# Review of Nanotechnology Applications in Sericulture

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# Abstract

# Recent decades have witnessed the transformative impact of nanotechnology across various technology and industry sectors, ushering in significant improvements and, in some cases, revolutionary changes. Sectors such as information technology, energy, environmental science and transportation are all benefiting from nanotechnological advancements. In the present, nanotechnology integrates the progress achieved in chemistry, physics, materials science, and biotechnology to craft innovative materials with distinctive properties, attributable to their structuring at the nanometer scale. This paper provides a comprehensive summary of the diverse applications of nanotechnology in Sericulture, highlighting its pervasive influence on multiple fronts.

# Keywords: Nanotechnology, Agricultural field, *Spirulina,* Titanium dioxide, Silkworm.

# Introduction

# Role of nanoparticles in agricultural field

The nanoparticle bound drug could not able to circulate broadly, its negative consequences are limited and a high localized concentration can be achieved where it is needed (Panyam and Labhasetwar, 2003). In view of the large surface area per unit mass of nanoparticles the drug loading can be relatively high (Han *et al*., 2007). Nanoparticle bound drugs can be readily suspended in liquids and are able to penetrate deep in organs and tissues.

Nanoparticles can be created through a variety of physicochemical processes  
(Cao, 2004; Sepeur, 2008), their synthesis of nanoparticles through safe and eco-friendly biological processes including agricultural fields are fascinating, particularly if they are intended for invasive medical applications. The biological or biogenic synthesis of nanoparticles from salts of the relevant metals, a number of methods have been developed (Singaravelu *et al*., 2007; Ray *et al*., 2011).

Titanium dioxide nanoparticles are commercially available as nanomaterials and have very strong antibacterial properties in agricultural field. These materials are used in a wide range of applications including food packaging, medical instruments and cosmetics (Chai *et al.,* 2006). There are several methodologies for NPs synthesis, including conventional methods such as chemical and physical approaches as well as modern methods like biological synthesis using natural living organisms (Lengke *et al.,* 2006). In chemical methods, the main components were the precursor metals and the stabilizing and reducing agents (zhang *et al.,* 2016).

Since, they have a longer half-life *in vivo*, longer circulation times and can deliver a potent drug in high concentrations where they are needed, drugs that are bound to nanoparticles have several advantages (Sahoo *et al*., 2007). To obtain the desired delivery characteristics, the size of the drug nanoparticle and its surface properties can be changed (Chen *et al*., 2007).

In recent years, a variety of synthetic techniques, including chemical techniques, microwave techniques and laser ablation techniques have been used to produce nanomaterials (Murphy *et al*., 2011).

According to Dhillon *et al.* (2012) shape, size, activity, conductivity and many other attributes of nanomaterials differed from those of bulk materials, illuminating the profound impact of nanotechnology on the environment and society. Currently, it is used in a wide range of applications, such as water treatment, building, energy sensors and bio- applications.

Nanotechnology is an innovative field that focuses on the synthesis, characterization, and emergence of improved nanomaterials. NPs can be classified based on a variety of factors, including shape and dimension, phase composition, nature, and origin. NPs were classified chemically into inorganic and organic NPs, which include metal and metal oxide NPs such as silver, gold, platinum, titanium dioxide, iron oxide, copper oxide, and zinc oxide NPs. Carbon and chitosan NPs were examples of organic NPs (Mubarakali *et al.,* 2013).

Titanium dioxide nanoparticles are widely used in the pharmaceutical industry as a new type of biomaterial. Especially in orthopaedic and dental fields, titanium dioxide nanoparticles are used to support the ossification process (Ni *et al.,* 2015).

Tian *et al.* (2016) reported that applications of titanium dioxide nanoparticles in nutrition fields exhibited which is activating the signalling pathway of insulin and promoted the protein, fat and carbohydrate metabolisms.

The natural sources used in NP biofabrication range from unicellular to multicellular organisms. Additionally, this process can be performed in the presence of single proteins, enzymes, pigments and so on (Asmathunisha *et al.,* 2012). The unicellular organisms include bacteria, cyanobacteria and diatoms, while multicellular organisms include algae and plant (Aritonang *et al.,* 2019).

# Green synthesis of TiO2 nanoparticles

TiO2 nanoparticles were synthesized by thermal decomposition of a precipitate obtained from a precursor solution of titanium isopropoxide (IV) and isopropyl alcohol. The synthesized nanoparticles were morphologically, texturally and structurally characterized using Thermo gravimetric differential thermal analysis (TGA- DTA), gas adsorption, SEM, XRD and FT-IR (Viana *et al.,* 2010).

Gunasundari *et al.* (2016) synthesized silver, chromium, iron, lead and zinc metal nanoparticles using the green synthesis method. The formation of metal nanoparticles were characterized through UV-visible spectroscopy, FTIR and scanning electron microscopy. Antimicrobial activity of synthesized different metallic nanoparticles were studied. Among these, different metallic nanoparticles chromium and zinc metallic nanoparticles showed good antimicrobial activity against *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli* compared to other metallic nanoparticles.

Dharanipriya and Thangapandiyan (2019) reported that mulberry leaves treated with chemically mediated Ag nanoparticles and *Spirulina* by different concentration, *viz.,*100 ppm, 300 ppm, 500 ppm, Ag nanoparticles with *Spirulina* and *Spirulina* alone. The obtained results indicated that significant differences were noted in economic traits also nutrient efficacy were significantly increased in Ag nanoparticles and *Spirulina* treated groups.

Aravind *et al.* (2021) synthesized TiO2NPs through chemical and green synthesis method. In green synthesis jasmine flower extract was used as reducing and stabilizing agent. The properties were analyzed using UV-Visible spectroscopy, XRD and SEM.

# Nanoparticle characterization

The characterization of nanoparticle is performed to learn more about the average size, size distribution and changes that occur when the particles are stored (for example, crystal growth and agglomeration). Nanoparticles and nanomaterials characteristics include their size, shape, surface area and disparity (Jiang *et al*., 2009). The most common methods for characterising nanoparticles are UV-Visible Absorbance Spectroscopy (UV), Particle Size Analyzer (PSA), Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (Mittal *et al*., 2013).

# Ultraviolet-Visible Absorbance Spectroscopy

The green synthesis method is an effective, non-toxic and eco-friendly method used for the preparation of metal nanoparticles such as AgNPs, CrNPs, PbNPs, ZnNPs and FeNPs loaded on the ultrasonic assisted *Spirulina platensis* using the same algal extract as a reducing agent. The formation of nanoparticles was confirmed with FTIR, UV visible spectroscopy and SEM (Gunasundari *et al.,* 2016).

Subhapriya *et al.* (2018) biosynthesized TiO2 NPs using aqueous leaf extract of *Trigonella foenumgraecum* and characterized by FT-IR, UV, XRD, HR-SEM and HR-TEM. TF-TiO2 NPs also used to study the antimicrobial activity which showed the significant results against all the tested microorganisms.

# Particle Size Analyzer

Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca* showed the negative zeta potential value of −34 mV suggesting that the nanoparticles are highly stable in colloidal solution (Kumar *et al.,* 2013).

The green synthesized *Azadirachta indica* mediated with TiO2NPs showed the zeta potential in the rate of -24 mV and particle size distribution was 124 nm (Sankar *et al.,* 2015).

# Fourier Transform Infrared (FTIR) Spectroscopy analysis

Infrared radiation (IR) is heat radiation, and its frequency ranges from 1014 to 1011 Hz on the electromagnetic spectrum. The energy differences between various molecular vibrational modes are equivalent to the energy differences of infrared radiation. The vibrational absorption spectra are clearly made up of bands rather than lines.

The IR spectral bands are narrower in the solid state and at low temperatures. The spectrum of infrared light is plotted as a function of wave number, which is the reciprocal of wavelength.

Wave number = 1/λ cm-1

The FT-IR method measures the vibrations of bonds within chemical functional groups and produces a spectrum that can be interpreted as the sample's biochemical or metabolic "fingerprint." It may be possible to detect minor changes in primary and secondary metabolites by obtaining IR spectra from plant samples (McCann *et al*., 1992).

Meenakshi *et al*. (2012) reported the FT-IR spectrum of *S. wightii* and *U. lactuca* samples, with the wave number of peaks lying between 449.32 cm-1 and 3495.89 cm-1 and 462.89 cm-1 and 3407.05 cm-1, respectively.

Devi and Gayathri (2014) demonstrated FTIR analysis of TiO2NPs to determine their functional groups. The results indicated the spectrum of titanium dioxide nanoparticles in which the peaks at 3400 and 1631.78 cm -1 spectra were due to stretching and bending vibrations of the –OH groups. The peaks such as 435.91 cm-1, 466.77 cm-1 to 700 cm-1 showed the bending and stretching mode of titanium dioxide.

Kannan (2014) determined the frequency of functional groups in brown algae (*S. wightii*) and red algae (*Gracilaria corticata*) using the FT-IR technique. N-H/O-H, C-H, and C-O stretching vibrations in various amines, hydroxyl groups, and carboxylic groups are represented by the bands at 3371 cm-1,  
 2924 cm-1, and 2358 cm-1, respectively.

Krishnasamy *et al*. (2015) used FTIR spectroscopy to determine the substances responsible for the formation and stabilisation of titanium nanoparticles using *Azadirachta indica*. The FTIR spectrum of the materials produced by biosynthesis peaks in the concentration of titanium nanoparticles was found at 3421.63, 1640.53, 1083.76, and 775.38. 3421.63, and 1637.73 can refer to either the C=C groups of aromatic rings or the C=O stretching of amide. O-H stretching of phenolic compounds and alcohols were indicated at 3421.63, and these values are both possible for amide. The absorption band at 1083.76 indicates that alcohols and carboxylic acids have C-O vibrations.

Thakur *et al*. (2019) reported *A. indica* leaf extract was used to create titanium dioxide nanoparticles, which were then analysed using FTIR. The results showed peaks at 3581.96, 1166.92, 1091.86, and 709.62 in the FTIR spectrum of the titanium dioxide nanoparticles. Aromatic ring C=C groups are responsible for the peak at 1166.92, while the peak at 3581.96 is caused by alcohols and phenolic compounds stretching their O-H bonds. Peak 709.62 represents the stretching vibration of titanium dioxide nanoparticles (Ti-O-Ti), whereas Peak 1091.86 represents the vibration of carboxylic acid (C=O).

# Powder X-ray diffraction (XRD) analysis

The nanocomposites' structures were characterised using the X-ray diffraction (XRD) technique. XRD is used to identify phases and characterize the crystal structure of nanoparticles (Sun *et al.,* 2000). To obtain structural information, X-rays are penetrated into the nanomaterial and the resulting diffraction pattern is compared to standards.

The spaces between structural layers of the silicate can be determined using Bragg's law, sin = n/2d, where is the measured diffraction angle, is the wavelength of the X-ray radiation used in the diffraction experiment, and d is the spacing between diffractional lattice planes (Porter *et al*., 2008).

X-ray powder diffraction (XRD) was rapid analytical technique that can provide information on unit cell dimensions and is primarily used for phase identification of crystalline materials. The fundamental principle of X-ray diffraction is the constructive interference of monochromatic X-rays with a crystalline sample. The X-rays produced by a cathode ray tube are filtered to produce monochromatic radiation, collimated to concentrate them, and directed at the sample. Bragg's Law is used to evaluate the obtained interference in order to determine various properties of the crystal or polycrystalline material (Pattabhi and Gautham, 2009).

Shameli *et al.* (2010) utilised XRD analysis to demonstrate the formation of Ag-NPs on the surface of talc layers in the path of ion impregnation, with Ag+ ions bound only on the talc external layer space's external surfaces and edges. The parallel lamellar structure of the mineral clay was not disrupted by the formation of nanocomposites because the intensities of reflections and half-widths of Ag/Talc NCs remained constant.

The XRD patterns of Talc and TPP (Talc/polypropylene) show the corresponding 2 and d spacing values, with a peak at 2 = 9.50 (9.302) observed for both T and TPP, indicating that the crystal structure of talc remains, though the intensity of this peak has decreased (Mashael, 2013).

Rajeshkumar (2019) synthesised titanium dioxide nanoparticles from *Cassia fistula* leaves and investigated their antibacterial activity. X-ray diffraction was used to confirm the crystalline nature and particle size of the biologically mediated NPs. An XRD grid was used to determine the crystallite size of a fine nanoparticle sample. Different Bragg's reflections were obtained in the TiO2NPs XRD pattern, which corresponds to (101), (102), (110), (103), (112), (201), (004), (202) on the set of lattice planes. Depending on Bragg's reflection, the TiO2 NPS synthesized is face-centred cubic (FCC) and essentially crystalline in nature. The (112), (201), and (202) exhibit very weakly broadened wurtzite structures, according to Vijayakumar *et al*., (2017).

# Scanning Electron Microscopy (SEM) analysis

Electron microscopy is another popular method for characterising nanoparticles (Cao, 2004). Microscopy techniques can be used to investigate a wide range of materials with particle sizes ranging from nanometer to millimetre. Optical light microscopes, scanning electron microscopes (SEM), transmission electron microscopes (TEM) and atomic force microscopes are examples of microscopy-based research instruments (AFM).

An SEM is a useful tool when high-resolution images of the surface are required. The microscope dealings the electrons scattered from the sample. Electrons are accelerated by a shorter wavelength electric potential than photons. It can magnify images up to 20,000 times, allowing for high-resolution surface imaging and determining the size distribution of nanoparticles.

Scanning electron microscopy and transmission electron microscopy are used to characterise the morphology of materials at the nanometer to micrometre scale (Schaffer *et al*., 2009).

According to Kalantari *et al*. (2013), high magnification SEM analysis of the external surface of talc/Fe3O4 nanocomposites shows that the presence of small Fe3O4 nanoparticles that aggregate together and form large particles causes the external surface to gradually become shinier.

Santhoshkumar *et al.* (2014) investigated *Psidium guajava* extract-based green synthesis of titanium dioxide nanoparticles. The FESEM images were measured, and the surface study was used to perform topographical analysis. The synthesised TiO2NPs were smooth and spherical in shape. The images revealed physical morphology, particle size, and aspect ratio of the synthesized nanoparticles at various magnifications.

# Transmission Electron Microscopy (TEM) analysis

Transmission electron microscopy (TEM) has a 10000-fold higher resolution than scanning electron microscopy (SEM) (Eppler et al., 2000). The TEM images and size distribution of talc/Fe3O4 nanocomposites revealed that the nanoparticles had a mean diameter ranging from 1.2 to 3.2 nm  
(Kalantari *et al*., 2013).

Shameli *et al.* (2010) reported that the mean diameters and standard deviations of talc and Ag-NCs for 1.0 per cent, 2.0 per cent, and 5.0 per cent, respectively, were approximately 7.60 2.62, 11.30 4.06, and 13.11 4.58 nm. The initial talc and the Ag/talc-NCs had no morphologic differences.

TEM analysis of biosynthesized neem leaf mediated titanium dioxide NPs was described by Krishnasamy *et al*. (2015). The TEM images revealed that the majority of the titanium nanoparticles were spherical in shape with smooth surfaces, with sizes ranging from 15 to 45 nm.

# Effects of *S. platensis* mediated TiO2NPs on silkworm growth and development

Govindaraju *et al.* (2011) reported *S. platensis* synthesized with AgNPs showed the strongest antiviral activity and immunological studies made on silkworm *B. mori* disclosed that the significant increase in the total haemocyte count and differential haemocyte count due to *S. platensis* synthesised AgNPs supplementation.

Titanium dioxide nanoparticles can mediate the synthesis of ecdysone which affects the growth and development of silkworms and increases the antiviral ability of silkworms (Jiang *et al.,* 2014). Similarly, feeding the silkworms with titanium dioxide nanoparticles can increase the feeding efficiency and silk protein synthesis (Ni *et al.,* 2015).

Kumar and Balasubramanian (2014) reported that the *Spirulina* exhibits the presence of certain growth stimulant activity and can be used to increase the feed efficacy in commercial silkworm rearing. The study proved that the larval male (3.86g) and female (3.89g) weight, cocoon male (2.50g) and female (2.53g) weight, pupal male (1.58g) and female (1.60g) weight, shell weight (0.84g) and shell ratio (33.00%) enhanced with the supplementation of *Spirulina* at 5 % concentration compared to control.

Ni *et al.* (2014) studied the effects TiO2 NPs at low concentration (5 mg/L) in silkworm. The results showed faster development of the ovaries and testes and more gamete differentiation and formation, with an average increase of 51 eggs per insect after the feeding of TiO2 NPs.

Zhang *et al.* (2014) reported that TiO2NPs can enhanced the food conversion efficiency of silkworms and which in turn increased the quality and yield of cocoon and silk filament.

Cai *et al.* (2015) reported that TiO2 NPs improved breaking strength and elongation break of modified silk can be improved upto (548±33) MPa and (16.7±0.8) per cent, respectively, by adding 1% nano anatase into the artificial diet in that TiO2 (1%) modified silk shows well-improved ultraviolet resistant properties as the breaking strength only decreased 15.9 per cent after exposing to ultraviolet light for 3h.

Li *et al.* (2016) reported that low concentration of TiO2 could improve feed efficiency and increase cocoon mass, cocoon shell mass, and the ratio of cocoon shell (5 and 10 mg/L) was more effective for weight gain being obtained at 72 h. TiO2 NPs at 20 mg/L or higher had certain inhibitory effects, with significant inhibition to B. mori growth being observed at 48 h. The feed efficiency was significantly improved at low concentrations of 5 and 10 mg/L for 14.6 and 13.1 %, respectively.

The feeding efficiency of silkworm larvae is very much important as it accounts for their growth rate and development. Pandiarajan *et al*. (2016) reported that exposure of silkworm larvae to the lowest doses of AgNPs (1 ppm) improved the growth of larvae and cocoon weight when compared to the highest doses of 10 ppm and 100 ppm.

Tian *et al.* (2016) studied the impact of 5 mg/L of TiO2NPs on the nutrient metabolism of fat bodies of silkworm and he observed the activation of insulin signaling pathway of silkworms thereby enhancing the carbohydrate, protein and fat metabolites when compared to the control group.

Yang *et al.* (2017) studied three different types of nanoparticles such as Cu, Fe and TiO2NPs were added to the silkworm diet. The analysed results showed that the amount of copper produced in silk was only two to three times higher than usual. Significant changes in the morphology, structures and diameter of silk products were observed. The results of this study exhibited that nanoparticles can be used to increase the strength of manufactured silk and silk products.

Alipanah *et al.* (2021) reported that TiO2NPs fed silkworms showed increased cocoon weight to 3.3 and 3.83 per cent respectively to concentrations of 5 and 10 mg/l. In addition to that TiO2NPs improved the feeding efficiency of silkworm larvae, silk protein synthesis and other economic traits of silkworm.

Soliman *et al.* (2021) determined the effects of enrichment of mulberry leaves with different concentrations of Spirulina extracts on some biological parameters of silkworm larval weight, pupal weight, cocoon weight, cocoon shell weight and silk ratio. The total haemolymph protein of the 5th larval instar of silkworm as a physiological parameter was also determined.

# Gut microbial diversity in silkworm

Silkworm gut microbes play an important role by interacting with the host's environmental habitat, diet, developmental stage and phylogeny.

Kalpana *et al.* (1995) reported *Micrococcus* sp. and *Staphylococcus* sp. were found to be present in the gut of silkworm crossbreed (PM × NB4D2).

Neelu Nangia *et al.* (1999) reported bivoltine breed, NB4D2 had a higher load of bacteria (16.5 × 104 cfu/g) than Pure Mysore and PM × NB4D2. Roy *et al.* (2000) reported the presence of bacteria in the gut of mulberry silkworm (*Bombyx mori*).

The bacterial flora associated with the Pure Mysore (multivoltine) and NB4D2 (bivoltine) silkworm midguts were studied during the third, fourth, and fifth instars. In midgut tissues of fifth instar larvae, pure mysore and NB4D2 races had the highest mean of 15 × 106 cfu/g and 28 ×106 cfu/g, respectively. The bacterial flora colonised in the midgut produced amylase, caseinase, gelatinase, lipase, and urease enzymes. Amylase producers compensated for the greatest proportion of isolates, followed by protein and lipid splitters (Chowdary *et al*., 2002).

Tandon and Mishra (2003) isolated *Enterobacter, E. coli* and *Bacillus* sp. from the gut of *Helicoverpa armigera* and described eight species of bacteria including *E. coli, Bacillus* sp. and *Staphylococcus* from the gut of indigenous multivoltine silkworm breeds.

Mohanraj *et al.* (2009) isolated the occurrence of *B. subtilis, S. aureus* and *Pseudomonas* sp. as common gut bacterial flora in several pure breeds and cross breeds of mulberry silkworms. The gut microflora from pure races revealed that bivoltine breed CSR2 observed higher load of bacteria than that of multivoltine breed and Pure Mysore.

Sekar *et al.* (2010) conducted a study to assess the colonization of bacterial strains adherent to the upper and lower sides of the mulberry leaves as well as in the intestinal zones of the silkworm *B. mori* fed on mulberry leaves of various ages (one, two and three weeks old) for 15 days and the region-wise abundance of gut bacteria. *Bacillus cereus* was the dominant species in all age groups of mulberry leaves. Other species found along with *B. cereus* included *Enterobacter, Lactococcus lactis* and *Staphylococcus lactis, Klebsiella pneumoniae* and *Escherichia coli* were also distributed. Among the gut regions such as foregut and midgut, the foregut zone was found to be inhabited by the greater number of bacteria (5.2 to 6.2 cfu × 108) and has been established to have its origin in mulberry phyllosphere microbes.

# Changes in gut microflora due to fortification

Khyade and Doshi (2012) reported various concentration of herbal drug treated with mulberry leaves and fed through 3rd to 5th instar silkworms reflected significant improvement in the levels of proteins (soluble and total) and velocities of biochemical reactions catalyzed by protease and amylase. The activities of midgut protease and amylase were increased by

21.444 to 83.706 % and 14.54 to 52.257 respectively.

Researches have revealed the digestion, absorption, growth, and immune function of *B. mori* under the experimental conditions, i.e., 96-hour exposure to 5 mg/L TiO2NPs (Ni *et al.,* 2015) and summarised the effects of TiO2NPs on the biological functions of *B. mori*.

Gunasundari *et al.* (2016) synthesized silver, chromium, iron, lead and zinc metal nanoparticles using the green synthesis method. Antimicrobial activity of synthesized different metallic nanoparticles were studied. Among these, different metallic nanoparticles chromium and zinc metallic nanoparticles showed good antimicrobial activity against *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli* compared to other metallic nanoparticles.

TiO2NPs increased related dominant bacterial strains at the genus level and significantly improved the expression of genes related to digestion, absorption, and detoxification after entering the *B. mori* intestine with food (Li *et al.,* 2016), assisting *B. mori* growth.

TiO2NPs enhanced resistance by reducing pathogenic bacteria (Xie *et al.,* 2014), thereby assisting the immune system and increasing bacteria involved in detoxification (Li *et al.,* 2020).

Ahmad *et al*. (2020) reported that anti-bacterial and anti-fungal activity of the green synthesized TiO2 NPs using leaf extract of *Mentha arvensis,* titanium tetraisopropoxide act as the precursor and leaf extract act as the reducing agent. The green synthesized TiO2 NPs were characterized and exhibited interesting antibacterial and antifungal activity against selected microorganism.

The green synthesized TiO2NPs were subjected for antibacterial activity against *Staphylococcus aureus* (gram-positive bacteria), *Klebsiella pneumonia* and *E-coli* (gram- negative bacteria). Among this, the green synthesized TiO2 NPs enhanced antibacterial activity compared to chemical synthesized TiO2 NPs which could be potential candidate for environmental and biomedical applications (Aravind *et al.,* 2021).

**Conclusion**

Nanotechnology holds the potential to revolutionize the realms of Sericulture and agriculture, as highlighted in this paper's review. The replication of natural systems emerges as a particularly promising avenue within this technology, yet scientists face the formidable task of comprehending the intricate complexities inherent in these systems. The field of nanotechnology and nanomaterials is experiencing rapid growth in research, presenting opportunities to leverage novel properties of materials at the nano-scale for industrial benefits. Numerous developments are on the horizon, capable of significantly altering the service life and life-cycle cost of construction infrastructure. In essence, this burgeoning area of study has the power to shape a new world in the future.

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