Crystalline Silica Particles Mediated Lung Injury[†]

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Abstract

Epidemiological studies have shown that the occupational exposure of crystalline silica can lead to silicosis and other lung injury. In this paper, we briefly summarize the various research works that have been conducted related to crystalline silica-induced toxicity. Firstly, a short description of the structure of quartz is presented, followed by the various types of silicosis that can be caused by inhalation of crystalline silica particles. Methods to characterize the particles and mechanisms of particle-cell interactions are also reported. The effect of physicochemical properties such as size, shape and surface functionality that influence the toxicity mechanisms are discussed, followed by a brief description on the effects of free radical generation due to quartz inhalation on biological targets (proteins, lipids and DNA). Finally, methods reported to modify the surface and reduce the quartz-induced toxicity are discussed.

Keywords: Quartz, Free radicals, Lung injury, Surface modifications, Toxicity

Introduction

The ancient Greeks¹⁾ first reported that inhaled crystalline silica can cause silicosis, which is one of the most important occupational diseases from silica inhalation. Reports on the toxicity of crystalline silica date back to the 1500s when metal diggers were known to wear protective equipments to avoid silica dust inhalation. The disease was initially referred to as ‘*pneumokoniosis*’ which means ‘*dust in the lung*’, but later replaced with ‘*silicosis*’ in 1870 by Visconti, who coined the term²⁾.

Quartz, one of the three crystalline forms of silica found in the earth’s crust, is arranged in a stacked-framework of Si-O tetrahedral units to make a 3d structure. Often, there are trace amounts of contaminating elements like aluminum, iron, lithium, magnesium, calcium, sodium and potassium associated

with the structure³⁾ which play a very important role to influence the biological activity of the material. Quartz was established as a Group I carcinogen by the International Agency for Research on Cancer (IARC) working committee in 1997⁴⁾. Despite various disagreements amongst the working group regarding this decision, it was concluded that there did exist sufficient evidence for carcinogenicity in experimental animals and humans caused by quartz inhalation. Moreover, it has also long been implicated with various other lung diseases⁵⁾, the progress of which has been shown to depend directly on the rate of quartz deposition in the lung⁶⁾.

However, carcinogenicity of quartz has also been shown to vary considerably across the different industries with similar quartz exposure⁷⁾. As per the views of the IARC working committee⁴⁾, the degree of quartz toxicity depends on the ‘inherent’ properties of the crystal or on other ‘external factors’ that can affect its biological activity. The ‘inherent properties’ of silica are determined by the state of its external surface (e.g. defects present, chemical functionalities) which again depends on the origin of the quartz. ‘External factors’ like contact, association

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and presence of contamination can trigger or inhibit silica carcinogenicity. Thus, the same quartz sample exhibits variable toxicity behavior, although the basic composition remains the same⁸⁻¹⁰. The potential influence of external factors¹¹ may be observed in the different events occurring from the deposition of quartz to the occurrence of silicosis and lung cancer.

Despite the substantial volume of research since the early 1950s, on the pathogenicity of quartz-induced diseases, the exact mechanism of action of crystalline silica is still debatable and inconclusive. There are several reports available from various medical investigations that describe the etiological and morphological facets of silica-induced diseases in detail. But quite frequently, these observations cannot be correlated to the insights obtained from chemical and biochemical processes of quartz toxicity.

The surface of quartz plays an important role in determining its biological effects, as cells and other biological molecules first come in contact with the surface^{7, 12}. Thus, in order to understand the basic molecular mechanisms of quartz-induced toxicity, it is crucial to first study the biochemical reactions associated with the surface chemistry of the inhaled crystalline silica particles. There are inherent difficulties in finding a clear-cut molecular mechanism mainly due to two reasons¹²:

- Most experimental studies on silicosis were conducted using silica samples which were not well-characterized. Thus although the available epidemiological data focuses on the quantitative aspects in details, frequently there is no correlation with qualitative issues like the physicochemical characteristics of the inhaled particles.
- Several particle-cell interactions occur during silica inhalation, leading to specific biological responses manifested by the surface. The varying molecular mechanism at each step lead to differences in surface functionalities. This again leads to variations in the physiochemical characteristics of the dust. Thus, the overall pathogenicity of the inhaled particle becomes more difficult to be interpreted¹³.

This communication is an attempt to summarize from the past and recent literature the effects of different physicochemical parameters that affect the pathogenic mechanism of silica-induced diseases in the lung, present a hypothesis on the toxicity mechanism of crystalline silica and gives a short discussion on some established ways that have been reported to reduce crystalline silica-mediated toxicity in the

lungs.

Structure of Silica in Relation to Cytotoxicity

Silicon dioxide (SiO_2) or silica is the earth's most abundant mineral, occurring in nature in several crystalline forms. The most common crystalline forms of silica are α and β -quartz, α and β -tridymite, α and β -cristobalite¹⁴. α -quartz is the most common and thermodynamically stable form at room temperature which is converted reversibly to β -quartz upon heating¹⁵. The different polymorphic forms are known to exhibit different levels of toxicity. The majority of the population is exposed to α -quartz and hence most toxicity experiments are conducted using this polymorph of silica¹².

Most quartz samples are associated with trace quantities of metal impurities that play a crucial role in determining its cytotoxic behavior^{3, 16}, as mentioned before. These impurities have a tendency to concentrate along certain crystallographic planes or defects inside the solid material, thereby affecting the relative ease of cleavage or fracture along those particular planes¹⁵. The differences in the relative frequency at which the cleavage planes occur on the surfaces of the polymorphic structures affect their toxicity behavior. Adsorption of impurities from the environment also influences the surface properties of the particles¹⁵. Moreover, the respirable particle sizes of the inhaled particles are also known to cause variation in the biological activity.

The basic difference between inhaling silica particles from other non-toxic particulates is that the latter is engulfed by the macrophages when it reaches the lung and transported through the lymphatic system to lymph nodes and rejected. But the silica particles when taken up by the macrophages can potentially kill the cell and lead to interstitialisation¹⁷. This phenomenon has been discussed in greater details in the subsequent sections. There are many *in vitro* and *in vivo* evidences that demonstrate the particle-cell interactions occurring in the lung^{3, 11, 18-21}. The toxicity investigations associated with silica inhalation in the last 25 years can be classified into the following main categories:

- (a) Acellular Tests – These investigations helped to ascertain the role of the different physiochemical parameters of the crystalline silica and correlated it to toxicity results obtained from cellular tests (in vitro and in vivo experiments). For example, the effect of surface-chemistry of well-character-

ized samples on the toxicity behavior has been reported by a number of investigators²². Although these tests are inexpensive, the results obtained are frequently inconclusive¹².

- (b) *In Vitro* Experiments – The effect of crystalline silica dusts on different cell lines (macrophages cells, epithelial cells and fibroblasts) have been studied in terms of cell viability, membrane integrity²³, inhibition of cell growth, transformation, release of cytokines^{21, 24} and other inflammatory mediators²⁵⁻²⁷. Although these *in vitro* models are not able to incorporate all variables from *in vivo* tests, they do provide valuable information that help in developing the process and determining optimal doses in a cost and time-effective manner.
- (c) *In Vivo* Experiments – Most *in vivo* tests have used rats, mice, hamsters and sheep to test for pathogenic activity in cells and tissues either after silica inhalation or by a comparatively faster and more economical process of intratracheal /intra-peritoneal instillation in the lungs^{18, 28}. A standard but not ‘so pure’ quartz sample – namely DQ 12 was developed in the 1970s to standardize and compare animal test results²⁹. Data generated from *in vivo* tests include: lung weight and histomorphology, weight of silica retained in the lung, amount of internalized silica, presence of lipids and proteins in the lung and fibrogenicity.
- (d) Epidemiology Studies – These studies consist of detailed scientific investigations into critical factors that affect the health and illness of a large set of population. Data obtained from such studies serve to provide the foundation and reason for administrative decisions made in the interest of public health and medicine. Recent epidemiological studies have shown a high risk of silicosis in workers with 30 years or more work experience in the ceramic industry³⁰.

Diseases associated with Silica Exposure

The Silicosis and Silicate Disease Committee has extensively studied the various diseases associated with silica inhalation. Silicosis is manifested as four pathologically distinct units¹² namely: chronic silicosis (nodular pulmonary fibrosis), acute silicosis (alveolar proteinosis), accelerated silicosis and complicated silicosis.

- Chronic silicosis (nodular pulmonary fibrosis): This ancient form of the occupational disease is associated with long period of exposure to low levels

of silica dust. This is the most common type of silicosis progressing slowly into a fibrotic disease that causes injury to the lung parenchyma. Chronic silicosis may become fatal due to insufficient gaseous exchange, heart failure or infections.

- Acute silicosis (alveolar proteinosis): This form of silicosis is observed after 1-3 years of exposure to high concentration of silica dust as in environments where mechanical processes like sandblasting, drilling etc are carried out. Acute silicosis is very often fatal due to the irreparable damage caused in alveolar lining cells from the accumulating surfactants, cell debris and proteinaceous matter.
- Accelerated silicosis: This form of silicosis is developed due to an exposure of high concentration of silica dust over a shorter period of time (~ 5 – 10 years). In this case, the process of inflammation, scarring, and development of symptoms occur much faster than in chronic silicosis.
- Asymptomatic silicosis: In this form of silicosis the early onset of the disease does not present any symptoms, thereby making it very difficult to diagnose.

Certain other outcomes of silica inhalation include an increased susceptibility to bacterial and fungal infections, an increase in the incidence of autoimmune diseases and lung cancer (bronchogenic carcinoma).

Methods to Characterize Silica Surfaces

When compared on the basis of equivalent weights or surface areas, toxicity of silica exceeds other non-fibrous mineral dusts¹⁵. Initially, a large number of theories based on the reactions of the silanol groups on the particle surface or hydrogen donation to form complexes with phospholipids in cell membranes, were formulated. These theories could not adequately explain the mechanism of toxicity in silica. The formation of silica free radicals on the particle surface is quite possibly the most important factor in the cytotoxicity of silica. The highest production of radicals occurs on a freshly fractured surface and with aging of the silica dust the radical formation decreases³¹. The toxicity effects of other surface properties like the charge, shape and surface area are yet to be extensively investigated.

In a recent study, to determine the crucial parameters that influence quartz toxicity²⁸, Warheit *et al* have shown that the toxicity behavior is dependent on surface characteristics in rats but not on particle size. Similarly, another critical analysis that considered all physicochemical characteristics like particle size,

specific surface areas, experimental uncertainties³²⁾ indicated a slight difference between the cytotoxicity of the three most common crystalline forms of silica (quartz, cristobalite and tridymite) when compared on the basis of equal number of particle number and surface area. The toxicity of crystalline silica can be markedly reduced by surface-modifications using a coating on the particle surface⁷⁾. Examples of coatings used for this purpose include: organosilane, surfactant, polyvinylpyridine-N-oxide (PVNO)^{12, 33, 34)} and a number of aluminum^{8, 12, 33)} compounds. Further details on the surface modification techniques to reduce the toxicity are described later.

A number of characterization techniques¹⁵⁾ have been developed to identify, quantify and make an attempt at correlating the measurable characteristics of crystalline silica particles to their cytotoxicity. These techniques include¹⁵⁾: electrophoretic mobility, thermally stimulated luminescence (TSL), electron spin resonance (ESR), composition profiling with energy dispersive X-Ray analysis (EDXA), laser microprobe mass analysis (LAMMA) and infrared spectroscopy (IR).

Interactions between Silica and Biological Molecules

The first step to establish the mechanism of action of any toxic material in the physiological environment is to determine the primary target cells that are injured or whose function gets altered. In this case, it has been well-established that the inhaled silica particles on reaching the terminal respiratory

tract and the alveolar space, activates the alveolar macrophages and an inflammatory response follows. The mechanism by which the alveolar macrophage participates in the inflammatory response, leading to fibrosis in the lung is still debatable.

At least two overall cellular mechanisms³⁵⁾ could be possible. Firstly, the alveolar macrophages may become cytotoxic after phagocytosis of the particles and release certain 'factors' that may alter or damage cells (e.g. epithelial cells) from the surrounding environment. It may also initiate an inflammatory response, which may become chronic due to the continuous release of the silica particles from the macrophage lysate followed by further engulfment by the macrophages to continue the lytic-cycle¹¹⁾. In the second model, the interaction between the silica particles and the alveolar macrophages is non-lytic. It may activate one or more intracellular signaling^{35, 36)} pathways that lead to the release of inflammatory 'mediators'. The two cellular models may not be mutually exclusive, i.e. one or both may be dominant at a given stage of inflammatory response.

For successful clearance, the macrophages engulf the silica particles and migrate to the mucociliary escalator to leave the lung with their particle-burden, while releasing certain inflammatory mediators in the process. Anything interfering with this chain of events, can lead to the phenomenon of *interstitialisation*¹⁷⁾. It is an unwanted outcome where the particles can no longer be cleared by the normal pathways and either remains back in the interstitium to chronically stimulate cells or transfer to the lymph nodes. This

Fig. 1 Schematic representing the uptake of quartz particles inside the lung, cleared through the normal pathway (left) and particles that are interstitialised (right). Reprinted from Reference 17 with permission from Elsevier.

has been schematically represented in **Fig. 1**¹⁷⁾. Generally, two events¹⁷⁾ can lead to the failed clearance of any particulate materials deposited in the lung: (a) Presence of toxic particles that may lead to macrophage toxicity (b) Particle Overload.

- (a) Presence of toxic particles – Presence of intrinsic toxic particles like quartz can stimulate the inflammatory mediators at a low dose and damage cells at higher doses, leading to acute and chronic inflammation¹⁷⁾.
- (b) Particle Overload¹⁷⁾ – If the number of respirable particles deposited in the lung is enormously high, due to high airborne mass concentrations, then the phenomenon of particle-overload occurs. Due to phagocytosis of a large volume of particles, the clearance ability of the macrophage gets affected. This leads to its inability to transport the large number of particles that gets deposited on the surface of the lung. The particles that remain left-behind start interacting with the epithelial cells and finally transfer across the epithelium (**Fig. 1**) and start accumulating in the interstitium. Continuous exposure under such conditions, shall lead to chronic inflammation, increase in the epithelial permeability, proliferation and clearance- retardation, fibrosis and finally cancer.

Effects of Physicochemical Parameters on Toxicity Behavior

Particle Size and Shape

Brown et al³⁷⁾ studied the particle size effect on toxicity of quartz in MET 5A mesothelial cells. The quartz particles used for the study varied between $0.8\ \mu\text{m}$ to $3\ \mu\text{m}$, consisting of three categories ultrafine particles (below $0.8\ \mu\text{m}$), fine particles ($0.8\ \mu\text{m}$ to $3\ \mu\text{m}$) and coarse particles (above $3\ \mu\text{m}$). It was reported that the toxicity behavior was highest for ultrafine particles and decreased with increase in particles size. In order to study the effect of particle agglomeration, the ultrafine particles were purposely agglomerated in cell-media to sizes above $3\ \mu\text{m}$. It was shown that the toxicity was reduced and became comparable to the coarse particles. An increase in percentage cytotoxicity values was observed with increasing particle concentration (25, 100 and $400\ \mu\text{g/ml}$) used for dosing the cells. The values of cytotoxicity for the different quartz particles at various particle concentrations, following 24 hour exposure, are shown in **Fig. 2**³⁷⁾.

Similar experiments were carried out using amor-

Fig. 2 LDH Values reported for quartz, after 24 hours exposure, with varying particle size and increasing particle concentration. Reprinted from Reference 37 with permission from Springer.

phous silica of two different shapes: silica rods and silica spheres. Although no significant differences in cytotoxicity was recognized in this case, the authors also report that this could be due to enhanced agglomeration in rod-shaped particles as compared to their sphere-shaped counterparts. It is generally agreed^{38, 39)} that the shape of various inhaled particles play a significant role to influence the toxicity. Thin and long fibers are typically more toxic and bio-persistent. Variations from the equiaxed to acicular-shaped structure lead to an increase in toxicity which could evolve due to various forms of mechanical aggravation to cells and difficulties encountered by the cells to phagocytose the particles in the lungs.

Surface Functionalities

The surface reactivity¹²⁾ of crystalline silica particles has been shown to affect its biological activity. The particle surface on encountering the lung lining fluids, biological molecules and cells interact to generate free radicals in several ways. The silanol groups (Si-OH) and the ionized silanol groups (Si-O) on the surface play a crucial role in the interaction between the particles and the membranes. The regular Si-O tetrahedral arrangement on the surface of the particle is interrupted if the quartz is comminuted, generating both homolytic and heterolytic cleavages of the SiO bonds that constitute the basic quartz structure. Homolytic cleavages generate the Si^* and Si-O^* radicals whereas heterolytic cleavage produces the charged Si^+ and Si-O^- groups. Inside the lung lining fluid or in the tissue fluid in the physiological environment, the products of homolytic cleavages give rise to reactive oxygen species (ROS) like hydroxyl radical ($\text{OH}^\bullet$) and hydrogen peroxide (H_2O_2) while

products of heterolytic cleavages interact with the membranes.

Presence of various common metals like iron, aluminum and copper may help to decrease the toxicity of quartz on one hand, but can also add to the oxidative stress by taking part as catalysts in Fenton Reactions on the other. Several investigators have demonstrated that aluminum salts present in minerals found together with quartz can lower quartz toxicity. Moreover, inhaling 300 mg/m³ aluminum hydroxide for 30 minutes per day for an exposure period of 12 months could successfully attenuate the fibrogenic response in rats. Similarly, in the sheep instillation model of silicosis, lungs of sheep instilled with quartz along with aluminum lactate were found to demonstrate reduced pathology scores and lessened cellular activity in the bronchoalveolar lavage (BAL). Presence of iron salts along with quartz has also been shown to reduce the toxicity in erythrocytes by protecting them against the quartz-mediated injury. However, the state of the metal is an important factor. For example, metallic iron has the inhibitory effects, but when present as ferrous or ferric salt contamination could lead to damage due to its role in assisting the ROS formation in Fenton Reaction. In that case, inhaling a mixture of freshly fractured quartz and iron will become more pathogenic than freshly fractured quartz all alone.

It is widely accepted that reactive oxygen species (ROS) and reactive nitrogen species (RNS) released from phagocytic cells are involved in inducing inflammation DNA damage and cancer. ROS and RNS generated due to exposure to quartz have long been implicated with lung diseases. Yamano *et al* reported an elevated level of the oxidative DNA lesions 8-hydroxydeoxyguanosine (8-OHdG) in the lung tissue obtained from rats exposed to quartz. Other investigators have found 8-OHdG induction more concentrated in the lung alveolar regions, and a possible role of isolated neutrophils in the induction of 8-OHdG in lung type II epithelial cells *in vitro*.

Surface Properties and Mechanism of Toxicity

Hydrogen Bonding – Hydrogen bonding has been implicated in inducing silica toxicity by several authors for a long time^{40, 41}. The silanol on the surface of the particle binds to macromolecules via H-bonding. The formation of the bond is determined by the matching between silanol group distribution on the surface and its matching to hydrogen molecule acceptor sites on the biomolecule. Macromolecules that bind

tightly onto the surface can mediate the toxicity in at least two ways: (a) May prevent any direct contact between the particle-surface and the biological molecules, thereby reducing the toxicity (b) May act to modify the surface, forming ‘non-self’ structures that act as antigens on which the immune system starts attacking. Thus there is enhanced toxicity in this case.

Surface Charges – Surface charges can be generated in different ways that could be mechanical and/or chemical¹⁴. The presence of charges on the surface of ground quartz dust has been observed from electron microscopic images. Generally, the positive charge on the quartz surface originates from mechanical treatments while low humidity atmospheres tend to increase the magnitude of charges¹². The tendency of the dust to stick tightly to the glass container can be reduced only by heating to very high temperatures (above 800°C). The silicon-oxygen bonds also get cleaved if the dust is ground¹². A heterolytic rupture on the dust results in the formation of a negative charge on the oxygen atom and a positive on silicon (**Fig. 3**). The presence of these charges can influence the adsorption of endogenous matter and contribute towards particle-cell adhesions.

Hydrophilicity /Hydrophobicity – The hydrophilicity of the surface contributes towards its membranolytic behavior⁴². The more hydrophobic a surface, less is its tendency to lead to cell membrane lysis. Moreover, hydrophobic moieties tend to get phagocytosed more readily by the macrophages and the process of phagocytosis is directly dependent on the relative hydrophobicity between the particle and the phagocyte in question. The surface of the crystalline silica particle contains patches of hydrophilic and hydrophobic areas¹². The silanol groups impart hydrophilicity while the siloxane groups contribute towards making the surface hydrophobic. The degree of hydrophilicity also differs between the different samples of quartz¹². For example, a completely hydrated silica surface is more hydrophilic while one which has been heated to higher temperatures (converts the silanes to siloxanes) becomes more hydrophobic^{43, 44}. Quartz and the other crystalline polymorphs of silica are more hydrophobic in comparison to its amorphous counterparts.

Freshly Fractured Surfaces⁴⁵ – Cleaving the silicon-oxygen bond, either by homolytic or a heterolytic cleavages, leads to the formation (**Fig. 3**) of dangling bonds^{12, 15} and surface charges¹⁵. These products are very reactive in nature and tend to recombine among themselves and form reactive bridges or react with

Fig. 3 Schematic to represent the formation of dangling bonds and surface charges from hemolytic and heterolytic cleavage of the silicon/oxygen bonds in crystalline silica particles. Figure taken from Reference 12 with permission.

the environment to form reactive oxygen species at the surface and in sub-surface layers. The potential reactions¹²⁾ that occur in the presence of oxygen due to reaction with quartz at the surface are:

If the process of grinding or cleaving the quartz surface is carried out in a liquid medium, the radicals generated depend on the reactivity between the solvent/ solute and the cleaved Si-O bonds¹²⁾. For example, presence of pure water prevents the formation of oxygen radicals whereas hydrogen peroxide tends to accelerate it and in some case induce the formation of hydroxyl radicals in parallel to the formation of other radicals.

Free Radical Release – It is hypothesized that the cytotoxicity and pathogenicity of silica dust is due to free radicals^{19, 46)} generated at the cleavage planes of silica, which make them more reactive to the lung tissues and thereby accelerates the onset of pulmonary diseases. In aqueous media, the Si[•] and Si-O[•] radicals formed on the quartz cleavage forms generate highly reactive hydroxyl radicals (OH[•]) which are detected and quantified using ESR spectroscopy¹⁵⁾, using a OH[•] spin trap, like DMPO (5,5 dimethyl-1-pyrroline-N-oxide)^{15, 47, 48)}. Many investigators have confirmed the release of OH[•] on the surface which can undergo a relatively fast decay to cause even greater damage⁴⁷⁾. This can be correlated to the acute lung injury caused in lungs of workers involved in mining, drilling activities etc. It is important to note that aged metal-free quartz

does not release any significant quantity amount of radicals. But as mentioned before, the presence of free metals, can lead to radical formation⁴⁷⁾ even on liquid-solid interfaces, that take part in Fenton-type reactions⁴⁸⁾.

Biological Targets of Particle-Cell Interactions

Certain biomolecular targets¹²⁾ that provide a simple and rapid approach to study the effect of crystalline silica on the cell membranes are:

- Membranolysis
- Induce Macrophage Cytotoxicity
- Lipid Peroxidation
- Free radical mediated DNA damage
- Free radical mediated Protein Modifications

- Membranolysis** – Recent investigations have shown that membranolysis is correlated to the number of silanol groups that occur on the surface of crystalline silica. On heating a cristobalite dust, there is a progressive condensation of silanol into siloxanes. On comparing the different samples, the fibrogenicity remained unchanged but there was a drastic increase in the retention of particles in the lung as well as particle uptake in cells for the sample heated to a high temperature. The site for membranolytic activity is the lysosomal membrane in macrophages, which on phagocytosis of silica particles form the phagolysosomes. The adsorption of proteins and lipids form a coating that can protect the outer cell membrane from the reactive sites on the particle surface. This coating is stripped off by the lysosomal enzymes during internalization, thus exposing the phagolysosomal membrane to the reactive particle-surface. The membrane is then lysed due to molecular interactions between the particle and membrane components.
- Induced cytotoxicity in macrophages:** It is important to study the cytotoxic effects of silica in macrophages, as they form the first line of defense mechanism and play an important role in the pathogenic process. Lactate dehydrogenase (LDH) release from macrophage cells is a good indicator of cytotoxicity, especially from crystalline polymorphs of silica. Several investigators have demonstrated the acute toxicity of quartz using % LDH as a scale. This test is extremely effective for quartz and there are various instances when quartz is used as a positive control⁴⁹⁾ for

LDH experiments on other particles.

- (c) **Lipid Peroxidation:** Lipid peroxidation^{47, 50} is the oxidative degradation of lipids in cell membranes in the presence of oxygen and transition metal ions or enzymes. It is an autocatalytic process by which free radicals extract electrons from the lipids in cell membranes resulting in cell damage. It has been established as a biomarker of cellular oxidative stress. The three stages of lipid peroxidation are: initiation, propagation and termination. Initiation consists of extraction of H atoms by ROS like $\text{OH}^\bullet$, from fatty acids in the cell membrane leaving behind an unpaired electron⁵¹. During propagation, the lipid radical combines with oxygen to form more radicals, leading to a series of autocatalytic chain reactions. The free radicals produced finally combine together during termination. Lipid peroxidation is quantified by measuring the breakdown products.
- (d) **Free-radical mediated DNA damage:** Generally, amino acid sequences of cellular proteins occur in the nuclear DNA (nDNA) or in the mitochondrial DNA (mtDNA) double helix. Although DNA is a very stable molecule, the mtDNA and nDNA can undergo gradual chemical decomposition over its lifetime. Indirect evidences have implicated that oxidative damage from ROS generation^{47, 50} in nDNA and mtDNA can lead to inflammation, neurodegenerative diseases, apoptosis, cancer and aging. ROS can cause base modifications (oxidation and deamination), base loss, single and double strand breaks, and cross-linking in DNA. $\text{O}_2^{\bullet -}$ and hydrogen peroxide (H_2O_2) do not react readily with DNA, but $\text{OH}^\bullet$ reacts readily with all 4 DNA bases. The ability of crystalline silica to cause DNA damage has been investigated using DNA strand breakage assay.
- (e) **Free-radical mediated Protein Modifications:** The main amino acid residues of oxidative degradations are histidine, proline, tryptophan, cysteine, tyrosine and to a lesser extent arginine, lysine and methionine⁵². $\text{OH}^\bullet$ is the main radical that initiates the radical chain reactions in proteins by extracting hydrogen atom⁵⁰. In addition to protein fragmentation, $\text{OH}^\bullet$ can also facilitate the generation of protein-protein cross-linkages and induce the formation of higher molecular weight compounds. Methods to detect and measure the oxidative damage in proteins include: protein carbonyl assays and amino acid analysis.

Surface-Modifications to Reduce the Toxic Potential of Silica

It has been noticed that modifying the quartz surface using polymeric coatings like PVPNO^{12, 33, 34}, aluminum salts^{8, 12, 33}, tetrandine and other surface functionalization techniques can reduce the toxicity of quartz. Moreover, thermal treatments have also been shown to lead to a general loss of biological activity.

- **Polymer Coating:** An effective inhibitor of quartz induced silicosis is PVPNO (poly-vinyl-pyridine-N-oxide)^{12, 33, 34}. The unique cytotoxicity of silica is attributed to the silanol groups ($-\text{SiOH}$) on the particulate surface which are good hydrogen donors and can easily form hydrogen bonds with various biological macromolecules that can efficiently accept hydrogen due to the presence of hydrogen acceptors like nitrogen or oxygen groups with lone pair electrons. So Schlupkotter⁵³ and collaborators postulated that it is possible to mask the $-\text{SiOH}$ groups on the particle surface by using hydrogen acceptor molecules like PVPNO. The coated silica would then be theoretically rendered non-toxic, thereby no longer being able to interact strongly with biological molecules. PVPNO, with a chemical name $(\text{C}_7\text{H}_7\text{NO})_n$ has a molecular weight ranging from 30,000 – 200,000 kDa and can effectively reduce the *in vitro* cytotoxicity of crystalline silica and its *in vivo* fibrogenicity.
- **Aluminum Salts:** It has been shown that the administration of aluminum salt^{8, 12, 33} can effectively reduce the adverse effects of crystalline silica. Haldane⁵⁴, in 1917, put forth that the non-fibrogenicity of some ‘quartz containing dusts’ could be due to the presence of certain clay silicates that help to reduce the toxicity. A couple of decades later, it was reported in the study of Denny *et al*⁵⁵ that inhalation of aluminum dusts can inhibit the pathogenic response of silica. The reduction in the solubility of quartz was demonstrated by a number of investigators^{12, 33} using aluminum powder and aluminum lactate⁵⁶⁻⁶⁰.
- **Tetrandine:** This chemical has been reported to retard and reverse the fibrotic lesions formed due to silicosis in rats⁶¹. Tetrandine has been in use in China for a long time as an ‘antifibrotic’ drug, although its mechanism of action is quite unclear. Current studies have indicated that it possesses anti-oxidant properties that can effectively inhibit lipid-peroxidation and act as a scavenger for hy-

droxyl and superoxide radicals inside the body⁶²⁾, thereby protecting the cells from oxidative damage.

○ *Thermal treatments*¹²⁾: As mentioned before, the silanol groups present on the surface of quartz are converted to siloxane bridges, when the structure is heated to high temperatures. Cristobalite samples were obtained by heating a sample of pure quartz dust to 800°C and found to be fibrogenic in property. But the same samples were non-fibrogenic when heated to 1300°C. Probably, heating to 800°C was not sufficient to eliminate all hydrophilic patches that exist on the surface of quartz¹²⁾. Loss of the pathogenic potential of silica occurred due to removal of all hydrophilic regions, when heated to 1300°C.

○ *Other surface functionalization techniques*¹²⁾: The surface properties of quartz can be engineered to attach certain organosilanes with terminal hydrophobic groups⁶³⁾. The modified surfaces show an improvement in the fibrogenic properties. This decrease in fibrogenicity has also been observed in p-amino groups. It is obvious that surface functionalization techniques cannot be used to prevent or treat silicosis but could help us to understand silica pathogenicity better.

Summary

Inhalation of crystalline silica has been shown to cause silicosis from results of epidemiological studies. Physicochemical factors associated with silica dust contribute towards the development of chronic inflammation that may subsequently lead to acute silicosis. The surface activity of crystalline silica particles can influence the generation of free radicals as well as affect particle uptake by macrophage cells in lungs. The undesirable effect of the generated free radical is cell-specific and depends on the form and concentration present in lungs. If not regulated properly, an excess of free radical can disrupt the normal cell functioning and continued exposure can result in pulmonary injuries. Surface modification of crystalline silica particles with polymer coatings, aluminum and tetrandine, thermal treatments as well as other functionalization techniques can reduce *in vitro* and *in vivo* toxicity.

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Reference

- 1) Rosen, G. "The History of Miner's Disease. A Medical and Social Interpretation." New York: Schuman, 1943, pp. 459-476.
- 2) Rovida, C.L. in *Annali di Chimica*, 1871.
- 3) Donaldson, K. and P. J. A. Borm, "The quartz hazard: A variable entity," *Annals of Occupational Hygiene*, vol. 42, pp. 287-294, 1998.
- 4) IARC, M. *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Silica, some silicates, coal dust and para-aramid fibrils*, vol. Vol 68. Geneva: IARC Press, 1997.
- 5) Craighead, J.E. "Diseases Associated with Exposure to Silica and Nonfibrous Silicate Minerals," *Archives of Pathology & Laboratory Medicine*, vol. 112, pp. 673-720, 1988.
- 6) Driscoll, K.E. and G. D. Guthrie, *Crystalline Silica and Silicosis*, vol. 8. New York: Elsevier, 1997.
- 7) Schins, R.P.F., R. Duffin, D. Hohr, A. M. Knaapen, T. M. Shi, C. Weishaupt, V. Stone, K. Donaldson, and P. J. A. Borm, "Surface modification of quartz inhibits toxicity, particle uptake, and oxidative DNA damage in human lung epithelial cells," *Chemical Research in Toxicology*, vol. 15, pp. 1166-1173, 2002.
- 8) Fubini, B., I. Fenoglio, R. Ceschino, M. Ghiazza, G. Martra, M. Tomatis, P. Borm, R. Schins, and J. Bruch, "Relationship between the state of the surface of four commercial quartz flours and their biological activity in vitro and in vivo," *International Journal of Hygiene and Environmental Health*, vol. 207, pp. 89-104, 2004.
- 9) Fubini, B., G. Zanetti, S. Altilla, R. Tiozzo, D. Lison, and U. Saffiotti, "Relationship between surface properties and cellular responses to crystalline silica: Studies with heat-treated cristobalite," *Chemical Research in Toxicology*, vol. 12, pp. 737-745, 1999.
- 10) Hemenway, D.R., M. P. Absher, B. Fubini, and V. Bolis, "What Is the Relationship between Hemolytic Potential and Fibrogenicity of Mineral Dusts," *Archives of Environmental Health*, vol. 48, pp. 343-347, 1993.
- 11) Fubini, B., "Surface chemistry and quartz hazard," *Annals of Occupational Hygiene*, vol. 42, pp. 521-530, 1998.
- 12) Legrand, A.P. "The Surface Properties of Silicas." New York: John Wiley and Sons, 1998, pp. Health Effects of Silica.
- 13) Fubini, B., V. Bolis, A. Cavenago, and M. Volante, "Physicochemical properties of crystalline silica dusts

- and their possible implication in various biological responses," *Scandinavian Journal of Work Environment & Health*, vol. 21, pp. 9-14, 1995.
- 14) Iler, R.K. "The Chemistry of Silica," John Wiley & Sons, 1979, pp. 15-29.
- 15) Bolsaitis, P.P. and W. E. Wallace, "The Structure of Silica Surfaces in Relation to Cytotoxicity," in *Silica and Silica-Induced Lung Diseases*, V. Castranova, V. Vallyathan, and W.E. Wallace, Eds.: CRC Press Inc, 1996, pp. 79-89.
- 16) Castranova, V., V. Vallyathan, D. M. Ramsey, J. L. McLaurin, D. Pack, S. Leonard, M. W. Barger, J. Y. C. Ma, N. S. Dalal, and A. Teass, "Augmentation of pulmonary reactions to quartz inhalation by trace amounts of iron-containing particles," *Environmental Health Perspectives*, vol. 105, pp. 1319-1324, 1997.
- 17) Donaldson, K., X. Y. Li, and W. Macnee, "Ultrafine (nanometre) particle mediated lung injury," *Journal of Aerosol Science*, vol. 29, pp. 553-560, 1998.
- 18) Albrecht, C., R. P. F. Schins, D. Hohr, A. Becker, T. M. Shi, A. M. Knaapen, and P. J. A. Borm, "Inflammatory time course after quartz instillation - Role of tumor necrosis factor-alpha and particle surface," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, pp. 292-301, 2004.
- 19) Donaldson, K. and C. L. Tran, "Inflammation caused by particles and fibers," *Inhalation Toxicology*, vol. 14, pp. 5-27, 2002.
- 20) Zhang, D.D., M. A. Hartsy, and D. B. Warheit, "Time course of quartz and TiO₂ particle-induced pulmonary inflammation and neutrophil apoptotic responses in rats," *Experimental Lung Research*, vol. 28, pp. 641-670, 2002.
- 21) Hetland, R.B., P. E. Schwarze, B. V. Johansen, T. Myran, N. Uthus, and M. Refsnes, "Silica-induced cytokine release from A549 cells: importance of surface area versus size," *Human & Experimental Toxicology*, vol. 20, pp. 46-55, 2001.
- 22) Fubini, B., E. Giamello, M. Volante, and V. Bolis, "Chemical functionalities at the silica surface determining its reactivity when inhaled. Formation and reactivity of surface radicals," *Toxicol Ind Health*, vol. 6, pp. 571-98, 1990.
- 23) Pozzolini, M., S. Scarfi, U. Benatti, and M. Giovine, "Interference in MTT cell viability assay in activated macrophage cell line," *Analytical Biochemistry*, vol. 313, pp. 338-341, 2003.
- 24) Driscoll, K.E. "In-Vitro Evaluation of Mineral Cytotoxicity and Inflammatory Activity," *Reviews in Mineralogy*, vol. 28, pp. 489-511, 1993.
- 25) Scarfi, S., U. Benatti, M. Pozzolini, E. Clavarino, C. Ferraris, M. Magnone, L. Valisano, and M. Giovine, "Ascorbic acid-pretreated quartz enhances cyclooxygenase-2 expression in RAW 264.7 murine macrophages," *Febs Journal*, vol. 274, pp. 60-73, 2007.
- 26) Barrett, E.G., C. Johnston, G. Oberdorster, and J. N. Finkelstein, "Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-alpha-induced oxidant stress," *American Journal of Physiology Lung Cellular and Molecular Physiology*, vol. 276, pp. L979-L988, 1999.
- 27) Barrett, E.G., C. Johnston, G. Oberdorster, and J. N. Finkelstein, "Silica binds serum proteins resulting in a shift of the dose-response for silica-induced chemokine expression in an alveolar type II cell line," *Toxicology and Applied Pharmacology*, vol. 161, pp. 111-122, 1999.
- 28) Warheit, D.B., T. R. Webb, V. L. Colvin, K. L. Reed, and C. M. Sayes, "Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics," *Toxicol Sci*, vol. 95, pp. 270-80, 2007.
- 29) Robock, K. *The Annals of Occupational Hygiene*, pp. 63-66, 1973.
- 30) Cavariani, F., A. DiPietro, M. Miceli, F. Forastiere, A. Biggeri, P. Scavalli, A. Petti, and P. Borgia, "Incidence of silicosis among ceramic workers in central Italy," *Scandinavian Journal of Work Environment & Health*, vol. 21, pp. 58-62, 1995.
- 31) Castranova, V., N.S. Dalal, and V. Vallyathan, "Role of Surface Free Radicals in the Pathogenicity of Silica," in *Silica and Silica-Induced Lung Diseases*, V. Castranova, V. Vallyathan, and W. W.E., Eds.: CRC Press, 1996.
- 32) Pandurangi, R.S., M. S. Seehra, B. L. Razzaboni, and P. Bolsaitis, "Surface and Bulk Infrared Modes of Crystalline and Amorphous Silica Particles - a Study of the Relation of Surface-Structure to Cytotoxicity of Respirable Silica," *Environmental Health Perspectives*, vol. 86, pp. 327-336, 1990.
- 33) Brown, G.E. and K. Donaldson, "Modulation of quartz toxicity by aluminum," in *Silica and Silica-Induced Lung Diseases*, V. Castranova, V. Vallyathan, and W. W.E., Eds.: CRC Press, 1996.
- 34) Castranova, V. "Suppression of cytotoxicity and fibrogenicity of Silica with PVPNO," in *Silica and Silica-Induced Lung Diseases*, V. Castranova, V. Vallyathan, and W. W.E., Eds.: CRC Press, 1996.
- 35) Jabbour, A.J., R. Iyer, R. K. Scheule, and A. Holian, "Potential Intracellular Messengers Involved in Silica Stimulation of Alveolar Macrophages," in *Silica and Silica-Induced Lung Diseases*, V. Castranova, V. Vallyathan, and W. W.E., Eds.: CRC Press, 1996.
- 36) Castranova, V. "Signaling pathways controlling the production of inflammatory mediators in response to crystalline silica exposure: Role of reactive oxygen/nitrogen species," *Free Radical Biology and Medicine*, vol. 37, pp. 916-925, 2004.
- 37) Brown, S.C., K. A. Mohammed, A. Banmuratov, P. Sharma, N. Nasreen, V. B. Antony, and B.M. Moudgil, "Influence of Shape, Adhesion and Simulated Lung Mechanics on Amorphous Silica Nanoparticle Toxicity," *Advanced Powder Technology*, vol. 18, pp. 69-79, 2007.
- 38) Vu V.T. and D. Y. Lai, "Approaches to characterizing human health risks of exposure to fibers," *Environmental Health Perspectives*, vol. 105, pp. 1329-1336, 1997.

- 39) Warheit, D.B., K. L. Reed, and T. R. Webb, "Man-made respirable-sized organic fibers: What do we know about their toxicological profiles?" *Industrial Health*, vol. 39, pp. 119-125, 2001.
- 40) Hobza, P., J. Sauer, C. Morgeneyer, J. Hurych, and R. Zahradnik, "Bonding Ability of Surface Sites on Silica and Their Effect on Hydrogen-Bonds - a Quantum-Chemical and Statistical Thermodynamic Treatment," *Journal of Physical Chemistry*, vol. 85, pp. 4061-4067, 1981.
- 41) Sauer, J., P. Ugliengo, E. Garrone, and V. R. Saunders, "Theoretical-Study of Van-Der-Waals Complexes at Surface Sites in Comparison with the Experiment," *Chemical Reviews*, vol. 94, pp. 2095-2160, 1994.
- 42) Bolis, V., B. Fubini, L. Marchese, G. Martra, and D. Costa, "Hydrophilic and Hydrophobic Sites on Dehydrated Crystalline and Amorphous Silicas," *Journal of the Chemical Society-Faraday Transactions*, vol. 87, pp. 497-505, 1991.
- 43) Fubini, B., V. Bolis, A. Cavenago, E. Garrone, and P. Ugliengo, "Structural and Induced Heterogeneity at the Surface of Some SiO₂ Polymorphs from the Enthalpy of Adsorption of Various Molecules," *Langmuir*, vol. 9, pp. 2712-2720, 1993.
- 44) Fubini, B., V. Bolis, A. Cavenago, and P. Ugliengo, "Ammonia and Water as Probes for the Surface Reactivity of Covalent Solids - Cristobalite and Silicon Carbide," *Journal of the Chemical Society-Faraday Transactions*, vol. 88, pp. 277-290, 1992.
- 45) Castranova, V., N. S. Dalal, and V. Vallyathan, "Role of Surface Free Radicals in the Pathogenicity of Silica," in *Silica and Silica-Induced Lung Injury*, V. Castranova, V. Vallyathan, and W. W.E., Eds.: CRC Press Inc, 1996.
- 46) Castranova, V. and V. Vallyathan, "Oxygen/Nitrogen Radicals and Silica-Induced Diseases," in *Oxygen/Nitrogen Radicals: Lung Injury and Diseases*, vol. 161-173, V. Castranova, V. Vallyathan, and X. Shi, Eds. New York: Marcel Dekker, Inc, 2004.
- 47) Halliwell, B. and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*. New York: Oxford University Press, 1999.
- 48) Kohen, R. and A. Nyska, "Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification," *Toxicol Pathol*, vol. 30, pp. 620-50, 2002.
- 49) Zhang, Q.W., Y. Kusaka, K. Sato, K. Nakakuki, N. Kohyama, and K. Donaldson, "Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: Role of free radicals," *Journal of Toxicology and Environmental Health-Part a-Current Issues*, vol. 53, pp. 423-438, 1998.
- 50) Wallace, K.B. *Free Radical Toxicology*: Taylor & Francis, 1997.
- 51) Parke, D.V. *Nutritional Antioxidants and Disease Prevention: Mechanisms of Action*. Oxon, UK: CAB International Publishing, 1999.
- 52) Roberfroid, M. and P. B. Calderon, *Free Radicals and Oxidation Phenomena in Biological Systems*. New York: Maecel Dekker Inc, 1995.
- 53) Schlipkoter, H.W. and A. Brockhaus, *Dtsch. Med. Wochenschr*, pp. 920-923, 1960.
- 54) Haldane, J.S. *Trans. Inst. Min. Engng.*, vol. 78, pp. 124-128, 1917.
- 55) Denny, J.J. W. D. Robson, and D. A. Irwin, "The Prevention of Silicosis by Metallic Aluminum," *Canadian Medical Association*, vol. 37, pp. 1-8, 1937.
- 56) Begin, R., S. Masse, M. Martel, L. Lebouffant, and H. Daniel, "Aluminothrapy after Initial Quartz-Induced Lung Injury," *American Review of Respiratory Disease*, vol. 133, pp. A196-a196, 1986.
- 57) Begin, R., S. Masse, M. Rolapleszczynski, M. Martel, Y. Desmarais, M. Geoffroy, L. Lebouffant, H. Daniel, and J. Martin, "Aluminum Lactate Treatment Alters the Lung Biological-Activity of Quartz," *Experimental Lung Research*, vol. 10, pp. 385-399, 1986.
- 58) Begin, R., S. Masse, P. Sebastien, M. Martel, M. Geoffroy, and J. Labbe, "Late Aluminum Therapy Reduces the Cellular Activities of Simple Silicosis in the Sheep Model," *Journal of Leukocyte Biology*, vol. 41, pp. 400-406, 1987.
- 59) Begin, R., F. Possmayer, M. A. Ormseth, M. Martel, A. Cantin, and S. Masse, "Effect of Aluminum Inhalation on Alveolar Phospholipid Profiles in Experimental Silicosis," *Lung*, vol. 167, pp. 107-115, 1989.
- 60) Dubois, F., R. Begin, A. Cantin, S. Masse, M. Martel, G. Bilodeau, A. Dufresne, G. Perreault, and P. Sebastien, "Aluminum Inhalation Reduces Silicosis in a Sheep Model," *American Review of Respiratory Disease*, vol. 137, pp. 1172-1179, 1988.
- 61) Liu, B.C., C. Q. Zou, and Y. R. Li, "Studies on the Contents of Glycosaminoglycans from Lungs of Silicotic Rats and Tetrandrine-Treated Silicotic Rats," *Ecotoxicology and Environmental Safety*, vol. 7, pp. 323-329, 1983.
- 62) Shi, X.L., Y. Mao, U. Saffiotti, L. Y. Wang, Y. Rojana-sakul, S. S. Leonard, and V. Vallyathan, "Antioxidant Activity of Tetrandrine and Its Inhibition of Quartz-Induced Lipid-Peroxidation," *Journal of Toxicology and Environmental Health*, vol. 46, pp. 233-248, 1995.
- 63) Wiessner, J.H., N. S. Mandel, P. G. Sohnle, A. Hasegawa, and G. S. Mandel, "The Effect of Chemical Modification of Quartz Surfaces on Particulate-Induced Pulmonary Inflammation and Fibrosis in the Mouse," *American Review of Respiratory Disease*, vol. 141, pp. 111-116, 1990.

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