Int J Pharma Bio Sci 2018 Oct; 9(4): (P) 92-95

Original Research Article Nanotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

ANTIBACTERIAL PROPERTIES OF REACTIVE OXYGEN SPECIES IN CARBON SUPPORTED SILICA

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ABSTRACT

Radicals possess enhanced antibacterial activity due to their oxidation potential. This is well known since most inorganic disinfectants exploit this effect. Due to the instability of radicals, and following studies in the field, peroxyl radicals present at the interface of carbon-supported silica were prepared from the pyrolysis of Kraft lignin onto high surface area silica (nanosilica).This material has stabilized peroxyl radicals that are formed by the reaction of the defective pyrolytic carbon with oxygen present in air. With this material it is possible to vehiculate radical oxygen species (ROS) directly in the cells, due to the presence of nanosilica as a support. In the present study, the activity of these supported radicals has been tested against gram +ve and gram –ve bacteria. In particular, *Bacillus subtilis* and *Klebsiella pneumonia* were used as a test organism showing a zone of inhibition 09±1 and 19±2 respectively for a drug concentration of 120 µM. The antibiograms obtained showed a good activity as antimicrobial agent, especially with *K. pneumonia*. The antibacterial activity was compared to that of doxorubicin. From the comparison, the material in this study has a comparable or superior activity when is used at the same concentration of doxorubicin. These encouraging results suggested us to move on the effect of the material in the eukaryotic cells. Infact, this could be interesting for anti-cancer treatment. The enhanced activity of peroxyl radicals at the interface of carbon supported silica over prokaryotic cells opening the avenue for the emerging research. The use of stabilized radicals could in fact be a second choice with respect the use of drugs. In fact, radical oxygen species are a specific and microorganism cannot develop resistance to radicals, as it happens in antibiotics.

KEYWORDS: ***Reactive oxygen species radical, carbon, inhibition and bacteria.***

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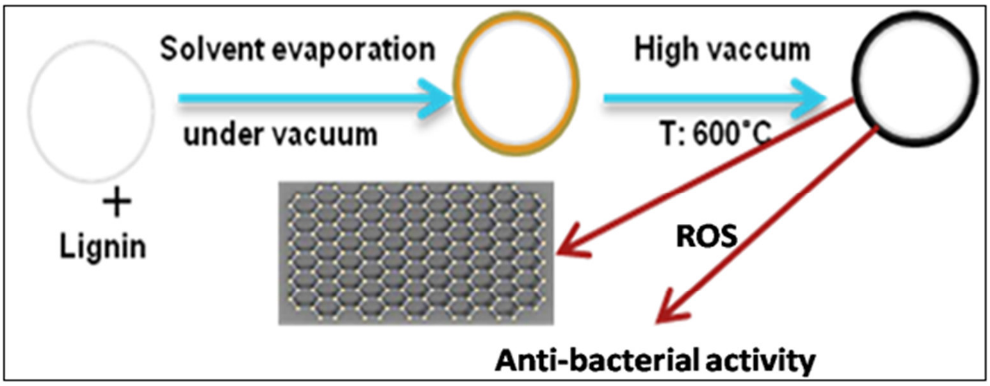
Received on: 22-09-2018
Revised and Accepted on: 13-11-2018
DOI: http://dx.doi.org/10.22376/ijpbs.2018.9.4.p92-95

INTRODUCTION

Reactive oxygen species (ROS) has diverse range of targets to exhibit antibacterial activity because of their flexibility towards the host defense against a broad spectrum of pathogens[1-2].The bio-targets for these ROS are specially DNA, RNA, proteins and lipids. In the case of ROS mediated DNA damage, oxidized DNA in vitro and in vivo includes more than 20 known products, the damage further includes all four bases and thymine-tyrosine cross-links[3].Modifications in genetic material causes adverse effect that lead to a loss of functions in membranes, proteinsand block DNA replication of mutagenic cells affecting mitosis[4]. Some nanomaterials have exhibited strong antioxidative properties, and their use may alleviate ROS-induced injuries and reduce ROS damage in biological systems. In particular, carbon nanomaterials, whose structures are based primarily on sp^2-hybridized C–C bonding, and include fullerenes[5], graphenes[6], carbon nanotubes[7] and their derivatives[8], exhibit strong ROS-scavenging properties. Carbon nanomaterials possess such properties due to their conjugated π-system, which permits the scavenging of free radicals attached to double bonds[9–11]. As Cheng, Ni, Wu *et al*. reported, fullerene derivatives are thought to be promising antioxidants that could have many important biological applications[12]. Graphene oxide flakes can be used to protect implanted mesenchymal stem cells from ROS-mediated death and thereby improve the therapeutic efficacy of mesenchymal stem cells[13]. Carbon is the basic element for life which is extensively used from ancient times for multiple purposes in day today life through the properties of organic materials. Nowadays graphene is trending allotropes of carbon which has diverse properties because of its unique structural features and chemical reactivity even in biology. Biomedical applications such as the development of potential new drugs reducing the size of the materials from micro to nano are vital in some cases of grapheme if it is used as a drug carrier. Moreover, graphene or graphene oxide sheets have been almost exclusively employed as drug carriers but the effect and influence in the overall drug action is ignored [14]. So understanding the carbon material influence and their properties plays a vital role. In the present study, we tried to make graphene-like carbon over silica by using lignin and zeosil silica as a starting material by using high vacuum pyrolytic technique at 600°C. Lignin is second richest organic material and zeosil silica is a high surface area, cheapest substrate. Surprisingly, there is existence formation of particularly peroxyl radical by reaction with atmospheric oxygen at the interface of synthesized carbon supported silica (PCS). The pyrolytic carbons were characterized by various techniques that are: Raman spectroscopy, electron paramagnetic resonance (EPR), thermogravimetric analysis (TGA) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-IR). The reactivity of the PCS was also investigated by reaction with nitrogen monoxide[15].Anti-bacterial activity of this PCS is measured by using both gram +ve and gram –ve bacterial strain to understand their biological activities.



Scheme 1
Schematic representation of preparation of stabilized peroxyl radicals over SiO_2.

MATERIALS AND METHODS

Materials and synthesis

Silica Zeosil 1165 (Rhodia, Italy) was used as received. For the preparation of carbon-supported silica, 50 mg of Kraft lignin[16] were dissolved in 50 mL of deionized water. Subsequently, 1 g of Zeosil silica was added and the solvent was removed under vacuum. Samples were pyrolyzed at 600 °C under high vacuum (10^{-3} Pa). The material was then brought into contact with air for the development of peroxyl radicals as described in a previous paper[15]**.** The size of the PCS is around 20 nm[15]. The bacterial strain in this study was procured from IMTECH, Chandigarh as reference strains.

Anti-bacterial activity tests

The antibacterial susceptibility of prepared compounds against Gram-positive and Gram-negative bacterial strains *B. Subtilis*and *K. pneumonia* were evaluated by disk diffusion/Kirby–Bauer method. Briefly, a 100µl sample of freshly-grown bacterial suspension (with a concentration of ~10^4 and ~10 colony forming unit (CFU)/ ml of *B. subtilis* and *K. pneumonia*, respectively) cultured in Luria Bertani (LB) medium was spread on the nutrient agar plates. Small sterile paper discs of uniform size (10 mm) were impregnated with prepared colloidal samples PCS and silica and then placed on the nutrient agar plates. Disks impregnated with DOX (as standard) and pure water was also placed on nutrient agar for positive and negative controls, respectively. Plates were then incubated at 37°C for 24 hrs. The resulting bacterial colonies' distance inhibition zones around the disks were recorded.

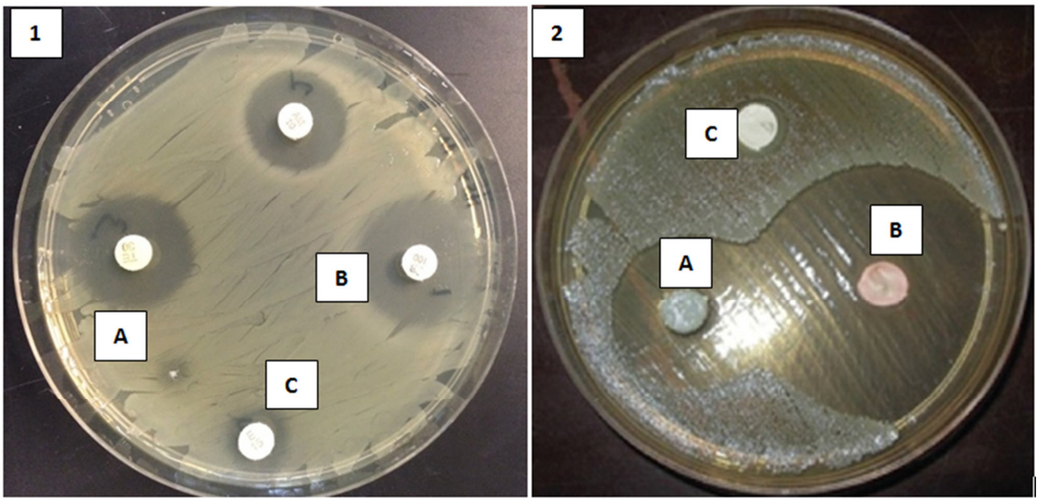
RESULTS

Characterization of PCS

The synthesized PCS is characterized by Raman, electron paramagnetic resonance, infrared and thermo gravimetric analysis[14].

Anti-bacterial studies

These anti- bacterial studies revealed the effect of materials PCS and silica in prokaryotic (*Bacillus subtilis*and *Klebsella pneumonia gram +ve and gram –ve*bacterial strains respectively). The bacterial strains were tested against synthesized PCS and pristine silicausing doxorubicin (DOX) as a control.The test results, made by using the disk-diffusion method are shown in Table 1 and Table 2, where columns 3 and 4 indicates the activity of PCS with respect to their concentration.



1) *B. subtilis* and 2) *K. pneumonia*.In both figures
A) Standard, B) carbon supported silica at 600°C, C) pristine silica.

Figure 1
Anti- bacterial activity of PCS against

Table 1
Antibacterial activity of synthesized compound. Standard deviations are calculated on the average of three measurements.

Compound	Pathogenic bacteria	Concentration (µM)	Zone of inhibition (mm)
PCS	*B. subtilis*	60	07±0.06
		120	09±0.14
		180	11±0.09

Values are mean ±SD; (n=3)
P< 0.02 when compared to control

Table 2
Antifungal activity of synthesized compound against K. pneumonia

Compound	Pathogenic fungus	Concentration (µM)	Zone of inhibition (mm)
PCS	*K. pneumonia*	60	09±0.3
		120	19±0.5
		180	20±0.6

Values are mean ±SD; (n=3)
P< 0.03 when compared to control

DISCUSSION

Hence, the activity of PCS itself has an inhibition effect over selected organisms, but in the case of pristine it is very low almost non-characteristics having DOX as a standard. This inhibition phenomenon having coherency with the concentration of PCS used shows that the presence of ROS has a strong impact on bacterial strains. In Figure 1 (2), the effect of PCS is really very high when compared Figure 1 (1) by using DOX as a standard these effects purely due to the ROS mediated inhibition of prokaryotic cells. The level of significance regarding PCS is improved purely because of the existence of peroxyl radical. These encouraging results suggested us to move onto the advanced studies in the eukaryotic cells even in human cells especially for anti-cancer treatment. Since most of the anticancer drugs following ROS mediated drug release.

STATISTICAL ANALYSIS

Statistical data which was obtained from anti-bacterial study.first we calculated the mean value by using excel ? we calculated the standard deviation for each experiment and the probability value (P) was obtained for gram +ve bacterial study is 0.02 and gram –ve bacterial study is 0.03 respectively considered to be statistically significant.

CONCLUSION

PCS is prepared by using kraft lignin and cheap high surface area silica in vacuum pyrolytic technique pyrolysis at 600°C. The study focused on the assessment of stabilized PCS as an active drug for bacterial infection because of peroxyl radicals already present in a material which was characterized by Raman, IR, EPR, and TGA.PCS showed good inhibition effects on selected bacteria. This basic study proved its ROS activity against bacterial stain, which can be further investigated for anti-cancer applications due to the availability of ROS PCS could be a cancer cell suppressors.

AUTHORS CONTRIBUTION STATEMENT

DhanalakshmiVadivel prepared the sample of PCS.NityaMeenakshi Raman carryout the anti-bacterial study.IlanchelianMalaichamy and Daniele Dondi supervising the research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

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