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Rapid Evaluation of Antibacterial Activity by Microtiter Well Coating.

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RAPID EVALUATION OF ANTIBACTERIAL ACTIVITY BY MICROTITER WELL COATING

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The colonization of clinical and industrial surfaces with microorganisms, including antimicrobial-resistant strains, has promoted increased research into the development of effective antibacterial and antifouling coatings. In this study the preparation of metal (Ag^{+}) and metal oxide (Cu^{2+}, Zn^{2+}) doped methyltriethoxysilane (MTEOS) coatings and the rapid assessment of their antibacterial activity is described. The wells of polystyrene microtiter plates were coated using various volumes of the sol-gels and cured at different temperatures for varying time periods. Curing parameters were analyzed using by thermogravimetric analysis (TGA) and visual examination. The optimum curing temperature in the microtiter wells was determined to be 50-60º C. when the wells were coated using a sol-gel volume of 200 µl. The coated wells were challenged with cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli.* Silver showed the highest antibacterial activity followed by zinc and copper. DLS showed that the size of the silver ions was smaller than that of the zinc and copper. The silver doped sol-gel had broad spectrum antibacterial activity making it potentially useful as a coating for biomaterials. The use of microtiter plates enabled a variety of sol-gel coatings to be screened for their antibacterial activity against a wide range of bacteria in a relatively short time. This route is a pre-screening technique that then applied to the surface of indwelling items (e.g. ear-rings), in which the continuous release of metal and metal oxides has ability to protect skin surfaces from microbial infection.

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Introduction

This work presents results for the rapid evaluation of the antibacterial action of metal ions in nanostructured sol gel coatings using microtiter plates. The technique facilitates multiple assays using standard dilution micro methods, measuring optical densities, determining minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The antibacterial activities of silver, copper and zinc doped sol-gels against a range of Gram-positive and Gram-negative organisms, including multi drug resistant (MDR) ones, are determined.

Experimental

Synthesis of metal doped methyltriethoxysilane (MTEOS) sol-gels
⇩
Determination of the sizes of metal species by Dynamic Light Spectroscopy (DLS)
⇩
Coating curing parameters and analysis: Volume, Temperature and Time
⇩
Assessment of antibacterial activity of coated plate

Sol-Gel Synthesis:
Under continuous stirring, MTEOS was hydrolysed under acidic conditions in the presence of a metal nitrate salt of interest and allowed to stir for 24 hours. All metal concentrations are quoted in w/w%.

Characterisation:
Thermogravimetric analysis (TGA) of the sols was carried out using a Shimadzu DTG 60 under a nitrogen flow of 40 mL.min^{-1}. Dynamic light spectroscopy was performed using a Malvern nanosizer.

Microtiter Coating:
Various sols volumes were dropped into a 96 well microtiter plate and cured at a TGA determined temperature

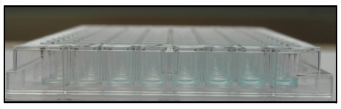
Antibacterial Activity:

The antibacterial activity of metal-doped sol gel coated microtiter well were assessed against Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* CSF 41498), Gram-negative (*Escherichia coli* ATCC 25922) and clinical isolates MDR *Enterobacter* WT6 and MDR *Pseudomonas* WTZ using standard dilution micro methods, measuring optical densities and determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) by streak out.

Results and Discussion

Analysis of curing Parameters

The wells of polystyrene microtiter plates were coated using various volumes of the sol-gels (Fig:1.) and cured at set temperatures for varying time periods. The optimum curing parameters were determined using TGA (Fig:2.) followed by visual examination.



50µl 100µl 150µl 200µl

Fig:1. Coated microtiter wells with different volume of Ag^{+}, Cu^{2+} and Zn^{2+} doped MTEOS at different concentrations.

To avoid deformation of the microtiter plate, temperatures above 70°C were avoided. Low volumes of sols were found to have poor film forming properties with cracking occurring below 100 µl.

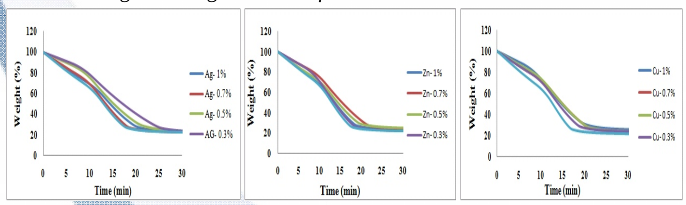


Fig:2. Thermogravimetric analysis (TGA) of Ag^{+}, Cu^{2+} and Zn^{2+} doped MTEOS of different concentrations under a nitrogen atmosphere at 50° C

The optimum volume for the microtiter well coating was 200 µl and the curing temperature was determined to be 50-60^{0} C for 5 hours.

From DLS analysis of different concentrations of metal species in the sols it was observed that the silane species are reproducible at 1-3 nm in diameter, but there was no evidence of metal based nanoparticles for silver or zinc. This was confirmed by the absence of any yellow colouring as would be expected. Non reproducible evidence of copper nanoparticles was observed, however the sols retained a blue colour indicative of Cu^{2+} chemistry.

Assessment of antibacterial activity

The antibacterial activity of the coating was determined by measuring the optical density at 600 nm of the challenge organisms (100µl of 10^{6} CFU/ml) after 24 hours of incubation at 37 ºC.

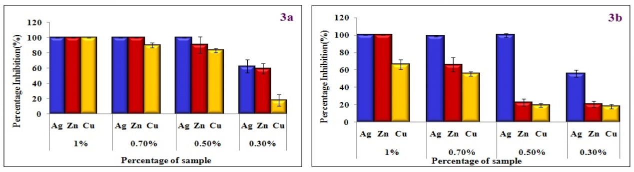
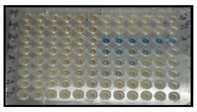


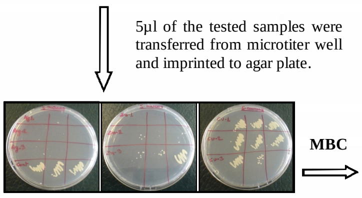
Fig:3. MIC of Ag^{+} ,Cu^{2+} and Zn^{2+} doped MTEOS coatings against (3a) *E. coli* and (3b) *Enterobacter*WT6.

The results in Fig:3. indicate that silver has the highest antibacterial activity of the metals against both test organisms. The MIC of silver for *E. coli* was 0.5%, 0.7% for zinc and 1% for copper. For *Enterobacter* WT6, the MICs were 0.5%, 1% and >1% for silver, zinc and copper respectively.



S. aureus culture in coated microtiter well after 24 h of incubation.

5µl of the tested samples were transferred from microtiter well and imprinted to agar plate.



MBC

Fig:4. Flow diagram shows modified imprint method for the determination of MBC of different concentrations of metal doped sol-gel against *S. aureus.*

Samples	Culture	MBC
Ag	*E. coli*	0.5%
Zn		0.7%
Cu		1%
Ag	*P. aeruginosa WTZ*	0.5%
Zn		1%
Cu		>1%
Ag	*Enterobacter WT6*	0.5%
Zn		1%
Cu		>1%
Ag	*S. epidermidis*	0.7%
Zn		1%
Cu		1%
Ag	*S. aureus*	0.5%
Zn		1%
Cu		>1%

Table:1.. MBC of Ag^{+} ,Cu^{2+} and Zn^{2+} doped MTEOS coatings against all test organisms.

Conclusion

The rapid evaluation of metal doped sol gel coatings in microtiter wells has been presented. The technique gave highly reproducible MIC data where the silver doped sol-gel coatings exhibited significant antibacterial activity against all the test organisms, followed by zinc and copper respectively.

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