CONCISE REVIEW ON PATHOPHYSIOLOGY AND ANIMAL MODELS OF SILICOSIS

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Abstract- **Silicosis is a type of pulmonary fibrosis, which is brought on by inhaling minute particles of silica, a common mineral present in quartz, sand, and many other kinds of rock. Workers exposed to silica dust in occupations like mining and construction are most susceptible to silicosis. Breathing difficulties may arise from lung scarring brought on by prolonged exposure to silica particles. Numerous animal models have already been created and used in research investigations to examine the pathophysiology and molecular underpinnings of the illness and to facilitate the search for therapeutics. There is an ongoing hunt for a better model because each experimental animal model that has been used thus far has pros and cons. Finding a better model will not only speed up basic research but also help with clinical issues and drug development. Rodent models are still a vital resource for researching human silicosis, despite certain anatomical and physiological variations. So, in this review, we can see a general insight into silicosis and various induction methods for modeling silicosis in rodents.**

Keywords: **Silicosis, Pulmonary fibrosis, quartz, Pro-inflammatory cytokines, TGF-β1.**

1. INTRODUCTION:

Inhaling respirable crystalline silica dust causes silicosis, a disease that eventually results in progressive, deadly lung fibrosis and inflammation. Respiratory dysfunction is linked to the physiologically active crystalline silicon dioxide (SiO_2) that results in the deposition of inorganic solid waste in the bronchi, lymph nodes, or pulmonary parenchyma.[1,2,3] There are two types of silica: crystalline and amorphous. Since there isn't much research indicating harmful health impacts, amorphous forms are typically regarded as having minimal toxicity. [4,5,6]

Despite the severe toxicity of silica crystals inhaled to lung tissue, humans are unlikely to suffer major health issues from a single, brief exposure. Normally, crystalline particles of varying sizes are inhaled together, while larger particles can be trapped and removed by natural defenses via coughing, sneezing, and mucus production mechanisms. Unfortunately, tiny crystalline particles can build in the lung without producing obvious discomfort, eventually leading to disease.[4,7]

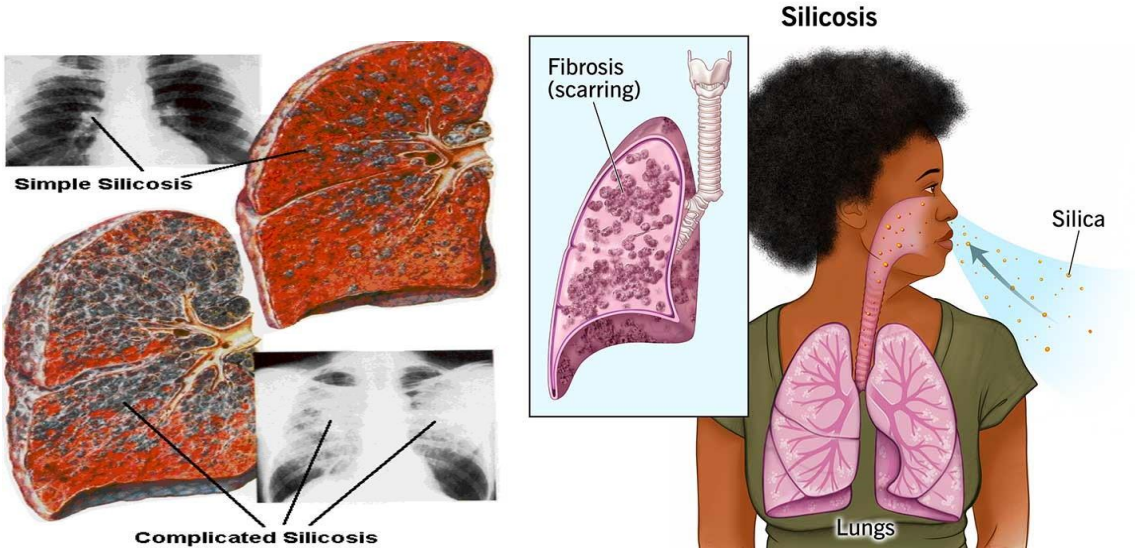

Figure 1. Silicosis

Millions of workers worldwide, particularly in developing countries, are still exposed to silica despite efforts are made to limit this exposure. silicosis poses a serious risk to workers in the construction and mining industries, especially to younger workers.[8-10] Although radiologic findings and occupational history are used to diagnose silicosis, X-rays are not a reliable method of identifying the disease in its early stages.[11,12]

Based on factors like the length of exposure, onset of symptoms, lung function, and results from chest imaging, there are four kinds of silicosis.[4,13,14] They are,

1. Simple Chronic silicosis
2. Complicated Chronic silicosis
3. Accelerated Silicosis
4. Acute silicosis

1.1 SIMPLE CHRONIC SILICOSIS:

Develops after 10–30 years from initial exposure and usually the consequence of prolonged exposure (10 years or longer) to comparatively low amounts of silica dust. And this is the most prevalent form of silicosis. Individuals with this kind of silicosis, particularly in the early stages, might not exhibit overt symptoms, but x-rays could reveal abnormalities. Common observations include exertional dyspnea (shortness of breath) and a persistent cough. On radiography, a large number of tiny (less than 10 mm in diameter) opacities, usually rounded and primarily located in the upper lung zones, are indicative of chronic uncomplicated silicosis.[15,16]

1.2 COMPLICATED CHRONIC SILICOSIS:

The development of severe scarring (progressive massive fibrosis, or conglomerate silicosis), in which the small nodules progressively become confluent and reach a size of 1 cm or higher, can make silicosis "complicated". Compared to a mild illness, PMF (Progressive Massive Fibrosis) is linked to more severe symptoms and respiratory impairment. Other lung conditions include tuberculosis, non-tuberculous mycobacterial infections, fungal infections, some autoimmune illnesses, and lung cancer can also exacerbate silicosis. Compared to the chronic form, accelerated silicosis is more likely to cause complicated silicosis.[15,16,17]

1.3 ACCELERATED SILICOSIS:

Similar to Chronic simple silicosis, develops five to ten years after initial exposure to greater amounts of silica dust. Symptoms appear earlier and tend to worsen more quickly. PMF and other complex diseases are more common in patients with accelerated silicosis.[14,15,17]

1.4 ACUTE SILICOSIS:

A few weeks to five years after being exposed to high amounts of respirable silica dust, silicosis develops. Another name for this type is silicoproteinosis. Acute silicosis symptoms include more quickly developing & severe incapacitating dyspnea, coughing, weakness, and weight loss, which frequently results in mortality. A diffuse alveolar filling with air bronchograms, also referred to as a "ground-glass appearance" on x-rays, is typically seen. This filling is comparable to pneumonia, pulmonary edema, alveolar hemorrhage, and alveolar cell lung cancer.[14-17]

2. PATHOPHYSIOLOGY OF SILICOSIS:

When minute silica dust particles are inhaled, they penetrate deeply into the tiny alveolar sacs and ducts in the lungs. The activation of alveolar macrophages in response to foreign material produces a large amount of reactive oxygen species (ROS), which in turn directly damages the lung parenchyma surrounding it.[1,7]

Cellular damage and increased expression of inducible Nitric Oxide Synthase (iNOS) in the bronchoalveolar results in the release of Pro-inflammatory cytokines such as IL-1β, IL-4 and TNF-α, and augmentation of cell signaling pathways. When these factors come together, lung tissue experiences aberrant collagen deposition and fibroblast proliferation.[12-14]

The fibrotic process is significantly triggered by oxygen-based free radicals produced by silica exposure, and the chronic inflammation that results plays a crucial part in the pathophysiology of Silicosis.[1,19,20]

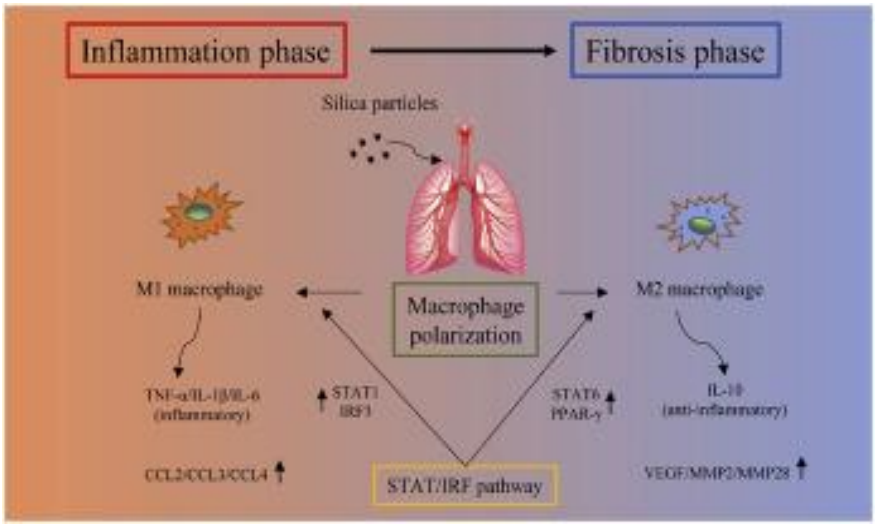

Figure 2. Pathophysiology of silicosis

Numerous cellular cytokines, such as platelet-derived growth factor (PDGF), transforming growth factor-beta1 (TGF-β1), and connective tissue growth factor (CTGF), have been implicated in the development of fibrosis in different organs, according to earlier research.[21-23]

Among them, the transforming growth factor β1 (TGF-β1) plays a crucial role in fibrogenesis resulting in the fibrosis by upsetting the microenvironment of homeostasis and promoting cell invasion, migration, differentiation, or hyperplasia primarily via the TGF-β1/Smad signaling pathway. [1,14]

It has been proven that oxidative stress is a harmful factor that is linked to profibrogenic actions of TGF-β1. It is clear that there is a definite link between TGF-β1 and oxidative stress. TGF-1-induced fibrosis is related to an increase in ROS-producing enzymes and/or a decrease in ROS-scavenging enzymes. The Nrf2/ARE signaling pathway has been revealed to be involved in the dynamic mechanism of fibrosis formation in these situations.[1,24]

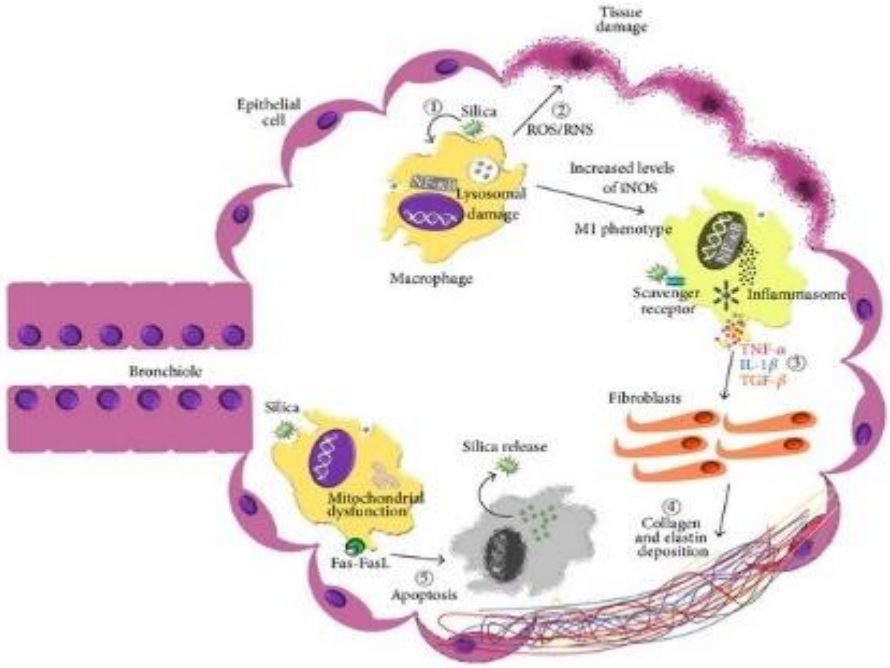

Figure 3. Pathophysiology of silicosis

On repeated silica exposure and ingestion, alveolar macrophages undergo necrosis, autophagy, and release of nondegraded intracellular silica. As a result, more macrophages are drawn in, resulting in the production of even more cytotoxic oxidants and proteases, inflammatory cytokines, and arachidonic metabolites. This vicious cycle continues to exacerbate alveolar inflammation and fibrosis.[15,25]

The activation and recruitment of type II pneumocytes and fibroblasts results in the continuous production of excess collagen and fibronectin. These alterations result in reduced gas-exchange surfaces and lung parenchymal deformation. [15,16,26]

3. EPIDEMEOLOGY:

Workers in a multitude of sectors and vocations may be at risk for silica dust exposure. Work in high-risk industries includes rock extraction, road maintenance, concrete manufacture, coal mining, and bricklaying.[8-10]

The Occupational Safety and Health Administration reports that over two million workers are regularly exposed to respirable silicon dioxide in one form or another. Over the past few decades, increased knowledge and workplace safety precautions have significantly reduced the incidence of silicosis; yet precautions are still insufficient, and new occurrences of the disease continue to occur.[10,25]

4. HISTOPATHOLOGY:

The presence of silicotic nodules is a characteristic of nodular silicosis. On the outside, silicotic nodules are spherical, hard, and distinct lesions with varying amounts of black pigment. The nodules usually appear in the subpleural and Para septal regions, around the respiratory bronchioles and tiny pulmonary arteries. Obliteration of pulmonary arteries and tiny airways results from progressive expansion. The latter are identified by hyalinized collagen bundles stacked concentrically and encircled by varying numbers of histiocytes loaded with dust.[25,27,28]

Fibroblasts and histiocytes with acellular collagen lamellae are noticeable in early nodules. Nodules frequently contain small, polarizable, doubly refractile, round or oval particles. Although they are not unique to silicosis and may occasionally be absent, their presence aids in the confirmation of the diagnosis.[25,30]

It is possible to see granulomatous reaction in the silicotic nodule capsule. Its existence increases the likelihood that mycobacterial infection will coexist. Necrosis in the center is unusual. Although dust macules and pigment-laden macrophages are typically observed around small airways, the surrounding lung may be unremarkable. Patients with persistent silicosis have occasionally been observed to have a nonspecific kind of interstitial fibrosis.[25,29]

Microscopic observations in acute silicosis resemble pulmonary alveolar proteinosis. In contrast to typical alveolar proteinosis, there is typically a varied quantity of pigment, interstitial inflammation and fibrosis, or uneven hyaline scars. Most of the time, silicotic nodules are either nonexistent or poorly developed. [29,30]

A microscopic examination can be useful in identifying some problems, such as non-TB infections and developing severe fibrosis or tuberculosis. A nodular fibrosis more than one centimeter in size is a hallmark of progressive enormous fibrosis. Usually, the lung parenchyma is obliterated and contracts due to a massive, amorphous mass of fibrous tissue made up of conglomerated nodules. This lesion can also be seen in other Pneumoconiosis, such as mixed dust fibrosis, asbestosis, and pneumoconiosis in coal miners.[25,29,30]

5. ETIOLOGY OF SILICOSIS:

One of the most common minerals in the earth's crust, silica, can be found in a range of natural environments. Silicosis is usually caused by breathing in respirable crystalline silica particles, usually quartz. The workers most at risk are those who transport or blast rock and sand (miners, quarry workers, stonecutters, and construction workers) or who use silica-containing rock or sand abrasives (sand blasters, glass makers, foundry, gemstone, and ceramic workers, potters). There have also been reports of severe silicosis outbreaks among workers in the manufactured stone industry. [10,11]

The frequency and severity of silicosis are influenced by the following factors:

- ❖ The duration and intensity of exposure,
- ❖ The form and surface characteristics of silica particles,
- ❖ The non-crystalline form of silica, known as amorphous silica, is present in glass and diatomaceous earth and does not cause silicosis.

6. SIGNS & SYMPTOMS: [31-33]

- Years of exposure is typically required for silicosis symptoms to manifest. The early stages are characterized by modest symptoms like as coughing up phlegm and gradually becoming dyspnoeic.
- Once the lung scarring has become more severe, a number of symptoms may manifest.
- An abnormal chest X-ray and a slowly emerging cough may be the first serious signs of a problem as the scarring worsens.
- Breathing difficulties, shortness of breath, and a persistent cough are typical signs of bronchitis.
- Other symptoms include weakness, exhaustion, fever, sweats at night, swelling in the legs, and bluish discolouration of the lips.
- Patients with silicosis are susceptible to acquiring renal illness, COPD, lung cancer, and tuberculosis because the disease compromises the immune system.

7. COMPLICATIONS:

Patients with silicosis are at risk of other disorders:[31]

- Tuberculosis (TB)
- Chronic obstructive pulmonary disease (COPD)
- Lung cancer
- Systemic rheumatic (autoimmune) diseases

Because of their weakened lung immunity, workers with silicosis—both radiological and sub-radiological—are more likely to contract silico-tuberculosis, a form of tuberculosis. Simultaneous occurrence of TB and silicosis may obscure silicosis shadows on chest radiographs with tuberculosis shadows, making identification more challenging. Additionally, a study found that a larger percentage of silicosis patients (20 out of 40) had multidrug-resistant pulmonary tuberculosis than did non-silicosis patients. [35,36]

According to the ICMR-NIOH study, silicotic lung damage can also be categorized as mild, moderate, or severe based on the lung damage scoring (LDS) of chest radiography and the blood level of CC16 that correlates with it. It is recommended that workers with a history of silica dust exposure employ serum CC16 for screening purposes in order to detect silicosis early on, based on the aforementioned findings and pertinent prior research. Furthermore, if silicotic lung damage is identified with serum CC16, additional screening with CB-NAAT/True-NAAT would identify silico-tuberculosis early on, which is a condition that should not be disregarded. In India, prioritising the control of silicosis is imperative due to the significant burden of both sub-radiological and silicosis.[11,34]

Co-occurring silicosis raises the risk of treatment failure, treatment cessation, death, relapse, and drug resistance in tuberculosis patients, according to another article which is published in the year 2023 article. Therefore, if India's goal is to "eliminate TB by 2025," then controlling silicosis is crucial.

8. DIAGNOSIS OF SILICOSIS:

8.1 IMAGING TESTS:[4,15,37]

- These consist of high-resolution Computed Tomography (CT) scans and chest X-rays.
- A chest x-ray will establish the existence of tiny (< 10 mm) lung nodules, particularly in the upper lung zones, in cases of simple silicosis.
- A computed tomography scan, or CT scan, can also identify cavitation from a concurrent mycobacterial infection and offer a more thorough examination of the lungs.

8.2 LUNG FUNCTION TESTS: [15,16,38]

- These examinations gauge the lung's capacity for healthy breathing and blood oxygen uptake. Diffusion capacity and spirometry are the two different tests used to make these measurements. They are also employed in assessing the extent of lung injury.
- Results from a pulmonary function test could indicate restricted deficiencies, decreased diffusion capacity, airflow limitation, mixed defects, or even normal results (particularly in the absence of a complex illness).

8.3 SPUTUM TEST: [31]

- Collecting coughed up mucus for evaluation.

8.4 BRONCHOSCOPY:[31,40]

- A bronchoscope, which is a tiny, flexible tube with a video camera attached at the end, will be inserted into the lung and windpipe through the mouth or nose. This instrument can be used to obtain lung tissue samples for additional analysis.

8.5 LUNG BIOPSY:[15,37]

- This test is seldom involves taking a tiny sample of tissue from your lungs.
- Tissue biopsies are not usually required for the diagnosis of silicosis, but they might be in some circumstances, mainly to rule out other illnesses.

8.6 LABORATORY TESTS:[31]

- These tests might be performed to rule out other illnesses, such as certain infections. A skin test for tuberculosis may be part of this. There is no laboratory test to confirm a possible case of silicosis.

8.7 BRONCHOALVEOLAR LAVAGE: [4,31,39]

- In this test, the lungs are "washed out," and the extracted fluid is examined.

8.8 PREVENTIVE MEASURES:[31,41-43]

- Reducing or eliminating exposure is the first step in primary preventative actions.
- A thorough exposure management program should be implemented in addition to respiratory masks, as they offer only a restricted level of protection.
- Early disease detection can be facilitated by secondary prevention using medical surveillance.
- Vaccinations against influenza, COVID, and pneumococci are preventive strategies to prevent consequences.

- Clinicians need to be aware of the danger of nontuberculous mycobacterial infections and tuberculosis in patients exposed to silica, particularly miners.
- Annual interferon-gamma release assay or tuberculin skin tests should be performed on workers exposed to silica.
- In 2016, the Occupational Safety and Health Administration (OSHA) released a revised Respirable Silica Standard in response to the continued prevalence of silicosis.
- The standard mandates pre-employment and ongoing medical surveillance of workers exposed to silica, which includes chest x-rays, lung function testing, and questionnaires. It also reduces the permissible exposure limit (PEL).

9. INDUCTION OF SILICOSIS IN RODENTS BY VARIOUS METHODS/ROUTES:

An animal model is a nonhuman species that is studied in detail to shed light on biological processes or characteristics in the hopes that the knowledge gleaned from this organism will aid in the understanding of the behaviour of other people. Today, all human disease types can be replicated in animals using animal models, which facilitates both the search for new treatment targets and a better understanding of the underlying pathological and physical processes.[4,44]
Despite the fact that silicosis is a lung disease that affects many different species, not all of them can be used as model organisms in lab experiments to mimic the disease because of problems related to handling, manipulation, and/or genetic modification.[4]
The features of the disease under study, as well as the socio-economic and administrative capabilities of the experimental unit, influence the choice of experimental animal. The utilization of the murine model has increased recently especially male animals are frequently used in research because it has been demonstrated that gender affects both the development of fibrosis and the degree of lung injury.[4,45,46]
Furthermore, research has demonstrated a clear link between the severity of silicotic lung illness and the duration and dosage of exposure to silica crystalline dust and the degree of silicotic lung illness is determined by the amount of material injected, the type of silica, the exposure method, whether the animal is exposed once or repeatedly, and its genetic susceptibility. [4,47-50]

9.1 INTRATRACHEAL INSTILLATION

This procedure requires highly competent and qualified professionals to do the experimentation because it is invasive and has a high failure rate involving the animals. The animal should be given a suitable anaesthetic prior to intubation, and the degree of anaesthesia in mice should be greater than that of given in oropharyngeal instillation. After that, the animal needs to be restrained at an angle and placed on a clean and disinfectant wood plate. The animal's neck should be cleaned of all hair, and the ventral side of the neck should be slightly incised. Subsequently, the cannula is inserted into the tracheal lumen and between the vocal cords. The animal's mouth is widely opened, and a direct light source can offer illumination. Through the use of a catheter, inserted into the trachea, the appropriate volume of silica is administered. Throughout the entire process, close attention should be paid to the rhythm and pace of breathing. Following injection, the animal should be raised to a 45-degree angle for 30 seconds before being given the opportunity to recuperate. With this technique, a substance is quickly delivered to the lungs; however, the distribution of silica across the left and right lungs is not equal. [14,51-54]

9.2 OROPHARYNGEAL ASPIRATION

The animals were placed on a board that was 60 degrees incline and supported by elastic bands after being moderately sedated with an appropriate anaesthetic medication. To view the base of the tongue and the pharynx, the nares were squeezed with curved forceps, and the tongue was carefully removed from the mouth with blunt forceps. Next, using a micro pipettor, the silica suspension was placed on the posterior pharynx. Before the tongue and nares were freed, breathing was observed to make sure the suspension had been completely aspirated. Once the animal had recovered from anaesthesia, it was put back in its own cage. During the duration of the investigation, the animals were observed every day.[4,52,55]

9.3 INTRANASAL INSTILLATION

Compared to intratracheal or oropharyngeal silica instillations, this route is the simplest for giving particle material, such as silica dust, but it results in a less severe sickness. The crystalline silica is administered intranasally once or twice at a predetermined interval after the animals have been given the proper anaesthesia. Between nostril injections and during the treatment, the animal is closely observed for indicators of distress, such as excessive struggle, colour changes in the mucous membranes, and changes in breathing. After that, the rat is placed back in its cage and checked for any instantly noticeable symptoms of distress.[4,8,56,57]

9.4 NASAL DROPS

It is a non-intrusive technique. Dropping the liquid on one side of the alar while keeping the mouse's breathing rate constant during anaesthesia is the most important stage in this procedure. The animal is trained to inhale and exhale at the same time, and the silica dispersion is added one drop at a time until the desired volume is attained.[4,58]

9.5 INHALATION METHOD

For two hours, the silica was broken up in an agate jar, and then it was baked at 180ºC for six hours to remove any endotoxins. The animals were housed in an inhalation chamber and, for 2, 4, 8, 12, 16, and 24 weeks, they were exposed to 50±10 g/m3 of silica for three hours each day. Temperature and humidity ranges of 20–25ºC, -50 to +50 Pa of pressure, 20% oxygen content, and 3.0–3.5 mL/min SiO_2 mixture flow rate was all maintained in the chamber atmospheres. Every two weeks, the device's SiO_2 concentration was measured to maintain it. [4,50,59,60]

CONCLUSION:

There is currently no treatment for silicosis lung disease that can change how the illness progresses or undo lung damage. Reducing and managing exposure to SiO2 dust is one of the best ways to prevent crystalline silica lung illness. Basic understanding of the biology of the illness and the molecular underpinning of its etiology is necessary for the development of pharmacological treatments.

Intratracheal instillation and oropharyngeal aspiration require highly skilled and qualified professionals to perform the experimentation because they are invasive and have a high failure rate involving the animals. In contrast, intranasal instillation is the simplest method of all the inducing methods for silicosis, but it results in a less severe sickness. The inhalation approach most closely resembles silica exposure in humans. Nevertheless, it necessitates specialized tools, substantial quantities of potentially useful material, and repeated exposures over extended periods of time.

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