Silica Radical-induced DNA Damage and Lipid Peroxidation

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In recent years, more attention has been given to the mechanism of disease induction caused by the surface properties of minerals. In this respect, specific research needs to be focused on the biologic interactions of oxygen radicals generated by mineral particles resulting in cell injury and DNA damage leading to fibrogenesis and carcinogenesis. In this investigation, we used electron spin resonance (ESR) and spin trapping to study oxygen radical generation from aqueous suspensions of freshly fractured crystalline silica. Hydroxyl radical (•OH), superoxide radical ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$) were all detected. Superoxide dismutase (SOD) partially inhibited •OH yield, whereas catalase abolished •OH generation. H_2O_2 enhanced •OH generation while deferoxamine inhibited it, indicating that •OH is generated via a Haber–Weiss type reaction. These spin trapping measurements provide the first evidence that aqueous suspensions of silica particles generate $O_2^{\bullet-}$ and $^1O_2$. Oxygen consumption measurements indicate that freshly fractured silica uses molecular oxygen to generate $O_2^{\bullet-}$ and $^1O_2$. Electrophoretic assays of *in vitro* DNA strand breakages showed that freshly fractured silica induced DNA strand breakage, which was inhibited by catalase and enhanced by H_2O_2. In an argon atmosphere, DNA damage was suppressed, showing that molecular oxygen is required for the silica-induced DNA damage. Incubation of freshly fractured silica with linoleic acid generated linoleic acid-derived free radicals and caused dose-dependent lipid peroxidation as measured by ESR spin trapping and malondialdehyde formation. SOD, catalase, and sodium benzoate inhibited lipid peroxidation by 49, 52, and 75%, respectively, again showing the role of oxygen radicals in silica-induced lipid peroxidation. These results show that in addition to •OH, $O_2^{\bullet-}$ and $^1O_2$ may play an important role in the mechanism of silica-induced cellular injury. — Environ Health Perspect 102(Suppl 10):149–154 (1994)

Key words: silica-based radicals, hydroxyl radical, singlet oxygen, superoxide radical, DNA damage, lipid peroxidation, electron spin resonance, spin trapping, crystalline silica, silicosis, carcinogenesis

Introduction

Epidemiologic and pathologic studies have established that inhalation of silica incites the development of acute and chronic pulmonary silicosis (*1,2*). In addition, increasing evidence in the recent years from epidemiologic and animal experimental studies has implicated crystalline silica as a potential carcinogen (*3*). However, the biochemical mechanisms involved in the silica-induced fibrogenesis and carcinogenesis are enigmatic. Indeed it is not known whether damage to DNA plays a role in the carcinogenesis process. It is thought that one event of primary importance is the perturbation of cell membranes due to silica particle interactions (*2,4*). This cell membrane damage releases certain lytic enzymes that cause additional cell injury and eventual fibrosis (*2*). Thus, considerable effort is currently devoted to understanding the mechanism whereby silica particles disrupt the cell membrane. Earlier studies (*4–7*) have suggested that the silicon-based free radicals (Si•, SO•, and SiOO•) on the surface of freshly fractured silica and the associated generation of H_2O_2 and hydroxyl radicals (•OH) might be directly or indirectly involved in the mechanism of lipid peroxidation, leading to the loss of membrane integrity and eventual pulmonary fibrosis.

While our earlier studies (*4–7*) have demonstrated that freshly fractured silica are capable of generating •OH radicals upon reaction with aqueous media, recent studies suggest that superoxide radical ($O_2^{\bullet-}$) may also be generated (*8–11*). However, whether silica particles generate $O_2^{\bullet-}$ upon reaction with aqueous medium has not been firmly established.

In a previous study, Karmanova and co-workers (*12*) provided evidence for the emission of singlet oxygen ($^1O_2$) during the reconstruction of a freshly generated quartz surface bearing nonexcited adsorbed oxygen molecules. It is unclear, however, whether singlet oxygen can be produced from aqueous suspension of silica particles under biologically relevant conditions. The current study was undertaken to answer the following questions: *a*) Does an aqueous suspension of silica particles generate $O_2^{\bullet-}$; *b*) does an aqueous suspension of freshly fractured silica generate $^1O_2$; *c*) do $O_2^{\bullet-}$and other oxygen free radicals, generated by reactions of freshly fractured silica, play an important role in DNA damage and lipid peroxidation; *d*) is freshly fractured silica-induced DNA damage strongly dependent on molecular oxygen?

Materials and Methods

Chemicals

Iron(II) sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), *N-tert*-butyl-α-phenyl- nitrone (PBN), ethidium bromide, 2,2,6,6-tetramethyl-4-piperidone, superoxide dismutase (SOD), cis-9-cis-12-octadeca- dienoic acid (linoleic acid), xanthine, xanthine oxidase, deferoxamine, diethylenetriaminepentaacetic acid (DTPA), and sodium benzoate were purchased from Sigma Chemical Company (St. Louis, MO). Beef liver catalase was purchased from Boehringer Mannheim (Indianapolis, IN). DNA (λ

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Hind III digest marker fragments) was obtained from BRL (Gaithersburg, MD). Chelex-100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA). Phosphate buffer, pH 7.4, was treated with Chelex-100 to remove putative metal ion contaminants. DMPO solutions were purified using activated charcoal until free radical impurities disappeared as verified by ESR spectroscopy.

Silica Preparations

Crystalline silica particles of 0.2 to 5 mm in diameter were obtained from the Generic Respirable Dust Technology Center, State University, Pennsylvania University Park, PA. Crystalline silica was hand ground in an agate mortar with a pestle to produce freshly fractured silica in a size range smaller than 20 μm. The mortar ground silica exhibited a wide size distribution pattern that was considered roughly comparable to the dust produced in mining or milling processes (*5,6*).

Electron Spin Resonance Measurements

Electron spin resonance (ESR) spin trapping (*13,14*) was used to detect short-lived free radical intermediates. All ESR measurements were made using a Varian E4 spectrometer and a flat cell assembly. Hyperfine splittings were measured (to 0.1 G) directly from magnetic field separations using potassium tetraperoxochromate (K_3CrO_8) (*15*) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as standards. Reactants were combined in a total final volume of 250 μl and then transferred to a flat cell for ESR measurements. All measurements were made under ambient conditions.

Oxygen Consumption Measurements

Oxygen consumption experiments were performed at 25°C using a Clark oxygen electrode (Model 5300, Yellow Springs Instrument Co., Yellow Springs, OH). The sample contained 50 mg/ml freshly fractured silica in 10 mM phosphate-buffered solution (pH 7.4). Oxygen consumption was monitored over a period of 5 min.

DNA Strand Breakage Assays

DNA double-strand breakage assay was carried out according to the method described earlier (*11*). Briefly, reactions were carried out in 10 mM phosphate buffer, pH 7.4, in 1.5 ml polypropylene tubes at 37°C. Each reaction mixture contained 10 μg DNA (λ Hind III digest) in a total volume of 100 μl. Freshly fractured silica suspensions were added to each reaction mixture to a final concentration of 10 mg/ml. DNA damage was assessed for each reaction by removal of a 10-μl aliquot from the supernatant of each reaction tube after centrifugation. To each sample, 0.2 ml volumes of gel loading buffer (50 mM EDTA, 2.5% SDS, 0.1% bromophenol blue, and 6.25% glycerol) was added. Samples were then electrophoresed in 0.7% agarose at 1 to 2 V/cm in 40 mM Tris-acetate buffer containing 2 mM EDTA (pH 8.0). Gels were stained in ethidium bromide (5 μg/ml) for 10 min and photographed under ultraviolet transillumination.

Lipid Peroxidation Measurements

Lipid peroxidation of the model polyunsaturated lipid, linoleic acid, by freshly fractured silica was measured by monitoring the malondialdehyde (MDA) formed according to an earlier described procedure (*16,17*). A typical reaction mixture contained 2.5 mg/ml silica and 20 μl of 0.52 mM linoleic acid emulsion in a total volume of 0.5 ml. The mixture was incubated for 1 hr in a shaking water bath at 37°C. The reaction was terminated by the addition of 0.5 ml of 3% SDS and then 2.0 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstic acid, and 1.0 ml of 0.7% 2-thiobarbituric acid. The mixture was then heated for 30 min at 95 to 100°C. Thiobarbuturic acid reactive substances were extracted with 5 ml 1-butanol after cooling. This extraction was centrifuged at 3000*g* for 1 min and the butanol layer was separated. The fluorescence of the butanol layer was then measured at 515 nm excitation and 555 nm emission using Perkin-Elmer fluorospectrophotometer (model MPG-36). MDA standards were prepared from 1,1,3,3-tetramethoxypropane to obtain a calibration curve, which was used for calculating the amount of MDA produced.

Results

Superoxide Radical Generation

In our earlier studies (*5,6*), we provided evidence for the generation of •OH from an aqueous suspension of freshly fractured silica. In this study, •OH generation was measured with emphasis on the possible involvement of $O_2^{\bullet-}$ in the mechanism of •OH generation. Figure 1*A* shows a typical ESR spectrum obtained from aqueous suspension of freshly fractured silica (150 mg/ml) containing 100 mM DMPO. The analysis of this spectrum yields the hyperfine splittings of $a_N = a_H = 14.9$ Gauss, which are identical to those reported earlier for the DMPO/•OH adduct (*18*). These results are in agreement with our previous reports on •OH generation from aqueous suspensions of freshly fractured silica (*5,6*).

To investigate the possible involvement of $O_2^{\bullet-}$ in the mechanism of •OH generation, SOD (50 μg/ml) was used. As shown in Figure 1*B*, SOD decreased the intensity of the DMPO/•OH spin adduct, indicating that $O_2^{\bullet-}$ is involved in •OH generation. Figure 1*C* shows that catalase (5000 units/ml) suppressed the formation of DMPO/•OH. H_2O_2 (10 mM) significantly enhanced •OH generation, suggesting a role of H_2O_2 in the mechanism of •OH generation. The iron chelator deferoxamine also inhibited •OH generation (Figure 1*D*). The above results are consistent with a mechanism of •OH generation by the reaction of H_2O_2 with metal ions or with the reactive silica surface via a Fenton-like mechanism. As reported earlier H_2O_2 is generated by the reaction of the silica surface with water (*5,11,19*).

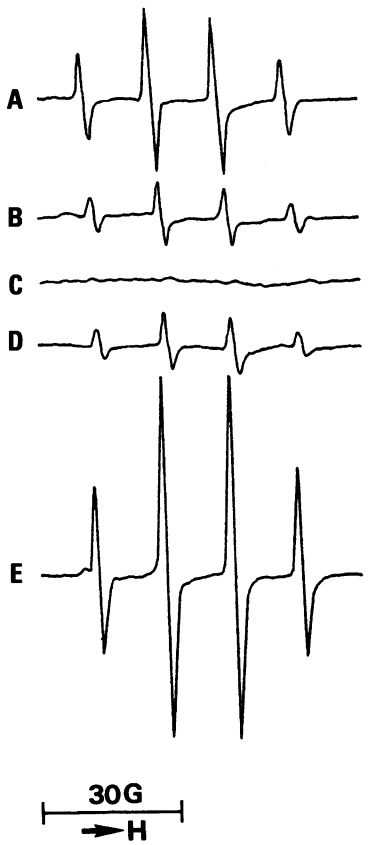


Figure 1. (*A*) ESR spectrum recorded 2 min after mixing 100 mM DMPO and freshly fractured silica (150 mg/ml) in a pH 7.4 phosphate-buffered solution. (*B*) Same as (*A*) but with 5 μg/ml SOD added. (*C*) Same as (*A*) but with 5000 units/ml catalase added. (*D*) Same as (*A*) but with 1 mM deferoxamine added. (*E*) Same as (*A*) but with 10 mM H_2O_2 added.

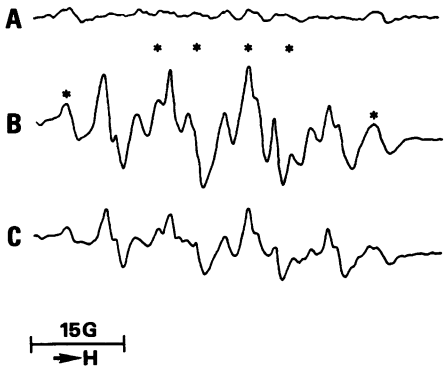


Figure 2. (*A*) ESR spectrum recorded from a phosphate-buffered solution containing 2 M DMPO. (*B*) Same as (*A*) but with 150 mg/ml freshly fractured silica. The spectrum was recorded 2 min after adding freshly fractured silica particles. (*C*) Same as (*B*) but with 50 μg/ml SOD added. Asterisks indicate DMPO/•R spin adduct signal.

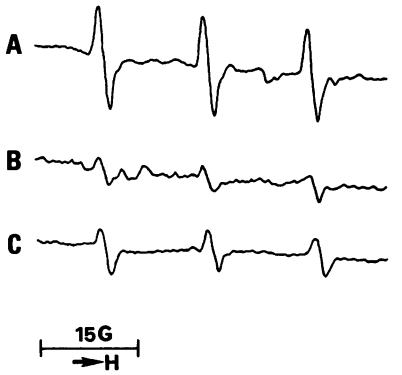


Figure 3. (*A*) ESR spectrum recorded 2 min after mixing 200 mM 2,2,6,6-tetramethyl-4-piperidone, 25 mM H_2O_2, and 150 mg/ml freshly fractured silica in a pH 7.4 phosphate-buffered solution. (*B*) Same as (*A*) but without freshly fractured silica particles. (*C*) ESR spectrum recorded 2 min after mixing 200 mM 2,2,6,6-tetramethyl-4-piperidone, 15 mM H_2O_2, and 2 mM $FeCl_2$.

Because SOD partially inhibited •OH generation from an aqueous suspension of silica particles, we deduced that $O_2^{\bullet-}$ may be involved. However, spin trapping measurement detected only the DMPO/•OH adduct (Figure 1*A*). It is known that the reaction rate of DMPO with •OH is much faster than that of DMPO with $O_2^{\bullet-}$ as shown by the following data:

$$\text{DMPO} + {}^{\bullet}\text{OH} \rightarrow \text{DMPO/}{}^{\bullet}\text{OH}$$
$$K_1 = 3.4 \times 10^9\ M^{-1}sec^{-1} \quad [1]$$
$$\text{or} \quad K_1 = 2.1 \times 10^9\ M^{-1}sec^{-1}\ (20)$$
$$\text{DMPO} + O_2 \rightarrow \text{DMPO/}O_2^{\bullet-}$$
$$K_2 = 10\ M^{-1}sec^{-1}\ (20) \quad [2]$$

If the relative concentration of $O_2^{\bullet-}$ is not higher than that of •OH, DMPO will react predominantly with •OH, and only the DMPO/•OH signal would be observable. In order to trap $O_2^{\bullet-}$, we used a relatively high concentration of DMPO. DMPO alone at concentration of 2 M did not generate any signal (Figure 2*A*). When freshly fractured silica particles (150 mg/ml) were added with 2 M DMPO, spin adduct signals were observed (Figure 2*B*). Computer simulation analysis showed that this spectrum is a composite of two spin adduct signals. The signal with hyperfine splittings of a_N = 16.7 G and a_H = 22.5 G (marked by asterisks) were assigned to a DMPO/•R spin adduct (•R represents a carbon-centered alkyl radical). This assignment was made based on the known hyperfine splittings of such adducts (*18*). The •R may result from the oxidation of DMPO. The other signal with hyperfine splittings of a_N = 14.2 G, a_H = 11.5 G and a_H^{λ} = 1.2 G was assigned to a DMPO/$O_2^{\bullet-}$ adduct. The broadening of the signals is likely due to the simultaneous trapping of •OH. Addition of SOD significantly inhibited the intensity of the spin adduct signal generated with 2 M DMPO and freshly fractured silica (Figure 2*C*). Xanthine and xanthine oxidase was used as a positive control for $O_2^{\bullet-}$ generation. In the presence of DMPO, xanthine and xanthine oxidase generated typical spectra of the DMPO/$O_2^{\bullet-}$ spin adduct (data not shown). The above results thus show that $O_2^{\bullet-}$ was indeed generated from freshly fractured silica.

Singlet Oxygen Generation

ESR spin trapping has been used for detecting $^1O_2$ employing sterically hindered 2,2,6,6-tetramethyl-4-piperidone (TMP) to generate a stable free radical nitroxide (*21,22*). Figure 3*A* shows the spectrum generated from an aqueous suspension of silica particles (150 mg/ml) containing 15 mM H_2O_2 and 200 mM TMP. The 3-line spectrum with 1:1:1 ratio was identical to that of TMP reacting with $^1O_2$ reported earlier (*21,22*). Aqueous suspension of freshly fractured silica with TMP without H_2O_2 did not generate detectable amounts of nitroxide radicals (data not shown). TMP solution containing H_2O_2 or containing H_2O_2 plus Fe^{2+} without freshly fractured silica generated nitroxide radical but at a much lower degree (Figure 3*B,C*). These results thus show that freshly silica in aqueous suspension containing H_2O_2 generate $^1O_2$.

To monitor the disappearance of dissolved molecular oxygen in association with the generation of $O_2^{\bullet-}$ and $^1O_2$, we employed a Clark-type oxygen electrode to measure oxygen consumption in an aqueous suspension of freshly fractured silica. As shown in Figure 4, the freshly fractured silica suspension rapidly consumed molecular oxygen, indicating the role of molecular oxygen in silica reactions that generate oxygenated species.

Lipid-derived Free Radical Generation from Linoleic Acid by Freshly Fractured Silica

Figure 5*A* shows an ESR spectrum obtained from a mixture containing linoleic acid (5 mM) and freshly fractured silica. Omission of freshly fractured silica resulted in the loss of the spin adduct signals (Figure 5*B*), indicating that free radicals generated from freshly fractured silica in the suspension reacted with linoleic acid to generate free radicals. Because of the broad peak of the spectrum, we have not identified the free radicals generated. We also directly monitored lipid peroxidation induced by freshly fractured silica using linoleic acid as a model lipid. As shown in Figure 6, freshly fractured silica induced lipid peroxidation in a dose-dependent

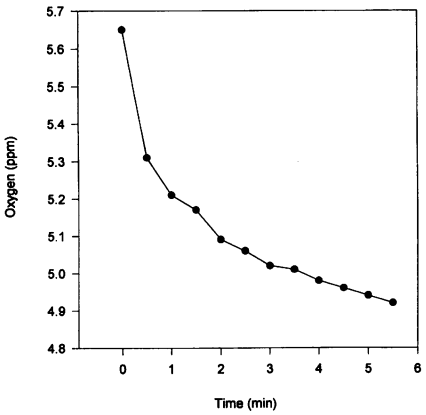


Figure 4. Dissolved oxygen concentration in parts per million (ppm) of an aqueous suspension of (50 mg/ml) freshly fractured silica in a pH 7.4 phosphate-buffered solution.

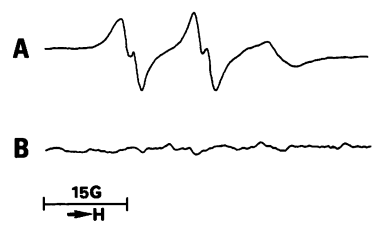


Figure 5. (*A*) ESR spectrum recorded 2 min after mixing 200 mM PBN, freshly fractured silica (20 mg/ml) and 1% linoleic acid in a pH 7.4 phosphate-buffered solution. (*B*) Same as (*A*) but without silica.

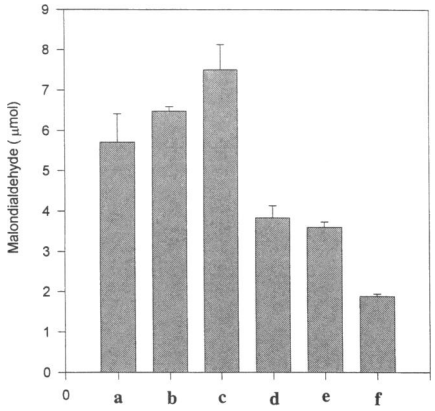


Figure 6. Silica-induced lipid peroxidation and its inhibition by scavengers of reactive oxygen species. Incubation mixture contained (*a*) 1.25 mg/ml, (*b*) 2.5 mg/ml, and (*c*) 5 mg/ml freshly fractured silica. For (*d*) to (*f*), same as (*c*) but with addition of 340 units/ml SOD, 1250 units/ml catalase, and 0.1 M sodium benzoate, respectively. Data presented are the means ± SD of a minimum of four sets of experiments in duplicate. Other experimental conditions are described in Materials and Methods.

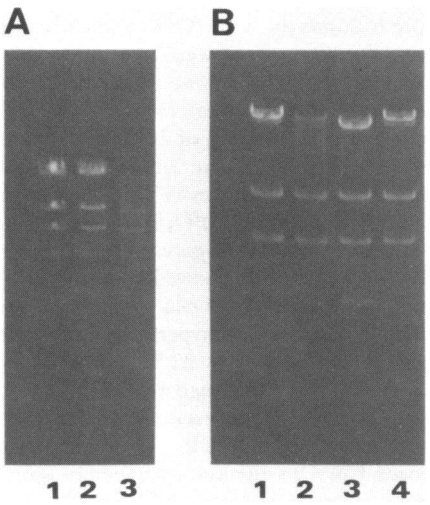


Figure 7. DNA damage by freshly fractured silica. (*A*), line 1, untreated control DNA; line 2, 10 mg/ml freshly fractured silica with λ Hind III digested DNA in a pH 7.4 phosphate-buffered solution; line 3, same as line 2 but with 1.5% H_2O_2 added. The samples were incubated for 24 hr. (*B*) line 1, untreated control DNA; line 2, 10 mg/ml freshly fractured silica with λ Hind III digested DNA in a pH 7.4 phosphate-buffered solution; line 3, same as line 2 but incubation was carried out under argon; line 4, same as line 2 but with 7500 units/ml catalase added. The samples were incubated for 3 weeks. Other experimental conditions are described in Materials and Methods.

manner. SOD and catalase inhibited the lipid peroxidation, as did sodium benzoate, an •OH scavenger.

DNA Damage by Freshly Fractured Silica and the Role of Molecular Oxygen

As shown in Figure 7*A*, lines 1 and 2, incubation of DNA with freshly fractured silica for 24 hr did not cause significant DNA double-strand breaks. H_2O_2 enhanced DNA double-strand breakage (Figure 7*A*, line 3). When the sample was incubated with freshly fractured silica for 3 weeks, a significant increase in DNA double-strand breaks was observed (Figure 7*B*, lines 1 and 2). When the incubation was carried out under argon for the same period of time, no DNA double-strand breaks were observed (Figure 7*B*, line 3). Addition of catalase also inhibited DNA double-strand breaks (Figure 7*B*, line 4) confirming the role of oxygen radicals.

Discussion

Earlier ESR studies have shown that aqueous suspension of freshly fractured silica generate •OH (*5–7*). This study demonstrates that freshly fractured silica in aqueous suspension is also capable of generating $O_2^{\cdot-}$ and $^1O_2$. The following experimental observations support this conclusion.

•OH generation was inhibited in the presence of SOD, suggesting that $O_2^{\cdot-}$ was not only generated but also involved in the mechanism of •OH radical generation. $O_2^{\cdot-}$ may function as a reductant to reduce metal ions or reactive centers on the surface of silica particles. These redox reactions may facilitate silica-mediated •OH generation from H_2O_2 according to Equations 3 to 5.

$$O_2^{\cdot-} + M^{n+1} \rightarrow M^{(n-1)+} + O_2 \quad [3]$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + {}^{\cdot}OH + OH^- \quad [4]$$

Overall,

$$O_2^{\cdot-} + H_2O_2 \xrightarrow{M^{n+}/M^{(n-1)+}} {}^{\cdot}OH + O_2 + OH^- \quad [5]$$

where M^{n+} represents metal ions or certain reactive centers on the surface of silica. The reactions described in Equations 3 to 5 are Haber–Weiss type reactions. The inhibitory effect of deferoxamine provides additional support for the presence of metal ions or reactive centers on the surface of silica particles.

The spin trapping measurements show that at a higher concentration of DMPO, the $O_2^{\cdot-}$ generated in an aqueous suspension of silica can be trapped. In addition, $O_2^{\cdot-}$ may react efficiently with metal ions or reactive centers on the surface of silica particles (Equation 3). $O_2^{\cdot-}$ may also be site-specifically generated and bound to the surface of silica particles. Higher concentration of DMPO is thus required to generate DMPO/$O_2^{\cdot-}$ and to prevent $O_2^{\cdot-}$ from reacting with other species.

Oxygen consumption measurements showed that an aqueous suspension of freshly fractured silica rapidly consumed molecular oxygen. While further investigations are needed to establish the mechanism of oxygen consumption, the following reactions (Equations 6–8) are proposed.

$$Si^{\cdot} + O_2 \rightarrow SiOO^{\cdot} \quad [6]$$

$$SiOO^{\cdot} + H_2O \rightarrow SiOH + HO_2^{\cdot} \quad [7]$$

$$HO_2^{\cdot} \rightarrow O_2^{\cdot-} + H^+ \quad [8]$$

$O_2^{\cdot-}$ may also be generated via metal autooxidation as reported earlier (*11,23*). The presence of Si• on the surface of freshly fractured silica has been reported in the literature (*24–28*).

Using ESR spin trapping, we have provided evidence for the generation of $^1O_2$ from aqueous suspension of freshly fractured silica in the presence of H_2O_2. This species is very reactive and can cause DNA damage. For example, it has been reported that $^1O_2$ can cause hydroxylation of the dG residue in DNA to generate 8-hydroxyldeoxyguanosine (*29–30*). It may be noted that $^1O_2$ generation in Cr(VI)-, Ni(II)-, and Co(II)-mediated reactions have been suggested to play an important role in carcinogenesis induced by these metal ions (*21,22,31*). Thus $^1O_2$ generated by freshly fractured silica may also play a significant role in the mechanism of silica-induced cellular injury and carcinogenesis.

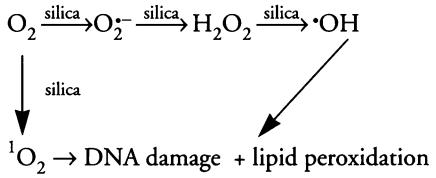
The results obtained in this study demonstrate that freshly fractured silica is able to cause DNA double-strand breaks. Catalase inhibited the DNA damage while H_2O_2 enhanced it. In our previous study, it was shown that SOD accelerated DNA strand breakage by silica in aqueous suspensions (*11*). On the other hand, the ESR data show that SOD decreases •OH formation. This apparent contradiction may be due to the following reasons: *a*) DNA strand breakage assays measure the cumulative effect of DNA damage by •OH, while ESR spin trapping measures instantaneous •OH generation; *b*) in the earlier DNA damage assays (*11*), aged silica containing lower concentration of free radicals was

used while in the current ESR study freshly fractured silica containing more free radicals was used; (*c*) the •OH may be generated site-specifically on the surface of silica particles. These site-specifically generated •OH radicals may efficiently attack DNA. In a very recent study, it was reported that DNA binds to the surface of silica particles (*32*), thus facilitating site-specific •OH reactions. The ESR spin trapping measurements used DMPO as a •OH probe: DMPO may not bind directly to the surface of silica particles. The current study also shows that DNA damage by freshly fractured silica is dependent on the presence of molecular oxygen. The above results point out an important role of oxygen radicals in the mechanism of silica-mediated DNA damage.

There is experimental evidence to support that crystalline silica is a potential carcinogen in rats (*3*). The development of neoplasms, predominatly adenocarcinomas close to silicotic lesions, provide further support to a cause and effect theory. There is also some epidemiologic evidence for silica/silicosis-associated lung cancer in humans (*33*). The role of host susceptibility factors for silica-induced carcinogenicity is currently a subject of active investigation (*34*). The observation that freshly fractured silica can cause DNA damage via oxygen-dependent, free radical-mediated reactions may significantly contribute to our understanding of the mechanism of silica-induced carcinogenesis and its prevention.

The results obtained from this study also demonstrate that freshly fractured silica reacts with linoleic acid to induce lipid peroxidation and can generate lipid-derived free radicals. SOD, catalase, and the •OH scavenger sodium benzoate all efficiently inhibit lipid peroxidation, supporting the role of oxygen radicals in silica-induced lipid peroxidation. This finding is particularly interesting since membrane damage via silica-induced lipid peroxidation is considered to be a primary step in the pathogenesis of silicosis (*4*).

Based on the results reported here and those in the literature (*4,11*), Scheme 1 is proposed for silica-induced cellular damage.



Scheme 1. Generation of oxygen radicals and singlet oxygen by silica and the relationship to cellular damage.

H_2O_2 is thought to be generated by reaction of H_2O with silicon-based radicals (*5,19*) and/or by $O_2^{\bullet-}$-related reactions involving surface metal oxidation (*11,23*).

In summary, the present results lead to the following conclusions. Aqueous suspension of freshly fractured silica generate $O_2^{\bullet-}$ and $^1O_2$, as demonstrated by ESR spin trapping. Aqueous suspension of freshly fractured silica causes DNA double strand breakage via oxygen-dependent, free radicals-mediated reactions. Freshly fractured silica cause lipid peroxidation and generate lipid-derived free radicals, which can be prevented by oxygen radical scavengers.

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