stand. 81 (1977) 273-281.
http://dx.doi.org/10.6028/jres.081A.017