Animal models of silicosis: fishing for new therapeutic targets and treatments

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Shareable abstract (@ERSpublications)
This review discusses the use of zebrafish as a model of silicosis and its similarities to other animal models, as well as the current state of the art of inflammatory and fibrotic zebrafish models that could be used in silicosis research. https://bit.ly/3XgdDgU

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Abstract

Silicosis as an occupational lung disease has been present in our lives for centuries. Research studies have already developed and implemented many animal models to study the pathogenesis and molecular basis of the disease and enabled the search for treatments. As all experimental animal models used to date have their advantages and disadvantages, there is a continuous search for a better model, which will not only accelerate basic research, but also contribute to clinical aspects and drug development. We review here, for the first time, the main animal models developed to date to study silicosis and the unique advantages of the zebrafish model that make it an optimal complement to other models. Among the main advantages of zebrafish for modelling human diseases are its ease of husbandry, low maintenance cost, external fertilisation and development, its transparency from early life, and its amenability to chemical and genetic screening. We discuss the use of zebrafish as a model of silicosis, its similarities to other animal models and the characteristics of patients at molecular and clinical levels, and show the current state of the art of inflammatory and fibrotic zebrafish models that could be used in silicosis research.

Silicosis and silica crystals

Silicosis is an irreversible occupational lung disease caused by intense and prolonged inhalation of silica dust with crystals up to 10 µm in diameter in various industries, such as cutting, crushing or grinding. It belongs to the group of interstitial lung diseases called pneumoconiosis [1]. Crystallised silicon dioxide (SiO_2), which is part of silica dust, is biologically active and the resulting deposition of inorganic solid waste in the bronchi, lymph nodes or pulmonary parenchyma is associated with respiratory dysfunction [2]. SiO_2 is found in nature in different forms of quartz as a crystalline deposit. It is also one of the most abundant elements in Earth's crust, accounting for 59%, and is the main constituent of over 95% of all rocks [3]. Silica can occur in two forms, namely crystalline and noncrystalline (amorphous) [4]. Amorphous forms are generally considered to have low toxicity, as there are few studies suggesting adverse health effects [4]. Although inhaled silica crystals are extremely toxic to lung tissue, brief or one-time exposure is unlikely to cause significant health problems in humans [5]. Normally, crystalline particles of different sizes are inhaled together, although larger particles can be captured and expelled by natural defences through the mechanisms of coughing, sneezing and mucus production. Unfortunately, smaller crystalline particles can remain in the lung and accumulate without causing immediate discomfort, ultimately leading to disease [6].

Silicosis is one of the oldest known causes of lung disease, characterised by lung dysfunction, persistent pulmonary inflammation, formation of silicotic nodules and irreversible pulmonary fibrosis leading to respiratory failure and eventually death, with more than 12 900 deaths worldwide in 2019 [5, 7]. The prevalence and incidence of this disease have been rising, especially in developing countries, although it is

also a high-profile occupational health problem in developed countries [8, 9]. The duration and intensity of exposure to crystalline silica dust are not the only determinants of its pathogenicity; host factors may also influence the susceptibility of an individual. Smaller particle size may increase the fibrogenicity of the dust. Depending on the severity of the disease, its onset and its speed of progression, silicosis is classified into chronic silicosis, accelerated silicosis, complicated silicosis and acute silicosis. The most common form is chronic silicosis, which occurs after 20 years of exposure to silica dust and is characterised by the presence of silicotic nodules that first develop in the hilar lymph nodes and then in the lungs, reaching 3 mm in diameter [10]. Each nodule has a well-demarcated central area of whorled hyalinised collagen fibres, with aggregates of dust-laden macrophages at the periphery. Over time, these lung nodules become confluent, erasing the intervening normal lung parenchyma and resulting in progressive massive fibrosis.

Accelerated silicosis occurs after 10 years of silica dust exposure and, although the changes are similar to those present in chronic silicosis, the clinical features appear sooner and the nodules develop earlier and are more cellular than fibrotic [11]. Finally, acute silicosis is characterised by different pathology from chronic silicosis, as it shows severe alveolitis and alveolar proteinosis. In addition, alveolar architecture may be preserved in this case, but with minimal fibrosis and rare small silicotic nodules [12, 13]. Patients with acute silicosis may become disabled within months of exposure.

In the early stages of the disease there may be no symptoms, meaning that the patient may be asymptomatic, with no pain and normal spirometric test results, but in the more advanced form of silicosis the person may tire easily with any kind of effort due to breathing difficulties and these symptoms may not appear until years after exposure. Over time, silica dust causes fibrous alterations in the lungs, decreasing respiratory capacity and loss of lung elasticity [14]. If the disease reaches the progressive massive fibrosis stage, exertional dyspnoea may occur [15].

The association between silicosis and tuberculosis has been known for a long time and patients with silicosis show a susceptibility to mycobacterial infections, called silicotuberculosis. The threefold increased risk of incidence is still not well understood. A possible reason may be damage to alveolar macrophages caused by silica crystals, which leads to inhibition of their ability to kill mycobacteria. Even prolonged exposure of silica, without causing silicosis, similarly increases the risk of tuberculosis [16, 17]. In addition to higher susceptibility to mycobacterial infections, patients with silicosis may present chronic bronchitis and airflow limitation, nontuberculous *Mycobacterium* infection, fungal lung infection, compensatory emphysema and pneumothorax as a pulmonary complication [18]. In addition, an association between silicosis and some autoimmune diseases, such as nephritis, scleroderma and systemic lupus erythematosus, especially in acute or accelerated silicosis, has been demonstrated [19]. Finally, in 1996, the International Agency for Research on Cancer classified silica crystals as a group 1 carcinogen with a strong potential to cause lung cancer in humans [20, 21].

To be diagnosed with silicosis, three key elements must be met. Initially, patient history should reveal exposure to enough silica dust to cause the disease. Next, characteristic radiological features such as chest imaging are necessary to reveal findings compatible with silicosis, usually by radiography or high-resolution computed tomography. There are standard approaches to the recognition and description of chest radiographic changes used in clinical practice. A widely accepted scheme was published by the International Labour Office, which provides a stepwise method for assessment and description of the shape, size, location and profusion of opacities that may have resulted from dust exposure; the patient's radiographs are classified after comparison with standard radiographs [22]. Finally, other conditions such as tuberculosis, sarcoidosis and histoplasmosis need to be excluded in order to determine that there are no other underlying diseases that are more likely to be causing the abnormalities [23]. Lung biopsy is rarely necessary but may be required to distinguish between progressive massive fibrosis and lung cancer. Physical examination adds nothing new but is a standard procedure. Although spirometry has most often been used as the method of first choice to assess pulmonary alterations in patients exposed to silica, it has limited sensitivity in detecting abnormalities before extensive damage occurs [24]. In addition, assessment of alveolar silica crystal load in bronchoalveolar lavage fluid may aid diagnosis.

Apart from the existing direct diagnosis of the disease, the validation of biomarkers by assessing their ability to indicate exposure, effect, disease or susceptibility has been proposed [25]. A large number of biomarkers in plasma or urine with potential to detect silicosis lung disease have already been investigated, including, among others, club cell protein 16 [26], heme oxygenase 1 [27], interleukin-6 [28], copper and zinc [29], selenium [30], and nephonectin [31]. However, it has not yet been possible to establish a single biomarker with the best potential, although it is very clear that there is a need to identify biomarkers to understand the pathogenesis of silicosis and the biological mechanisms underlying its progression.

Recently, exhaled breath condensate (EBC) has been used to assess the respiratory health of silicosis patients. The EBC is collected and then analysed for volatile and nonvolatile macromolecules, including oxidative stress, inflammatory derived biomarkers and other organic compounds [32, 33]. Interestingly, it has been shown that crystalline silica particles can be detected in the EBC of exposed patients [34]. EBC assessment is a noninvasive and highly accurate technique, making it an attractive option for the early detection of silicosis.

Treatment for silicosis

As silicosis is a chronic disease, there is no cure and once the damage is done it cannot be reversed [15]. Currently, the treatment options available are very limited and focus solely on relieving symptoms, preventing further progress of the disease and improving general condition, as well as reducing the risks of associated disorders. These include cessation of exposure to silica dust, cough suppressants, antibiotics for bacterial lung infection, chest physiotherapy to aid bronchial drainage of mucus, oxygen administration to treat hypoxaemia, bronchodilators to facilitate breathing and many others. Unfortunately, to date, pharmacological approaches have not shown a beneficial effect in halting or reversing the progression of fibrosis. The antifibrotic drugs pirfenidone and nintedanib, both currently in phase 2 of clinical trials for silicosis, are used in other fibrotic lung diseases such as idiopathic pulmonary fibrosis and are known to be effective in a wide range of progressive fibrotic lung diseases, by downregulating the production of growth factors and procollagens I and II, or inhibiting both nonreceptor tyrosine kinases (nRTKs) and receptor tyrosine kinases (RTKs), respectively [35–38]. Whole-lung lavage to remove retained dust is being investigated as a therapy for people with moderate-to-severe silicosis [39, 40]. In addition, lung transplantation, which essentially involves replacing damaged lung tissue, may be the most effective treatment. It has already been performed in some patients with advanced severe silicosis, but it is a limited resource. It is associated with serious risks inherent to lung transplant surgery, availability of ideal donor match, the consequences of long-term immunosuppression as well as the high cost of the whole procedure [41].

Several novel experimental approaches have been explored for pulmonary silicosis. Most of them consist of suppressing inflammasome activation, reducing oxidative stress by antioxidants, limiting caspase-1-driven inflammation, restoring the function of the autophagy–lysosomal system or blocking pro-inflammatory cytokines or pro-fibrotic factors [41, 42]. In addition, treatment with microRNA [43, 44], mesenchymal stem cells [45–47] or extracellular vesicles from mesenchymal stem cells [48, 49] has also shown favourable effects. Improvement of silicosis-dependent lung fibrosis has also been observed with agents increasing cyclic adenosine monophosphate (cAMP) or guanosine monophosphate (cGMP), such as inhibitors of phosphodiesterases [50, 51] or for corticosteroids [52–54]. As the importance of metabolic reprogramming of arachidonic acid, especially its derivatives prostaglandin D2 and thromboxane A2, in the progression of silicosis has been demonstrated, blocking its receptors by ramatroban may be a novel potential therapeutic therapy to inhibit the progression of silicosis [55]. In addition, several experimental studies have demonstrated a wide potential of natural plant-based compounds that may influence several of the pathological mechanisms of silicosis and thus may mitigate silica-induced inflammation as well as the process of fibrosis (table 1) [56–64].

Several methods are used to prevent exposure to respirable silica crystals and avoid the development of silicosis. First, the implementation of appropriate dust control measures in work environments is essential for prevention of the disease. Next, crystalline dust should be captured at the point of generation by enclosure and extracted by an extractor through ducts to suitable bag filters. In addition to ventilation, water-integrated tools should be used. The use of personal protective equipment as well as personal respirators that provide adequate respiratory protection must be used continuously to ensure protection at all times [65].

Animal models of silicosis

An animal model is a nonhuman species that is widely studied to understand biological process or characteristics, with the expectation that the findings obtained in this organism will allow understanding the action of other individuals. At present, animal models are widely used for the reproduction of all different types of human diseases, allowing the deeper study of physical and pathological mechanisms and the search for new therapies and treatments. Silicosis can occur spontaneously in nature and many cases have been reported in numerous mammalian species such as pigs [66], dogs [67], camels [68], horses [69, 70], nonhuman primates [71], river otters [72] and marsupials [73]. It has also been found in various species of birds like ground dwelling kiwi (*Apteryx* spp.) [73, 74]. Unfortunately, although this lung disease is common in many different species, not all of them can be used as experimental organisms to model silicosis in the laboratory because of difficulties in handling, manipulation and/or genetic modification, among others.

TABLE 1 Plant-based compounds used in different animal models of silicosis

Plant-based compound	Mechanism of action	Animal model	References
Sodium tanshinone IIA sulfonate	Reduction in collagen deposition in the lung and reduced ROS by decreasing malondialdehyde production	Rat	[57]
Kaempferol	Modulated silica-induced autophagy and significantly inhibited pulmonary inflammation as well as inhibited silica-induced pulmonary fibrosis	Murine	[58]
Astragaloside IV	Reduction of the expression of fibrotic markers	Rat	[59]
Dioscin	Anti-inflammatory and anti-fibrotic effects through alleviating the apoptosis of alveolar macrophages and promoting autophagy, reduced macrophage and lymphocyte lung infiltration and secretion, and prevented fibroblast activation and mitigation	Murine	[60, 61]
Oleanolic acid	Attenuated silica-induced fibrosis and reduced oxidative stress in the lungs	Rat	[62]
Hesperetin	Reduced alveolitis and pulmonary fibrosis, decreased levels of malondialdehyde, increased activity of antioxidant enzymes and total antioxidant capacity, inhibited synthesis and secretion of TGF-β1, decreased levels of pro-inflammatory cytokines, and elevated levels of anti-inflammatory factors	Rat	[63]
Emodin	Improved pulmonary function and reduced deposition of collagen I and α-SMA in the lungs, and reduced degree of alveolitis and inhibited apoptosis	Murine	[64]

ROS: reactive oxygen species; α-SMA: α-smooth muscle actin; TGF-β1: transforming growth factor β1.

There are many different animal models used for their resemblance to human lung silicosis, as summarised in table 2; however, recently, the murine model has been widely used. The choice of experimental animal depends on the characteristics of the disease to be studied as well as the economical and organisational capacity of the experimental unit. Importantly, it has been shown that gender influences the severity of lung injury as well as the development of fibrosis, which is why male animals are often chosen for the experiments whenever possible [75, 76]. In addition, studies have shown a direct correlation between the dose and time of exposure to the silica crystalline dust and the severity of the silicotic lung disease [77, 78]. In addition, the presentation and severity of the disease has been shown to depend on the genetic predisposition of the experimental animal chosen for the study [79, 80]. Furthermore, it is important to bear in mind while performing an experiment that the artificial induction of silicosis will also depend on the form of silica and the route of exposure, as well as whether the administration of crystals is single or repeated in the experimental animal [5, 81–83]. It has also been found that the volume of material instilled can influence the severity of the disease, with animals receiving the same dose in a larger volume showing more diffuse inflammation and more severe pathology [84–86]. Finally, when the method of silica administration requires a liquid carrier, PBS is often used in experiments as a control [84, 87] and, when administered in a gaseous state, pure air is used [88].

Zebrafish models of silicosis

In 1995, the World Health Organization initiated a campaign with the goal of eliminating silicosis from the world by 2030; however, unfortunately, over the years, silicosis remains a major health problem internationally [89]. Therefore, there is a continuous need to generate new tools as well as to implement new experimental animal models for the study of lung silicosis.

Since 1960, zebrafish (*Danio rerio*) has emerged as an experimental model organism to study vertebrate developmental biology, cancer, toxicology, drug discovery, molecular genetics and, most importantly, it is nowadays widely used in biomedicine to model many different human diseases [90]. As a small tropical freshwater fish, it is ideal for use as an experimental model in the laboratory, as it ensures ease and low cost of husbandry. In addition, its physical and developmental characteristics as a vertebrate and the development and function of zebrafish organs closely resemble those of humans. There is a long list of the advantages of using zebrafish to model lung silicosis *in vivo*. One very important advantage is that adult female fish can produce up to 200–300 offspring per week, which makes them very suitable for high-throughput genetic studies with high efficiency and robustness. What differentiates them from mammalian models is that their embryonic development is external and the larvae are completely transparent without any feeding requirements for up to 5 days post-fertilisation [91]. During the first weeks of life, all major organ systems are formed and start to function, which can be observed under low-power microscopy due to their transparency [92]. Zebrafish embryos and larvae are relatively small, reaching millimetres in length, allowing hundreds of individuals to fit into a single Petri dish. This makes them more economically profitable at a reasonable cost than maintaining bigger experimental animals that

TABLE 2 Animal models of silicosis

Animal model	Type of SiO_2 administration	Procedure/characteristics	References
Mouse	Transoral/oropharyngeal instillation	A drop of liquid is placed in the mouth of the mouse while simultaneously holding its tongue to inhibit the swallowing reflex and pinching its nose shut, forcing the mouse to breathe through its mouth and aspirating the silica containing liquid. This route of instillation distributes the silica crystals evenly and more homogenously than other surgical techniques and shows greater penetration of the silica crystals, with less variability and more pronounced alveolitis. In addition, this method is easier to learn, is less stressful for the animals and more reproducible. Silica-treated mice show pre- and post-fibrotic lung stages of silica injury, as well as lung inflammation with increased levels of cytokines. Severe alveolitis, perivasculitis and peribronchitis and high numbers of inflammatory cell infiltration in bronchoalveolar lavage fluid are the main aspects in this model. Transoral instillation of silica crystals shows a widely distributed pathology with numerous small fibrotic lesions extending to the peripheral and distal regions of the lung.	[82, 136]
Mouse	Intratracheal instillation	This method is technically challenging and requires a high degree of technical skill with a long training period beforehand and, as it is invasive, the animals can suffer a high failure rate. It requires instilling the liquid containing silica crystals directly into the lumen of the trachea. This requires the use of a catheter or needle, or surgical exposure of the trachea, which may be referred to as transtracheal instillation. Mice subjected to this technique need to undergo deeper anaesthesia compared to transoral instillations. The nonsurgical catheter method does not have the potential issues of animal recovery after surgery and the complication of inflammation, but can only be performed in species where the mouth can be opened wide enough to see the vocal cords. This method of silica instillation allows reproducible delivery of material to the lungs in a very short time and avoids exposure to skin or fur, which is an issue with whole-body inhalation. Unfortunately, in this method there is no uniform distribution of silica between the right and left lungs, which is especially important for protocols where both lungs are processed for different analysis. This model has shown significant inflammatory cell infiltration and severe lung parenchymal destruction in lung tissues, as well as diffuse pulmonary fibrosis and fibrous nodules. In addition, the results confirmed an increased expression of fibrosis markers such as fibronectin, collagen I and α-SMA, which was corroborated by histopathological changes in silica exposed mice.	[85, 137]
Mouse	Intranasal instillations	This route is the easiest method of administering particulate material such as silica dust but produces less severe disease than intratracheal or oropharyngeal silica instillations. Previously anesthetised mice are instilled intranasally with crystalline silica once or twice at a set time interval. Histopathological analysis of the silica-treated mice revealed profound inflammatory cellular changes and granulomas, as well as increased collagen deposition. In addition, the results confirmed the cytotoxic effect of silica and increased inflammation.	[138, 139]
Mouse	Nasal drops	It is a noninvasive method. The most crucial step in this process is to drop the liquid on one side of the alar while maintaining the mouse's respiratory rate during anaesthesia. As soon as the mouse is at the end of its exhalation and beginning its inhalation, the silica suspension is dropped one at a time until a target volume is reached. Silica administration *via* nasal drops caused weight loss with reduced locomotor activity in mice, induced dynamic histopathological changes and fibrosis with developmental time after 1 month. Damaged lung epithelial cells, pulmonary oedema and an increase of inflammatory cells in the lung of silica-treated mice were determined in relation to the control. In this model. silica crystals are found in macrophages under a polarising microscope, confirming the human pathology.	[140]
Mouse	Inhalation	This method best models human exposure to silica. However, it requires specialised equipment, large amounts of potentially valuable material and repeated exposures over long periods of time. In mice administered silica by inhalation, severe lung lesions and inflammation with infiltration of macrophages and polymorphonuclear neutrophils, as well as interstitial fibrosis and granulomas, were detected after a certain latency period, despite strong surface reactivity.	[141]

Continued

TABLE 2 Continued

Animal model	Type of SiO_2 administration	Procedure/characteristics	References
Mouse	Intravenous	Silica dust can be administered intravenously in the murine experimental model, showing the effect of the crystals on the liver, characterised by structural abnormalities, accumulations of mononuclear cells, presence of silicotic nodules and high collagen production.	[142]
Rat	Intratracheal instillation	A catheter with a special adapter or laryngoscope was inserted into the trachea and then a suspension of silicon dioxide dust and air was perfused into the trachea or bronchi. The rat was then immediately rotated to evenly distribute the injection into the lungs. Neutrophils, macrophages and lymphocytes significantly increased in silicosis-treated animals. Pulmonary inflammatory response along with inflammatory factors as well as oxidative stress markers were elevated in rats with silicosis. The lung structure was destroyed with increased cell proliferation and infiltration, a transparent silicotic nodule, thickened interstitial substance and fibroplasias. In addition, fibrosis was observed after silica instillation with increased collagen I, fibronectin and α-SMA.	[143, 144]
Rat	Inhalation	A HOPE MED 8050 exposure control device can be used as a noninvasive instrument consisting of a cabinet in which specific concentrations of dust can be set for inhalation by the experimental animals even for several weeks. As a result, inhalation of silica crystals promoted macrophage activation, myofibroblast differentiation and collagen deposition. In addition, sick animals showed silicotic lesions, inflammatory alveoli and enlarged lymph nodes.	[88, 145]
Guinea pig	Inhalation	The animals were exposed to SiO_2 aerosol by inhalation for 8 h a day for 5 days a week for 3 weeks. Although inhalation exposure does not allow for a precise determination of the dose level administered to each individual, it certainly represents a more normal route of exposure. This model showed lung cell damage combined with inflammation and signs of fibrosis.	[146]
Guinea pig	Intratracheal instillation	Diffuse alveolar septal infiltration with interstitial fibrosis and granulomatous infiltration with nodular fibrosis were observed in the tissues studied. In addition, mixed inflammatory exudate in the alveolar interstitium consisting of histiocytes, neutrophils, eosinophils and lymphocytes was observed in silica-treated animals. Total lung collagen and collagen synthesis increased in the lung tissue of experimental animals after silica exposure.	[147]
Rabbit	Inhalation	This method consists of exposing the animals to silica dust in cloud form to inhale the crystals. The dusting mechanism consisted of a cylinder of silica dust on a floating platform, the height of which could be changed by adding or removing water. The dust was removed from the cylinder by suction and injected into the room with compressed air. The rabbits did not show severe silicotic characteristics, as their respiratory rate is much lower than that of rodents, meaning that they would inhale far fewer silica crystals over the same period of time.	[148, 149]
Rabbit	Intratracheal instillation	The histological picture resembled that observed in other models, namely, phagocytosis of the silica, breaking down of the monocytes, fibroblast invasion and early area fibrous tissue reaction.	[150]
Rabbit	Intravenous	The study used crystals suspension of very finely divided and thoroughly washed silicotic hyaline tissue from human lungs, which caused pulmonary inflammation and fibrotic lesions in the rabbit model.	[151, 152]
Hamster	Intratracheal instillation	Silica dust suspension was instilled intratracheally into the hamster lungs. The diseased animals showed pathogenic effects with increased polymorphonuclear leukocytes, intra-alveolar haemorrhage and increased biochemical markers of inflammation. Silica exposure resulted in elevated levels of cytoplasmic and lysosomal enzymes, pulmonary oedema and increased cell numbers in lung lavage. In addition, macrophage phagocytosis was inhibited. In the hamster silicosis model, lung lesions showed a diffuse consolidation of alveolar tissue with few nodules. Silica crystals were not only concentrated in the nodules, but also diffusely distributed in the consolidated alveoli. Interestingly, although the stroma was argyrophyllic, it did not change into collagen because of lung inflammation, a pattern like that observed in lymph nodes. The different reaction in this model is thought to be due to less effective alveolar clearance and different tissue reactivity.	[153, 154]

Continued

TABLE 2 Continued

Animal model	Type of SiO_2 administration	Procedure/characteristics	References
Nonhuman primate	Inhalation	The study of the pathophysiology of silicosis was carried out in *Macacus cynomolgus*. The reasons for choosing this monkey for the experimental procedures were the assurance that this animal can undergo repeated lung lavages and it is possible to follow the course of events over a long period of time and, in case of availability of only a small number of animals, the monkey model has a very close phylogenetic relationship to humans. Exposure to inhalation of respirable dust of silica crystals in the form of natural quartz was performed 4 h·day^{-1}, 5 days·week^{-1} for 18 weeks. Monkey chest radiographs showed changes as early as 21 weeks into the experiment, suggesting a significant reaction to dust inhalation. In addition, lung autopsy revealed a massive fibrotic reaction, infiltration of inflammatory cells and the presence of microabscesses. The lesions obtained in the monkey animal model were similar to those commonly described in patients with silicosis, such as silicotic nodules or granulomas with inflammatory cells.	[71]
Sheep	Nasotracheal instillation	Silica exposures can be performed after nasotracheal intubation, with repeated slow infusions of the suspension into the trachea at intervals of several days. Lung lavage of silicotic sheep showed alveolitis with characteristics of cytotoxicity, lung matrix damage and fibrogenicity. The disease reproduced in the sheep model had the basic characteristics of human simple nodular silicosis and showed abnormal chest radiography. In addition, it was characterised by changes in lung lavage consistent with silica-induced alveolitis. Sheep with silicosis had significantly reduced and restrictive lung functions with altered and increased cellularity, phospholipids and hyaluronan.	[155, 156]
Sheep	Bronchoscopic catheterisation of the trachea	This method consists of slow infusion of the suspension into the trachea at several day intervals for 18 months. This multiple intratracheal instillation was shown to lead to a more uniform distribution of particles in the peripheral airspace of the lung. This model showed abnormal chest radiographs, decreased lung capacity, as well as increases in macrophages, lymphocytes and neutrophils in lung lavage after silica exposure.	[157, 158]
Dog	Intratracheal instillation	Dogs were rarely used to model lung silicosis, as they are reputed to have a low susceptibility to silica crystalline dust. Over a period of more than 3 years, animals were given intratracheal injections of SiO_2 in quartz form. The lesions present in silica-treated dogs were comparable to those seen in humans. Dust-laden phagocytes, frank silicotic nodules or even pseudotumours were present in the diseased animals. The gravity and severity of the disease and the silica-dependent lesions were due to the total amount of crystals received by the animal, the effect being intensified if applied in several doses.	[159, 160]
Yucatan miniature pig	Bronchoscopic catheter into right cranial lobe	Silica crystals were delivered into the right cranial lobe through the right apical bronchus *via* a bronchoscopic guided catheter to induce silicosis in this animal model. This disease model was characterised by pulmonary structural changes typical for patients with silicosis, which showed extensive remodelling accentuated by fibrosis and inflammation.	[161]
Zebrafish	Hindbrain injection	This method consists of direct injection of silica crystals into the hindbrain ventricle of 2 days post-fertilisation zebrafish larvae. For details see the main text of this review.	[95]

α-SMA: α-smooth muscle actin.

require more space and resources. Importantly, genetic conservation between zebrafish and humans is very high, as they share 70% of all genes and 84% of all human disease genes [93]. The zebrafish genome is fully sequenced and allows for easy genetic modification. Many new genome-editing technologies have emerged and are used for creating new transgenic and mutant lines. However, as a nonmammalian species, zebrafish also have certain disadvantages for modelling human diseases. Most importantly, fish lack some mammalian organs, such as lungs. Hence, modelling lung diseases may seem impossible in this model, but fortunately it has been overcome. For example, the hindbrain ventricle situated in the back of the zebrafish head, a cavity into which immune cells can be easily recruited, mimics the alveolar environment of the human lung. It has already been used to model mycobacterial infections, coronavirus disease 2019 and silicosis in zebrafish [94–97]. In addition, gene duplication can lead to gene subfunctionalisation and neofunctionalisation, and phenotypic characteristics of diseases caused by ortholog genes may be different in zebrafish models.

Hindbrain crystalline silica dust injection

The zebrafish model of silicosis has been established by injecting crystalline silica dust resuspended in sterile PBS into the hindbrain of zebrafish larvae. This neuroepithelium-lined cavity has been used to mimic the alveolar environment that can be found in human lungs and, as a convenient injection site, allows the study of the interaction between silica crystals and host immune cells (figure 1). Normally, the hindbrain lacks macrophages and can be used to assess the recruitment of those cells to the injection site. In the case of neutrophils, although they may be present in the cavity, additional stimuli are able to enforce neutrophil recruitment as well. Because of the rapid development of zebrafish larvae and changes in larval physiology, which basically include shrinking of the hindbrain ventricle over time as well as increased rigidity of the larval epithelium, needle penetration becomes difficult or even impossible. The optimal time for this procedure is around 30–48 h post-fertilisation in zebrafish larvae. In detail, at that age zebrafish larvae need to be dechorionated, anesthetised and placed on a Petri plate to be pulled by the suction device, which is a microprobe called a VAMP (Vacuum-Assisted MicroProbe) [98]. One by one, the larvae can be immobilised and the crystalline silica dust can be injected several times into the hindbrain until the

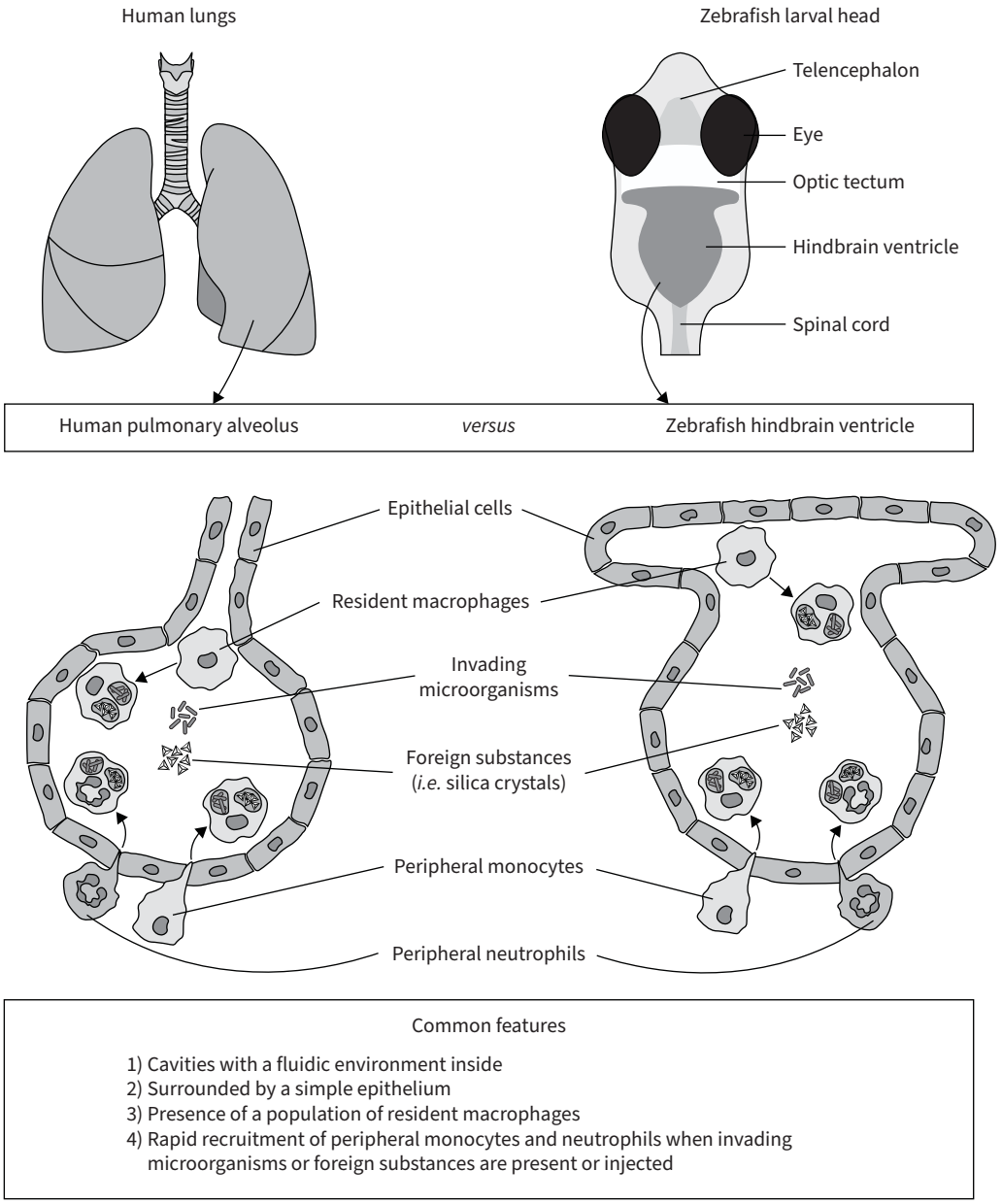

FIGURE 1 Similarities between human pulmonary alveolus and zebrafish hindbrain ventricle.

cavity is filled. Hindbrain injection can be done by directly injecting the needle into the cavity (however, this can generate a hole through which the injected liquid and crystals can escape) or it can be performed by penetration through the forebrain [95].

The zebrafish model of silicosis has shown similar characteristics at the molecular level to those seen in different experimental animal models, as well as in silicotic patients. SiO_2 crystals were able to induce both local and systemic inflammation in zebrafish [95]. The crystals induced significant recruitment of neutrophils and macrophages at the injection site, demonstrating that they can be recognised by the zebrafish immune system. The C-X-C motif chemokine ligand 8/C-X-C motif chemokine receptor 2 axis has been shown to be essential for the spreading of the inflammatory response induced by silica crystals. In addition, induction of proinflammatory cytokines and M1 polarisation of macrophages after silica injection was also observed in the experimental zebrafish model. This model has also revealed that silica crystals activate Toll-like receptor and Nlrp3-inflammasome signalling pathways in zebrafish. Moreover, neutrophilia and monocytosis were found in larvae injected with silica crystals, suggesting that SiO_2 crystals can activate emergency myelopoiesis. Interestingly, two signalling pathways have been shown to be activated by silica crystals in zebrafish: a Caspa/Gsdme (caspase a/gasdermin e) axis that regulates myeloid cell pyroptosis and inflammation, and a Caspa/Gata1 (gata-binding protein 1) axis that promotes emergency myelopoesis [95].

Fibrotic processes also appear to be present in the zebrafish model of silicosis as a result of silica crystal injection, which start after resolution of inflammation. Thus, 5 days post-injection of silica crystals, the fibrotic markers *tgfb1*, *acta2* (smooth muscle actin alpha 2), *col1a1a* (type I collagen alpha 1a), *col1a2* (type I collagen alpha 2) and *fn1a* (fibronectin 1a) were significantly elevated at the site of injection, as were some (*acta2*, *col1a2* and *fn1a*) in the whole body [95]. In addition, a histological study could be performed on silica injected fish, thus adding information about the pathophysiological mechanisms of fibrosis in this model.

Another interesting observation is the lower motility of the larvae injected with silica dust [95]. They remained in the centre of the Petri dish, in contrast to the control larvae, which were very motile and always explored the edges of the dish. In addition, the silica-injected fish showed an impaired escape response to a tactile stimulus, whereas the control larvae instantly reacted by swimming rapidly away when touched on the tail.

It should be noticed that silicotuberculosis can also be easily modelled in zebrafish by combining the already well-established mycobacterial infection model in zebrafish [94] with the new model of silicosis in zebrafish [95].

Inflammatory zebrafish transgenic reporter lines

The zebrafish model of silicosis generated by injecting silica dust into the hindbrain of zebrafish larvae can be used not only in wild-type fish, but also in many different transgenic or mutant lines, which may help to decipher the mechanisms orchestrating the pathology and constitute a unique platform for chemical screening in the future. An important characteristic of zebrafish as an experimental model is the wide variety of transgenic lines expressing fluorescent genes driven by a lineage-specific promoter, which allows real-time imaging of pathophysiological processes *in vivo* [99]. Of interest for the study of silicosis and its pathophysiology has been the development of stable transgenic markers for macrophages and leukocytes, as well as pro-inflammatory markers that allow simultaneous and real-time fluorescent detection of the processes occurring during the development of the disease. Here, we summarise the main representative zebrafish transgenic lines labelling different cell populations or molecules that can be used for characterisation of inflammatory events in silicosis (table 3).

The contribution of immune cells to the development of silicosis can be easily assessed. To study neutrophil recruitment, their total number or dispersion after silica crystal injection, the transgenic lines *Tg (mpx:eGFP)* and *Tg(lyz:dsRED)* have been generated, in which neutrophils express the fluorescent proteins eGFP or dsRED, respectively, under the control of the specific promoter myeloperoxidase (*mpx*) or lysozyme (*lyz*) promoters [100, 101]. Similarly, zebrafish transgenic lines *Tg(mpeg1:eGFP)* and *Tg(mpeg1:mCherry)* with the promoter of the macrophage-specific marker *mpeg1* (macrophage-expressed gene 1) [102] can be used to study macrophage recruitment or their total number in response to SiO_2 as a stimulus. From 3 days post-fertilisation, the downregulation of endogenous *mpeg1* gene expression in zebrafish was observed and an additional long-term transgenic line with fluorescent macrophages was generated. Thus, the *mfap4* (microfibril-associated protein 4) promoter has been used as a macrophage-specific marker leading to long-term expression of the red fluorescent protein mCherry in the line *Tg(mfap4:mCherry-F)* [103]. In addition, two other transgenic lines driving eGFP expression in macrophages have been

TABLE 3 Zebrafish transgenic lines useful for studying silicosis

Zebrafish transgenic line	Cell type/molecule labelled	Process	References
Tg(mpx:GFP)	Neutrophils	Inflammation	[100]
Tg(lyz:dsRED)	Neutrophils	Inflammation	[101]
Tg(mpeg1:EGFP) Tg(mpeg1:mCherry)	Macrophages	Inflammation	[102]
Tg(mfap4:mCherry-F)	Macrophages	Inflammation	[103]
Tg(irg1:eGFP)	Macrophages	Inflammation	[104]
Tg(fms:GAL4.VP16)	Macrophages	Inflammation	[105]
Tg(coro1a:eGFP)	Neutrophils and macrophages	Inflammation	[106]
Tg(lck:lck-eGFP)	T-cells	Inflammation	[107]
Tg(NFkB:eGFP)	NF-κB	Inflammation	[108]
Tg(il1b:GFP-F)	IL-1β	Inflammation	[109]
Tg(tnfa:eGFP-F)	TNF-α	Inflammation	[110]
Tg(acta2:eGFP)	Acta2	Fibrosis	[111]
Tg(krt19:col1a2-GFP)	Col1a2	Fibrosis	[112]

IL-1β: interleukin 1-β; TNF-α: tumour necrosis factor-α.

generated, although they are not as widely used as the previous ones. The first uses the promoter of the immunoresponsive gene 1 (*irg1*), which labels macrophages only after activation *Tg(irg1:eGFP)* [104]. The other, *Tg(fms:eGFP)*, uses a bacterial artificial chromosome transgene with the promoter of the zebrafish *fms* gene, encoding the macrophage colony stimulating factor 1 receptor, to label macrophages and skin xanthophores with eGFP [105]. Interestingly, the transgenic line *Tg(coro1a:eGFP)*, with eGFP driven by the promoter of coronin 1a, is expressed in a full repertoire of myeloid cells, as it is highly enriched in leukocytes [106]. In addition, the zebrafish transgenic line *Tg(lck:eGFP)* can be used to assess the role of T-cells in the pathogenesis of silicosis by expressing eGFP under the control of the T-cell-specific tyrosine kinase (lck) promoter [107].

The production of inflammatory markers can also be tracked *in vivo* by fluorescent proteins. To date, several transgenic reporter lines have been generated using the promoters of pro-inflammatory molecules that drive the expression of fluorescent proteins. The nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) is considered a master regulator of inflammation and immune responses. The NF-κB reporter system *in vivo*, consisting of the expression of the fluorescent protein eGFP under the promoter of the master transcription factor NF-κB, can serve as a useful tool to monitor temporal and spatial patterns of activation to stimuli [108]. In fact, this line has been successfully used to demonstrate the induction of NF-κB following silica crystal injection [95].

The pro-inflammatory cytokine interleukin-1β (IL-1β) is a key mediator of the inflammatory response and can provide an important information about the inflammatory status of the studied process. A *Tg(il1b:GFP-F)* zebrafish line has been generated, in which the expression of farnesylated GFP, which is membrane-targeted, is driven by the *il1b* promoter. It has been used to study the spatiotemporal behaviour of the cells that produce the *il1b* transcripts in resting conditions and after stimulus [109]. Similarly to *Tg(NFkB:eGFP)*, *Tg(il1b:GFP-F)* has been used to show that *il1b* increases locally after silica dust injection [95].

Another very useful line is the *Tg(tnfa:eGFP-F)* line, in which the expression of eGFP is driven after *tnfa* gene promoter stimuli [110]. This line can be used to track the polarisation of macrophages into M1 *in vivo* and to track $Tnfa^{+}$ cells upon stimuli [110]. In particular, silica crystal injection into the hindbrain of larvae caused M1 polarisation of macrophages [95].

Fibrosis zebrafish transgenic reporter lines

Zebrafish transgenic lines used to study the process of fibrosis *in vivo* have been widely used. The promoter of the zebrafish fibrosis marker *acta2* has been used to drive the expression of the green and red fluorescent proteins *Tg(acta2:eGFP)* and *Tg(acta2:mCherry)*, resulting in the labelling of vascular mural cells and visceral smooth muscle cells [111]. Another useful line for modelling fibrosis in zebrafish models of silicosis is *Tg(krt19:col1a2-GFP)*, which express Col1a2 fused to GFP under the promoter *krt19*, driving expression in the basal layer of epidermal cells, which are cells previously reported to be responsible for deposition of skin collagen I in early stages of zebrafish development [112]. The expression of Col1a2 is restricted to one cell type in this model and fluorescence became apparent within the skin at 2 days post-fertilisation and increased in intensity until 5 days post-fertilisation, being first observed in the dorsal

region of the head. GFP–collagen was detected intracellularly in occasional epidermal cells, although it was also apparent as extracellular wispy fibrils adjacent to these cells [112]. Finally, other zebrafish lines could be designed to drive expression of the fluorescent protein with the promoters of other markers of fibrosis, such as Col1a1a, Col1a2, Fn1 and others. These transgenic lines could be used to assess this pathological process *in vivo* by and for high-throughput antifibrotic drug screening.

Concluding remarks and future directions

To date, silicosis lung disease has no available cure that can alter the course of the disease and no medications that can reverse lung damage. Strategies to effectively combat crystalline silica lung disease are prevention by reducing and controlling exposure to SiO_2 dust. The development of drug therapies requires fundamental knowledge of the biology of the disease and the molecular basis of the pathogenesis. However, despite many inconveniences, drug development strategies to deal with lung silicosis can be classified into the following groups that offer many different drug targets, as follows: 1) targets to prevent the onset of inflammation by anti-cytokine therapy; 2) targets to prevent the onset of inflammation by blocking inflammasome activation; 3) targets to prevent the onset of inflammation by agents enhancing an autophagy–lysosomal system; 4) targets to prevent the onset of inflammation by antioxidants; 5) targets to prevent the onset of inflammation by corticosteroids; 6) use of agents that increase cAMP and cGMP; 7) use of agents that influence TGF-β-signalling; 8) use of microRNAs; and 9) targets to prevent the process of fibrosis, called anti-fibrotic drugs [50, 54, 87, 113–124]. With all these possibilities in mind, several treatments are in different stages of development, giving hope to patients with silicosis. In relation to this, the zebrafish is a great model for human health and can act as an intermediary between *in vitro* assays performed in cells and mammalian *in vivo* studies to evaluate efficacy, appropriate dose, toxicity and simultaneous assessment of different drugs [125, 126]. Unfortunately, all tests performed with cell-based methods provide limited information on the aspects of absorption, distribution, metabolism, excretion and toxicity of anti-silicotic compounds, whereas *in vivo* zebrafish assays often allow discovering these pharmacological aspects. The compounds tested must have the ability to be absorbed, reach the target tissue and avoid rapid metabolism and excretion in the *in vivo* zebrafish model. The great advantage of testing newly discovered compounds in zebrafish is that they can be rapidly tested in mammalian models *in vivo* with minimal optimisation of pharmacological aspects.

The entire drug discovery process is very time-consuming and the usual timeline for approval of a new therapeutic agent for any given disease can be as long as 10 years. The most promising and time-saving strategy for the search, identification and development of treatment for lung diseases is the use of drugs that have already been approved by the United States Food and Drug Administration (FDA), especially drugs involved in diseases with inflammatory and fibrotic lung processes [127]. This will shorten the path to clinical research, as the identified compounds have already been tested pharmacokinetically and undergone safety trials to be approved for human use. To date, many drug libraries are available for testing in *in vitro* and *in vivo* models [128]. Beyond the design of novel drugs and discovery and the testing of already FDA-approved drugs, screening natural products for anti-inflammatory and antifibrotic activity also seems to be a good approach [56]. Herbal compounds may be beneficial for pulmonary silicosis considering their biological relevance, characteristics and diversity. The "COlleCtion of Open NatUral producTs" (COCONUT) has been created, which contains structures and sparse annotations of more than 400 000 nonredundant natural products that can be tested in experimental animal models of human diseases [129]. In conclusion, all of the above approaches can easily be used in zebrafish models of many human diseases, such as the silicosis model, as they have already shown high efficacy in high-throughput screening of repurposed drugs [130–132] and natural or herbal products [133–135], as well as helping to discover specific therapeutic targets in humans.

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References

1 Delight N, Sachs H. Pneumoconiosis. Treasure Island, FL, StatPearls, 2022.

2 Skuland T, Ovrevik J, Låg M, *et al.* Role of size and surface area for pro-inflammatory responses to silica nanoparticles in epithelial lung cells: importance of exposure conditions. *Toxicol In Vitro* 2014; 28: 146–155.

3 Pavan C, Delle Piane M, Gullo M, *et al.* The puzzling issue of silica toxicity: are silanols bridging the gaps between surface states and pathogenicity? *Part Fibre Toxicol* 2019; 16: 32.
4 Merget R, Bauer T, Küpper HU, *et al.* Health hazards due to the inhalation of amorphous silica. *Arch Toxicol* 2002; 75: 625–634.
5 Leung CC, Yu IT, Chen W. Silicosis. *Lancet* 2012; 379: 2008–2018.
6 Rimal B, Greenberg AK, Rom WN. Basic pathogenetic mechanisms in silicosis: current understanding. *Curr Opin Pulm Med* 2005; 11: 169–173.
7 Chen S, Liu M, Xie F. Global and national burden and trends of mortality and disability-adjusted life years for silicosis, from 1990 to 2019: results from the Global Burden of Disease study 2019. *BMC Pulm Med* 2022; 22: 240.
8 Reilly MJ, Timmer SJ, Rosenman KD. The burden of silicosis in Michigan: 1988–2016. *Ann Am Thorac Soc* 2018; 15: 1404–1410.
9 Knight D, Ehrlich R, Fielding K, *et al.* Trends in silicosis prevalence and the healthy worker effect among gold miners in South Africa: a prevalence study with follow up of employment status. *BMC Public Health* 2015; 15: 1258.
10 Murray J, Webster I, Reid G, *et al.* The relation between fibrosis of hilar lymph glands and the development of parenchymal silicosis. *Br J Ind Med* 1991; 48: 267–269.
11 Hutyrová B, Smolková P, Nakládalová M, *et al.* Case of accelerated silicosis in a sandblaster. *Ind Health* 2015; 53: 178–183.
12 Ishii H, Trapnell BC, Tazawa R, *et al.* Comparative study of high-resolution CT findings between autoimmune and secondary pulmonary alveolar proteinosis. *Chest* 2009; 136: 1348–1355.
13 Duchange L, Brichet A, Lamblin C, *et al.* [Acute silicosis. Clinical, radiologic, functional, and cytologic characteristics of the broncho-alveolar fluids. Observations of 6 cases]. *Rev Mal Respir* 1998; 15: 527–534.
14 Li X, Wang Y, An G, *et al.* Bone marrow mesenchymal stem cells attenuate silica-induced pulmonary fibrosis *via* paracrine mechanisms. *Toxicol Lett* 2017; 270: 96–107.
15 Wagner GR. Asbestosis and silicosis. *Lancet* 1997; 349: 1311–1315.
16 Cowie RL. The epidemiology of tuberculosis in gold miners with silicosis. *Am J Respir Crit Care Med* 1994; 150: 1460–1462.
17 Hnizdo E, Murray J. Risk of pulmonary tuberculosis relative to silicosis and exposure to silica dust in South African gold miners. *Occup Environ Med* 1998; 55: 496–502.
18 Rees D, Murray J. Silica, silicosis and tuberculosis. *Int J Tuberc Lung Dis* 2007; 11: 474–484.
19 Pollard KM. Silica, silicosis, and autoimmunity. *Front Immunol* 2016; 7: 97.
20 Sato T, Shimosato T, Klinman DM. Silicosis and lung cancer: current perspectives. *Lung Cancer* 2018; 9: 91–101.
21 Partanen T, Pukkala E, Vainio H, *et al.* Increased incidence of lung and skin cancer in Finnish silicotic patients. *J Occup Med* 1994; 36: 616–622.
22 Muszyńska-Graca M, Dąbkowska B, Brewczyński PZ. [Guidelines for the use of the International Classification of Radiographs of Pneumoconioses of the International Labour Office (ILO): Substantial changes in the current edition]. *Med Pr* 2016; 67: 833–837.
23 Apte SH, Tan ME, Lutzky VP, *et al.* Alveolar crystal burden in stone workers with artificial stone silicosis. *Respirology* 2022; 27: 437–446.
24 Chao TP, Sperandio EF, Ostolin TLVP, *et al.* Use of cardiopulmonary exercise testing to assess early ventilatory changes related to occupational particulate matter. *Braz J Med Biol Res* 2018; 51: e6486.
25 Schulte PA. The use of biomarkers in surveillance, medical screening, and intervention. *Mutat Res* 2005; 592: 155–163.
26 Naha N, Muhamed JCJ, Pagdhune A, *et al.* Club cell protein 16 as a biomarker for early detection of silicosis. *Indian J Med Res* 2020; 151: 319–325.
27 Sato T, Saito Y, Inoue S, *et al.* Serum heme oxygenase-1 as a marker of lung function decline in patients with chronic silicosis. *J Occup Environ Med* 2012; 54: 1461–1466.
28 Liu SJ, Wang P, Jiao J, *et al.* Differential gene expression associated with inflammation in peripheral blood cells of patients with pneumoconiosis. *J Occup Health* 2016; 58: 373–380.
29 Beshir S, Aziz H, Shaheen W, *et al.* Serum levels of copper, ceruloplasmin and angiotensin converting enzyme among silicotic and non-silicotic workers. *Open Access Maced J Med Sci* 2015; 3: 467–473.
30 Muzembo BA, Dumavibhat N, Ngatu NR, *et al.* Serum selenium and selenoprotein p in patients with silicosis. *J Trace Elem Med Biol* 2013; 27: 40–44.
31 Lee S, Honda M, Yamamoto S, *et al.* Role of nephronectin in pathophysiology of silicosis. *Int J Mol Sci* 2019; 20: 2581.
32 Horváth I, Barnes PJ, Loukides S, *et al.* A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *Eur Respir J* 2017; 49: 1600965.
33 Sauni R, Oksa P, Lehtimäki L, *et al.* Increased alveolar nitric oxide and systemic inflammation markers in silica-exposed workers. *Occup Environ Med* 2012; 69: 256–260.

34 Leese E, Staff JF, Carolan VA, *et al.* Exhaled breath condensate: a novel matrix for biological monitoring to assess occupational exposure to respirable crystalline silica. *Ann Work Expo Health* 2017; 61: 902–906.
35 Richeldi L, du Bois RM, Raghu G, *et al.* Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014; 370: 2071–2082.
36 Taniguchi H, Ebina M, Kondoh Y, *et al.* Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2010; 35: 821–829.
37 Aimo A, Spitaleri G, Nieri D, *et al.* Pirfenidone for idiopathic pulmonary fibrosis and beyond. *Card Fail Rev* 2022; 8: e12.
38 Wollin L, Wex E, Pautsch A, *et al.* Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur Respir J* 2015; 45: 1434–1445.
39 Wilt JL, Banks DE, Weissman DN, *et al.* Reduction of lung dust burden in pneumoconiosis by whole-lung lavage. *J Occup Environ Med* 1996; 38: 619–624.
40 Chambers DC, Apte SH, Deller D, *et al.* Radiological outcomes of whole lung lavage for artificial stone-associated silicosis. *Respirology* 2021; 26: 501–503.
41 McEwen K, Brodie L. Lung transplantation for silicosis and recovery: an Australian case study. *Br J Nurs* 2021; 30: 178–183.
42 Lee CM, Park JW, Cho WK, *et al.* Modifiers of TGF-β1 effector function as novel therapeutic targets of pulmonary fibrosis. *Korean J Intern Med* 2014; 29: 281–290.
43 Das S, Kumar M, Negi V, *et al.* MicroRNA-326 regulates profibrotic functions of transforming growth factor-β in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2014; 50: 882–892.
44 Xu T, Yan W, Wu Q, *et al.* MiR-326 inhibits inflammation and promotes autophagy in silica-induced pulmonary fibrosis through targeting TNFSF14 and PTBP1. *Chem Res Toxicol* 2019; 32: 2192–2203.
45 Liu WW, Wang HX, Yu W, *et al.* Treatment of silicosis with hepatocyte growth factor-modified autologous bone marrow stromal cells: a non-randomized study with follow-up. *Genet Mol Res* 2015; 14: 10672–10681.
46 Chen S, Cui G, Peng C, *et al.* Transplantation of adipose-derived mesenchymal stem cells attenuates pulmonary fibrosis of silicosis *via* anti-inflammatory and anti-apoptosis effects in rats. *Stem Cell Res Ther* 2018; 9: 110.
47 Li X, An G, Wang Y, *et al.* Targeted migration of bone marrow mesenchymal stem cells inhibits silica-induced pulmonary fibrosis in rats. *Stem Cell Res Ther* 2018; 9: 335.
48 Choi M, Ban T, Rhim T. Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell. *Mol Cells* 2014; 37: 133–139.
49 Phinney DG, Di Giuseppe M, Njah J, *et al.* Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* 2015; 6: 8472.
50 Abdelaziz RR, Elkashef WF, Said E. Tadalafil reduces airway hyperactivity and protects against lung and respiratory airways dysfunction in a rat model of silicosis. *Int Immunopharmacol* 2016; 40: 530–541.
51 Kolb M, Raghu G, Wells AU, *et al.* Nintedanib plus sildenafil in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2018; 379: 1722–1731.
52 Barbarin V, Nihoul A, Misson P, *et al.* The role of pro- and anti-inflammatory responses in silica-induced lung fibrosis. *Respir Res* 2005; 6: 112.
53 Trentin PG, Ferreira TP, Arantes AC, *et al.* Annexin A1 mimetic peptide controls the inflammatory and fibrotic effects of silica particles in mice. *Br J Pharmacol* 2015; 172: 3058–3071.
54 Ferreira TPT, Lima JGME, Farias-Filho FA, *et al.* Intranasal flunisolide suppresses pathological alterations caused by silica particles in the lungs of mice. *Front Endocrinol* 2020; 11: 388.
55 Pang J, Qi X, Luo Y, *et al.* Multi-omics study of silicosis reveals the potential therapeutic targets PGD. *Theranostics* 2021; 11: 2381–2394.
56 Adamcakova J, Mokra D. Herbal compounds in the treatment of pulmonary silicosis. *Physiol Res* 2021; 70: S275–S287.
57 Zhu Z, Li Q, Xu C, *et al.* Sodium tanshinone IIA sulfonate attenuates silica-induced pulmonary fibrosis in rats *via* activation of the Nrf2 and thioredoxin system. *Environ Toxicol Pharmacol* 2020; 80: 103461.
58 Liu H, Yu H, Cao Z, *et al.* Kaempferol modulates autophagy and alleviates silica-induced pulmonary fibrosis. *DNA Cell Biol* 2019; 38: 1418–1426.
59 Li N, Feng F, Wu K, *et al.* Inhibitory effects of astragaloside IV on silica-induced pulmonary fibrosis *via* inactivating TGF-β1/Smad3 signaling. *Biomed Pharmacother* 2019; 119: 109387.
60 Du S, Li C, Lu Y, *et al.* Dioscin alleviates crystalline silica-induced pulmonary inflammation and fibrosis through promoting alveolar macrophage autophagy. *Theranostics* 2019; 9: 1878–1892.
61 Li C, Lu Y, Du S, *et al.* Dioscin exerts protective effects against crystalline silica-induced pulmonary fibrosis in mice. *Theranostics* 2017; 7: 4255–4275.
62 Peng HB, Wang RX, Deng HJ, *et al.* Protective effects of oleanolic acid on oxidative stress and the expression of cytokines and collagen by the AKT/NF-κB pathway in silicotic rats. *Mol Med Rep* 2017; 15: 3121–3128.
63 Li S, Shao L, Fang J, *et al.* Hesperetin attenuates silica-induced lung injury by reducing oxidative damage and inflammatory response. *Exp Ther Med* 2021; 21: 297.

64 Yang T, Wang J, Pang Y, *et al.* Emodin suppresses silica-induced lung fibrosis by promoting Sirt1 signaling *via* direct contact. *Mol Med Rep* 2016; 14: 4643–4649.
65 Kulkarni GK. Prevention and control of silicosis: a national challenge. *Indian J Occup Environ Med* 2007; 11: 95–96.
66 Roperto F, Damiano S, De Vico G, *et al.* Silicate pneumoconiosis in pigs: optical and scanning electron microscopical investigations with X-ray microanalysis. *J Comp Pathol* 1994; 110: 227–236.
67 Canfield PJ, Rothwell TL, Papadimitriou JM, *et al.* Siliceous pneumoconiosis in two dogs. *J Comp Pathol* 1989; 100: 199–202.
68 Hansen HJ, Jama FM, Nilsson C, *et al.* Silicate pneumoconiosis in camels (*Camelus dromedarius* L). *Zentralbl Veterinarmed A* 1989; 36: 789–796.
69 Schwartz LW, Knight HD, Whittig LD, *et al.* Silicate pneumoconiosis and pulmonary fibrosis in horses from the Monterey-Carmel peninsula. *Chest* 1981; 80: Suppl. 1: 82–85.
70 Arens AM, Barr B, Puchalski SM, *et al.* Osteoporosis associated with pulmonary silicosis in an equine bone fragility syndrome. *Vet Pathol* 2011; 48: 593–615.
71 Hannothiaux MH, Scharfman A, Wastiaux A, *et al.* An attempt to evaluate lung aggression in monkey silicosis: hydrolases, peroxidase and antiproteases activities in serial bronchoalveolar lavages. *Eur Respir J* 1991; 4: 191–204.
72 Suedmeyer WK, Johnson G, Lovell MA. Pulmonary silicosis in three North American river otters (*Lutra canadensis*). *J Zoo Wildl Med* 1999; 30: 564–572.
73 Brambilla C, Abraham J, Brambilla E, *et al.* Comparative pathology of silicate pneumoconiosis. *Am J Pathol* 1979; 96: 149–170.
74 Evans MG, Slocombe RF, Schwartz LD. Pulmonary silicosis in captive ring-necked pheasants: definitive diagnosis by electron probe X-ray microanalysis. *Vet Pathol* 1988; 25: 239–241.
75 Camp PG, Dimich-Ward H, Kennedy SM. Women and occupational lung disease: sex differences and gender influences on research and disease outcomes. *Clin Chest Med* 2004; 25: 269–279.
76 Poinen-Rughooputh S, Rughooputh MS, Guo Y, *et al.* Sex-related differences in the risk of silicosis among Chinese pottery workers: a cohort study. *J Occup Environ Med* 2021; 63: 74–79.
77 Callis AH, Sohnle PG, Mandel GS, *et al.* Kinetics of inflammatory and fibrotic pulmonary changes in a murine model of silicosis. *J Lab Clin Med* 1985; 105: 547–553.
78 Davis GS, Leslie KO, Hemenway DR. Silicosis in mice: effects of dose, time, and genetic strain. *J Environ Pathol Toxicol Oncol* 1998; 17: 81–97.
79 Mayeux JM, Escalante GM, Christy JM, *et al.* Silicosis and silica-induced autoimmunity in the diversity outbred mouse. *Front Immunol* 2018; 9: 874.
80 Brown JM, Archer AJ, Pfau JC, *et al.* Silica accelerated systemic autoimmune disease in lupus-prone New Zealand mixed mice. *Clin Exp Immunol* 2003; 131: 415–421.
81 Moore BB, Lawson WE, Oury TD, *et al.* Animal models of fibrotic lung disease. *Am J Respir Cell Mol Biol* 2013; 49: 167–179.
82 Lakatos HF, Burgess HA, Thatcher TH, *et al.* Oropharyngeal aspiration of a silica suspension produces a superior model of silicosis in the mouse when compared to intratracheal instillation. *Exp Lung Res* 2006; 32: 181–199.
83 Lacher SE, Johnson C, Jessop F, *et al.* Murine pulmonary inflammation model: a comparative study of anesthesia and instillation methods. *Inhal Toxicol* 2010; 22: 77–83.
84 Mayeux JM, Kono DH, Pollard KM. Development of experimental silicosis in inbred and outbred mice depends on instillation volume. *Sci Rep* 2019; 9: 14190.
85 Driscoll KE, Costa DL, Hatch G, *et al.* Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol Sci* 2000; 55: 24–35.
86 Helms MN, Torres-Gonzalez E, Goodson P, *et al.* Direct tracheal instillation of solutes into mouse lung. *J Vis Exp* 2010; 42: 1941.
87 Biswas R, Trout KL, Jessop F, *et al.* Imipramine blocks acute silicosis in a mouse model. *Part Fibre Toxicol* 2017; 14: 36.
88 Li T, Yang XY, Xu DJ, *et al.* OC-STAMP overexpression drives lung alveolar epithelial cell type II senescence in silicosis. *Oxid Med Cell Longev* 2021; 2021: 4158495.
89 Sharma N, Kundu D, Dhaked S, *et al.* Silicosis and silicotuberculosis in India. *Bull World Health Organ* 2016; 94: 777–778.
90 Santoriello C, Zon LI. Hooked! Modeling human disease in zebrafish. *J Clin Invest* 2012; 122: 2337–2343.
91 Garcia-Moreno D, Tyrkalska SD, Valera-Pérez A, *et al.* The zebrafish: a research model to understand the evolution of vertebrate immunity. *Fish Shellfish Immunol* 2019; 90: 215–222.
92 White RM, Sessa A, Burke C, *et al.* Transparent adult zebrafish as a tool for *in vivo* transplantation analysis. *Cell Stem Cell* 2008; 2: 183–189.
93 Howe K, Clark MD, Torroja CF, *et al.* The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013; 496: 498–503.

94 Cambier CJ, Takaki KK, Larson RP, *et al.* Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. *Nature* 2014; 505: 218–222.

95 Tyrkalska SD, Pedoto A, Martínez-López A, *et al.* Silica crystals activate Toll-like receptors and inflammasomes to promote local and systemic immune responses in zebrafish. *Dev Comp Immunol* 2023; 138: 104523.

96 Tyrkalska SD, Martínez-López A, Pedoto A, *et al.* The spike protein of SARS-CoV-2 signals *via* TLR2 in zebrafish. *Dev Comp Immunol* 2023; 140: 104626.

97 Tyrkalska SD, Martínez-López A, Arroyo AB, *et al.* Differential proinflammatory activities of spike proteins of SARS-CoV-2 variants of concern. *Sci Adv* 2022; 8: eabo0732.

98 Takaki K, Davis JM, Winglee K, *et al.* Evaluation of the pathogenesis and treatment of *Mycobacterium marinum* infection in zebrafish. *Nat Protoc* 2013; 8: 1114–1124.

99 Choe CP, Choi SY, Kee Y, *et al.* Transgenic fluorescent zebrafish lines that have revolutionized biomedical research. *Lab Anim Res* 2021; 37: 26.

100 Renshaw SA, Loynes CA, Trushell DM, *et al.* A transgenic zebrafish model of neutrophilic inflammation. *Blood* 2006; 108: 3976–3978.

101 Hall C, Flores MV, Storm T, *et al.* The zebrafish lysozyme C promoter drives myeloid-specific expression in transgenic fish. *BMC Dev Biol* 2007; 7: 42.

102 Ellett F, Pase L, Hayman JW, *et al.* mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. *Blood* 2011; 117: e49–e56.

103 Walton EM, Cronan MR, Beerman RW, *et al.* The macrophage-specific promoter mfap4 allows live, long-term analysis of macrophage behavior during mycobacterial infection in zebrafish. *PLoS One* 2015; 10: e0138949.

104 Sanderson LE, Chien AT, Astin JW, *et al.* An inducible transgene reports activation of macrophages in live zebrafish larvae. *Dev Comp Immunol* 2015; 53: 63–69.

105 Gray C, Loynes CA, Whyte MK, *et al.* Simultaneous intravital imaging of macrophage and neutrophil behaviour during inflammation using a novel transgenic zebrafish. *Thromb Haemost* 2011; 105: 811–819.

106 Li L, Yan B, Shi YQ, *et al.* Live imaging reveals differing roles of macrophages and neutrophils during zebrafish tail fin regeneration. *J Biol Chem* 2012; 287: 25353–25360.

107 Langenau DM, Ferrando AA, Traver D, *et al. In vivo* tracking of T cell development, ablation, and engraftment in transgenic zebrafish. *Proc Natl Acad Sci USA* 2004; 101: 7369–7374.

108 Kanther M, Sun X, Mühlbauer M, *et al.* Microbial colonization induces dynamic temporal and spatial patterns of NF-κB activation in the zebrafish digestive tract. *Gastroenterology* 2011; 141: 197–207.

109 Nguyen-Chi M, Phan QT, Gonzalez C, *et al.* Transient infection of the zebrafish notochord with *E. coli* induces chronic inflammation. *Dis Model Mech* 2014; 7: 871–882.

110 Nguyen-Chi M, Laplace-Builhe B, Travnickova J, *et al.* Identification of polarized macrophage subsets in zebrafish. *Elife* 2015; 4: e07288.

111 Whitesell TR, Kennedy RM, Carter AD, *et al.* An α-smooth muscle actin (acta2/αsma) zebrafish transgenic line marking vascular mural cells and visceral smooth muscle cells. *PLoS One* 2014; 9: e90590.

112 Morris JL, Cross SJ, Lu Y, *et al.* Live imaging of collagen deposition during skin development and repair in a collagen I–GFP fusion transgenic zebrafish line. *Dev Biol* 2018; 441: 4–11.

113 Rosenbloom J, Macarak E, Piera-Velazquez S, *et al.* Human fibrotic diseases: current challenges in fibrosis research. *Methods Mol Biol* 2017; 1627: 1–23.

114 Wollin L, Maillet I, Quesniaux V, *et al.* Antifibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. *J Pharmacol Exp Ther* 2014; 349: 209–220.

115 Piguet PF, Vesin C, Grau GE, *et al.* Interleukin 1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. *Cytokine* 1993; 5: 57–61.

116 Song L, Weng D, Dai W, *et al.* Th17 can regulate silica-induced lung inflammation through an IL-1β-dependent mechanism. *J Cell Mol Med* 2014; 18: 1773–1784.

117 Piguet PF, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 1994; 7: 515–518.

118 Zahid A, Li B, Kombe AJK, *et al.* Pharmacological inhibitors of the NLRP3 inflammasome. *Front Immunol* 2019; 10: 2538.

119 Caseley EA, Poulter JA, Rodrigues F, *et al.* Inflammasome inhibition under physiological and pharmacological conditions. *Genes Immun* 2020; 21: 211–223.

120 Tan S, Yang S, Chen G, *et al.* Trehalose alleviates apoptosis by protecting the autophagy-lysosomal system in alveolar macrophages during human silicosis. *Life Sci* 2020; 257: 118043.

121 Sun J, Song P, Wang Y, *et al.* Clinical efficacy of acetylcysteine combined with tetrandrine tablets in the treatment of silicosis and the effect on serum IL-6 and TNF-α. *Exp Ther Med* 2019; 18: 3383–3388.

122 Zhang J, Wang Y, Zhang S, *et al.* Effects of tetrandrine combined with acetylcysteine on exercise tolerance, pulmonary function and serum TNF-β1 and MMP-7 in silicosis patients. *Exp Ther Med* 2020; 19: 2195–2201.

123 Qu Y, Zhang L, Kang Z, *et al.* Ponatinib ameliorates pulmonary fibrosis by suppressing TGF-β1/Smad3 pathway. *Pulm Pharmacol Ther* 2015; 34: 1–7.

124 Sun J, Li Q, Lian X, *et al.* MicroRNA-29b mediates lung mesenchymal-epithelial transition and prevents lung fibrosis in the silicosis model. *Mol Ther Nucleic Acids* 2019; 14: 20–31.
125 Ding CB, Zhang JP, Zhao Y, *et al.* Zebrafish as a potential model organism for drug test against hepatitis C virus. *PLoS One* 2011; 6: e22921.
126 Cassar S, Adatto I, Freeman JL, *et al.* Use of zebrafish in drug discovery toxicology. *Chem Res Toxicol* 2020; 33: 95–118.
127 Yanagihara T, Chong SG, Vierhout M, *et al.* Current models of pulmonary fibrosis for future drug discovery efforts. *Expert Opin Drug Discov* 2020; 15: 931–941.
128 Corsello SM, Bittker JA, Liu Z, *et al.* The Drug Repurposing Hub: a next-generation drug library and information resource. *Nat Med* 2017; 23: 405–408.
129 Sorokina M, Steinbeck C. Review on natural products databases: where to find data in 2020. *J Cheminform* 2020; 12: 20.
130 Ganzen L, Ko MJ, Zhang M, *et al.* Drug screening with zebrafish visual behavior identifies carvedilol as a potential treatment for an autosomal dominant form of retinitis pigmentosa. *Sci Rep* 2021; 11: 11432.
131 Cousin MA, Ebbert JO, Wiinamaki AR, *et al.* Larval zebrafish model for FDA-approved drug repositioning for tobacco dependence treatment. *PLoS One* 2014; 9: e90467.
132 Rovira M, Huang W, Yusuff S, *et al.* Chemical screen identifies FDA-approved drugs and target pathways that induce precocious pancreatic endocrine differentiation. *Proc Natl Acad Sci USA* 2011; 108: 19264–19269.
133 Liang F, Han Y, Gao H, *et al.* Kaempferol identified by zebrafish assay and fine fractionations strategy from *Dysosma versipellis* inhibits angiogenesis through VEGF and FGF pathways. *Sci Rep* 2015; 5: 14468.
134 Brillatz T, Lauritano C, Jacmin M, *et al.* Zebrafish-based identification of the antiseizure nucleoside inosine from the marine diatom *Skeletonema marinoi*. *PLoS One* 2018; 13: e0196195.
135 Cheng MC, Lee TH, Chu YT, *et al.* Melanogenesis inhibitors from the rhizoma of *Ligusticum sinense* in B16-F10 melanoma cells *in vitro* and zebrafish *in vivo*. *Int J Mol Sci* 2018; 19: 3994.
136 Kato K, Zemskova MA, Hanss AD, *et al.* Muc1 deficiency exacerbates pulmonary fibrosis in a mouse model of silicosis. *Biochem Biophys Res Commun* 2017; 493: 1230–1235.
137 Peukert K, Steinhagen F, Fox M, *et al.* Tetracycline ameliorates silica-induced pulmonary inflammation and fibrosis *via* inhibition of caspase-1. *Respir Res* 2022; 23: 21.
138 Thakur SA, Beamer CA, Migliaccio CT, *et al.* Critical role of MARCO in crystalline silica-induced pulmonary inflammation. *Toxicol Sci* 2009; 108: 462–471.
139 Cassel SL, Eisenbarth SC, Iyer SS, *et al.* The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci USA* 2008; 105: 9035–9040.
140 Li B, Mu M, Sun Q, *et al.* A suitable silicosis mouse model was constructed by repeated inhalation of silica dust *via* nose. *Toxicol Lett* 2021; 353: 1–12.
141 Kawasaki H. A mechanistic review of silica-induced inhalation toxicity. *Inhal Toxicol* 2015; 27: 363–377.
142 James DM, Morris TG, Marks J. Drug treatment of experimental silicosis. *Br J Ind Med* 1960; 17: 36–40.
143 Feng F, Cheng P, Zhang H, *et al.* The protective role of tanshinone IIA in silicosis rat model *via* TGF-β1/Smad signaling suppression, NOX4 inhibition and Nrf2/ARE signaling activation. *Drug Des Devel Ther* 2019; 13: 4275–4290.
144 Jiao J, Li L, Yao W, *et al.* Influence of silica exposure for lung silicosis rat. *Dis Markers* 2021; 2021: 6268091.
145 Zhu Y, Yao J, Duan Y, *et al.* Protein expression profile in rat silicosis model reveals upregulation of PTPN2 and its inhibitory effect on epithelial-mesenchymal transition by dephosphorylation of STAT3. *Int J Mol Sci* 2020; 21: 1189.
146 Sjöstrand M, Rylander R. Enzymes in lung lavage fluid after inhalation exposure to silica dust. *Environ Res* 1984; 33: 307–311.
147 Dauber JH, Rossman MD, Pietra GG, *et al.* Experimental silicosis: morphologic and biochemical abnormalities produced by intratracheal instillation of quartz into guinea pig lungs. *Am J Pathol* 1980; 101: 595–612.
148 Marks GS, Marasas LW. Changes in the lung lipids of rabbits and guinea-pigs exposed to the inhalation of silica dust. *Br J Ind Med* 1960; 17: 31–35.
149 Schepers GW, Delahant AB, Schmidt JG, *et al.* The biological action of Degussa submicron amorphous silica dust (Dow Corning silica). III. Inhalation studies on rabbits. *AMA Arch Ind Health* 1957; 16: 280–301.
150 Fallon JT. Specific tissue reaction to phospholipids: a suggested explanation for the similarity of the lesions of silicosis and pulmonary tubercolosis. *Can Med Assoc J* 1937; 36: 223–228.
151 Mosinger M, Jouglard-Duplay J, Versino A, *et al.* [On experimental silicosis in the rabbit obtained by the intravenous administration of silica]. *Arch Mal Prof* 1963; 24: 503–521.
152 Mosinger M, Jouglard-Duplay J, Versino A, *et al.* [Electrophoretic changes in rabbits receiving intravenous silica injections (statistical study)]. *Arch Mal Prof* 1963; 24: 881–886.
153 Sato E, McDonald SA, Fan Y, *et al.* Analysis of particles from hamster lungs following pulmonary talc exposures: implications for pathogenicity. *Part Fibre Toxicol* 2020; 17: 20.

154 Beck BD, Feldman HA, Brain JD, *et al.* The pulmonary toxicity of talc and granite dust as estimated from an *in vivo* hamster bioassay. *Toxicol Appl Pharmacol* 1987; 87: 222–234.

155 Bégin R, Massé S, Dufresne A. Further information on aluminium inhalation in silicosis. *Occup Environ Med* 1995; 52: 778–780.

156 Bégin R, Cantin A, Massé S, *et al.* Aluminium inhalation in sheep silicosis. *Int J Exp Pathol* 1993; 74: 299–307.

157 Bégin R, Dufresne A, Cantin A, *et al.* Quartz exposure, retention, and early silicosis in sheep. *Exp Lung Res* 1989; 15: 409–428.

158 Lesur O, Melloni B, Cantin AM, *et al.* Silica-exposed lung fluids have a proliferative activity for type II epithelial cells: a study on human and sheep alveolar fluids. *Exp Lung Res* 1992; 18: 633–654.

159 Van Duffel J, Meersman F, Tosetti R, *et al.* [Experimental pulmonary silicosis in the dog]. *G Ital Mal Torace* 1967; 21: 11–23.

160 Mosinger M, Gras A, Fiorentini H. [Experimental demonstrations of silicosis in the dog]. *Ann Med Leg Criminol Police Sci Toxicol* 1961; 41: 550–551.

161 Hammond E, Newell JD, Dilger SK, *et al.* Computed tomography and magnetic resonance imaging for longitudinal characterization of lung structure changes in a Yucatan miniature pig silicosis model. *Toxicol Pathol* 2016; 44: 373–381.