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REVIEW ARTICLE

Radical Scavenging of Nanoceria in Minimizing the Oxidative Stress-Induced Loss of Residual Hearing: A Review

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Abstract | Among the several unresolved issues, profound/total loss of low-frequency residual hearing after cochlear implant fixation is the most frequent event. Even after several attempts such as modifications in the design of electrodes, improvement in the surgical procedures and use of protective drugs to minimize the trauma and its after-effects, residual hearing has been retained in less than 50% patients only. Surgical procedure and mechanical trauma at the electrode insertion site are thought to be responsible for excess generation of reactive oxygen species (ROS) following initiation of inflammatory cytokines resulting into loss of residual hearing due to programmed cell death of essential inner ear structures. Though very recent studies have reported the use of conventional antioxidants to preserve the residual hearing, they have their own limitations. With the emerging need of better and effective antioxidants, nanoceria has spurred immense research interest on utilizing its unique catalytic characteristics for ROS-associated diseases. Nanoceria has shown effective protection against several ROS-induced damages compared to conventional antioxidants such as vitamin C and vitamin E. The objective of the present work is to develop an understanding about the underlying mechanism of loss of residual hearing and propose a novel method based on delivery of nanoceria to minimize it. The first part of the article highlights the failure of cochlear implants, nature of failures and revised surgeries due to loss of residual hearing. Subsequently, the article explores the relation among surgical/mechanical trauma, excess generation of ROS at electrode insertion site, progressive death of hair cells and loss of residual hearing. Finally, effectiveness of radical scavenging characteristics of nanoceria along with controlling parameters and involved mechanisms has been reviewed. The present work also focuses on the limitations and challenges of nanoceria in clinical applications. Based on the literature review, it is hypothesized that the residual hearing loss is associated with excess generation of ROS and it is proposed that the delivery of effective antioxidants/radical scavengers having a good longevity and regenerative ability is expected to reduce the excess level of ROS and retain the residual hearing.

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1 Introduction

Cochlear implants are state-of-the-art devices that are considered to be the most effective and standard treatment solution when the degree of hearing loss is moderate to severe and it is due to the damage of hair cells while auditory nerves are still functioning. Since inception, the design and performance of cochlear implants have improved drastically[1–3]. However, there are some issues including preservation of residual hearing, which are still not resolved and need further investigations and research. The presence of preserved residual hearing improves the quality of sound recovery specifically music and voice quality. In addition, there is a betterment in terms of speech understanding in background noise when compared to hearing from cochlear implant alone[4]. However, in several cases, it has been reported that the residual hearing deteriorates progressively and ultimately to complete loss within a very few months after implantation[5, 6]. Even after several decades of active research, the mechanism of loss of residual hearing has not been fully understood. A number of studies have confirmed that a complete damage-free surgery is not possible during cochlear implantation, where it has been hypothesized that the damage during surgery initiated inflammation and excess generation of reactive oxygen species (ROS). Due to high reactivity, the ROS interacts with the surrounding tissues of the inner ear resulting into cell death of essential structures of the inner ear. Depending on the type of structures affected inside the cochlea, the loss of hearing post-cochlear implantation could be either loss of preoperative residual hearing alone or complete loss of hearing. Although, the cochlear implant bypasses the damaged portion of inner ear, still it requires the presence of healthy auditory nerves to transfer the electrical signals to brain[7–10].

Recently, nanoceria has emerged as a highly promising material in the field of biomedical applications such as bioanalysis, biomedicines, drug delivery devices and tissue engineering owing to its unique catalytic characteristics giving to its multi-enzyme like ability[11, 12]. Since the proposed solution to preserve the residual hearing in a human study is based on scavenging of excessively generated ROS, this review highlights the possible mechanisms, controlling parameters, major challenges and limitations of pharmacological ability of nanoceria.

2 Hearing loss

According to the WHO [2012], 5.3% of the world's population lives with disabled hearing, and among them, 91% are adults and 9% are children. In India, 2.21% of total population suffers different types of disability and 19% of them is due to hearing (Census of India 2012), and among them 67% are adults.

Hearing loss that is commonly encountered in clinical practice has been classified into three following groups, which are based on damaged segment of the auditory system[13]:

1. Conductive hearing loss
2. Sensorineural hearing loss
3. Mixed hearing loss

2.1 Conductive Hearing Loss

Conductive hearing loss is associated with disorders of the outer and middle ear, which interferes the passing of sound waves into inner ear. Some of the common causes for the same are as follows: excessive earwax, a punctured eardrum, ear infections, fluid build-up in middle ear, loss of ossicular continuity and abnormal bone growth in the middle ear. Treatment for conductive hearing loss includes bone conduction hearing aids, bone-anchored hearing devices and middle ear implants[13].

2.2 Sensorineural Hearing Loss

Sensorineural hearing loss occurs when the inner ear or the nerve pathways from cochlea to brain is damaged. In most of the cases, the hearing loss is associated with the inner ear rather than the hearing nerve. This type of loss can be either present at birth or can develop at a later stage. The first one is known as a congenital defect, which can be inherited either from family or by mother having rubella (a contagious viral infection) during pregnancy. The sensorineural hearing loss that occurs at a later stage is known as acquired sensorineural hearing loss and it can be caused by several factors such as exposure to loud noise, aging, certain disease, tumors, injury and side effects from certain drugs.

Treatment for sensorineural hearing loss depends on the severity of loss. In few cases, hearing aids have been used. However, the benefit from hearing aid is limited as sensorineural hearing loss involves not only a raised hearing threshold but also difficulty in speech recognition[14]. Today the most effective and successful solution to treat sensorineural hearing loss is the cochlear implantation[15]. The total number of registered cochlear implant exceeds approximately 324,200 worldwide with approximately 96,000 implants being fixed in the US alone (US Food and Drug Administration 2012).

2.3 Mixed Hearing Loss

A mixed hearing loss occurs when both sensorineural hearing loss and conductive hearing loss are present.

3 Cochlear Implant

A cochlear implant is an electronic medical device that directly stimulates auditory nerves bypassing the damaged portion of the inner ear and thus helps to provide or restore functional hearing in those people, who have moderate to profound sensorineural hearing loss. All the cochlear implant consists of an external unit that is worn on the ear and an internal unit that is surgically implanted under the skin. The external unit also known as a speech processor covers frequency range from 11.5 kHz to 70 Hz. It has three main components: a digital signal processing (DSP) unit, a power amplifier, and RF transmitter coil. The DSP unit contains a microphone that receives the sound. The control unit of DSP extracts information from the sound, and converts them into electrical signals that are sent wirelessly into the internal unit by the RF transmitter link, which is magnetically held in a position. The speech processing parameters can be controlled by a computer fitting program. The internal unit consists of RF receiver and a hermetically sealed stimulator. As the internal unit does not have any battery, RF link helps in transferring required power to the internal units. The charged up stimulator decodes the coded audio signals and convert them into electric currents which is then delivered to appropriate electrodes. All modern systems also contain a feedback circuit to transfer internal responses back to the external unit[16–19].

Materials for the parts of cochlear implant that come in direct contact with the body include silicon, platinum, platinum10% iridium and titanium to fulfill different requirements such as mechanical and long-term stability, electrical requirements and biocompatibility. Due to good flexibility and stability, silicon is used as a covering material over the electrode. The electrical properties of platinum and platinum10% iridium are used as wires and contact points, respectively. Titanium being low weight and high corrosion resistant material is utilized in making sealed enclosure to secure the electronic components[20]. The objective of the present work is an attempt to relate the loss of residual hearing with excess generation of reactive oxygen species and propose nanoceria as a potential solution, which is applicable irrespective of the selection of type of cochlear implant. Therefore, the material aspect of cochlear implant has not been discussed in detail in this review. However, the electrode part goes inside cochlea and, therefore, has been discussed in detail in the next section.

3.1 Cochlear Electrode Array

Electrode array is the portion of the electrode that goes inside the cochlea. The length of electrode varies in the range 15–31.5 mm for different models. Wires of platinum/10% iridium are connected to a tip, which is known as stimulating contacts and are made of platinum. The wires carrying the tips are then arranged such that these tips are at some distances apart. This whole assembly is encased within medical grade silicon in a way that only the contact points are exposed and the rest of the wire is inside the silicon casing, which is non-conducting. The number of wires or stimulating contacts varies in different designs from 12 to 22[17, 21]. Each wire carries distinct frequency of sound.

4 Failure of Cochlear Implants

The consistency in the performance of any implant over time is an important concern for both doctors and patient. Stefanescu et al.[22] performed reliability study of Med-El cochlear implants on 256 patients for a 5-year period throughout Romania, where failure rate of 6.64% and the average duration of functioning of the device before its failure were reported to be 22 months (range being 5–54 months). Furthermore, the failure was categorized into hard failure (59%), soft failure (12%) and failure due to infection (29%). Hard failure refers to detectable hardware problems whereas soft failure refers to underperformance, hearing and/or non-hearing-related problems, side effects, and discontinuous function of the device. The largest report available on the rate of revised surgeries and implant failure is given by Wang et al.[23], who performed the study on 2311 patients receiving a total of 2827 primary cochlear implants over a 30-year period at the Sydney Cochlear Implant Center. Their data revealed the removal of 4.8% (136/2827) of implants due to device-related failure among the total 8.3% (235/2827) failure leading to revised surgeries on both primary and revision implants. Remaining 3.5% (99/2827) implant removal was related to non-device failure, which was further categorized into (1) migration/extrusion (1.9%), (2) infection (1.4%) and (3) secondary pathology (0.53%). Migration/extrusion was defined as displacement of either electrode or the stimulator

from its original point. Infection complications included wound infection and others. Secondary pathology consisted of loss of residual hearing and others such as nonuser of the device and willingness of patient for removal. Figure 1 shows the rate of failure of cochlear implants in different studies during the period of 2001–2016.

4.1 Electric-Acoustic Cochlear Implants: A New Treatment Option

Since inception, the design and performance of cochlear implants have improved drastically. The more recent research focussed on utilizing the intact hair cells in low-frequency region and preservations of the preoperative hearing after cochlear implantation. Hearing capability before implant fixation is known as residual hearing. The earlier practice involved insertion of standard length electrode irrespective of whether or not patients had residual hearing. Later, it was realised that most of the age-related and noise-induced hearing loss were confined in the basal region and the patients had measurable low-frequency hearing. To cover the lower frequency range (up to 200 Hz), the insertion of electrode should be at least 540° distance measured from the round window. Studies have shown that electrode insertions cannot be made beyond a depth of 450° due to limitations on design and manufacturing[19]. An insertion depth of 400° or less has been achieved without much resistance beyond which, it results in more trauma and damage to inner ear structure due to increased resistance. Adunka and Kiefer[24] have studied the insertion trauma associated with partial, where first resistance was felt and full insertion of electrode using 21 human temporal bones implanted with cochlear electrode. The mean insertion depth for partial and full insertion was 20.3 mm (305°) and 30.8 mm (535°), respectively. The full insertion group showed the highest grade 4 level trauma with 77.8% due to fracture of the osseous spiral lamina compared to partial insertion group, where grade 4 trauma was reported to be about 16.7%.

Less damage with short length electrode and preserved residual hearing post-cochlear implantation in several patients made researchers believe that combining the electrical stimulation in the high-frequency region by partial electrode insertion and acoustic hearing in low-frequency region will improve the speech understating in patients. Gantz and Turner[1] studied the effect of integration of electrical stimulation and acoustic hearing on speech perception in patients who had only high-frequency hearing loss. It was found that the placement of up to 10 mm electrode did not damage the low-frequency inner hair cells and this technology improved word recognition in patients. Since electric-acoustic technique takes the advantage of intact hair cells in low-frequency region, therefore, any successful implementation will require preservation of the residual hearing.

4.2 Statistical Data on Failure Rate Due to Loss of Residual Hearing

Promising results of electro-acoustic stimulation compared to electrical stimulation alone have been achieved in many studies. However, there have been a number of cases, where post-implantation-hearing preservation was achieved only for a short duration and gradually deteriorated over months and finally to complete deafness in some cases (Fig. 2). Barbara et al.[5] measured the hearing threshold of patients with measurable preoperative residual hearing, 1 week post-implantation and hearing threshold at later stage (6–87 months) for eight cochlear recipients. All the patients retained the residual hearing 1 week after implantation. However, the residual hearing dropped to complete loss in 50% patients after a mean duration of 33.5 months from surgery. Gstoettner et al.[6] performed long-term hearing preservation study on 23 cochlear recipients having measurable preoperative low-frequency hearing. They found the preserved hearing in 39% cases (nine patients) over a mean duration of 29 months (range 7–70 months), partial preservation of hearing in 30% cases (seven patients), which was stable up to 6–70 months (average 25.0 months), delayed and complete loss of hearing in 22% cases (five patients) over 12.6 months (range 7–18 months) after surgery. Woodson et al.[4] reported complete loss of residual hearing in 10% patients (8/81), in which 2% experienced the loss within 1 month of surgery. Gantz et al.[2] reported a total loss of hearing in 2 ($N=87$) cochlear implant recipients within 1 month following surgery and in six patients between 3 and 24 months following implantation. The clinical trial data (Food and Drug Administration (FDA) USA 2014) of 50 cochlear implant recipients showed that 44% patient (22/50) experienced profound/total loss of low-frequency residual hearing. The loss was experienced within 6 months after cochlear fixation in 17 (34%) patients while remaining 10% (5/50) experienced the loss between 1 and 4 years. The more recent clinical trial data (FDA 2016) demonstrates profound loss of residual hearing in 10% (7/67)

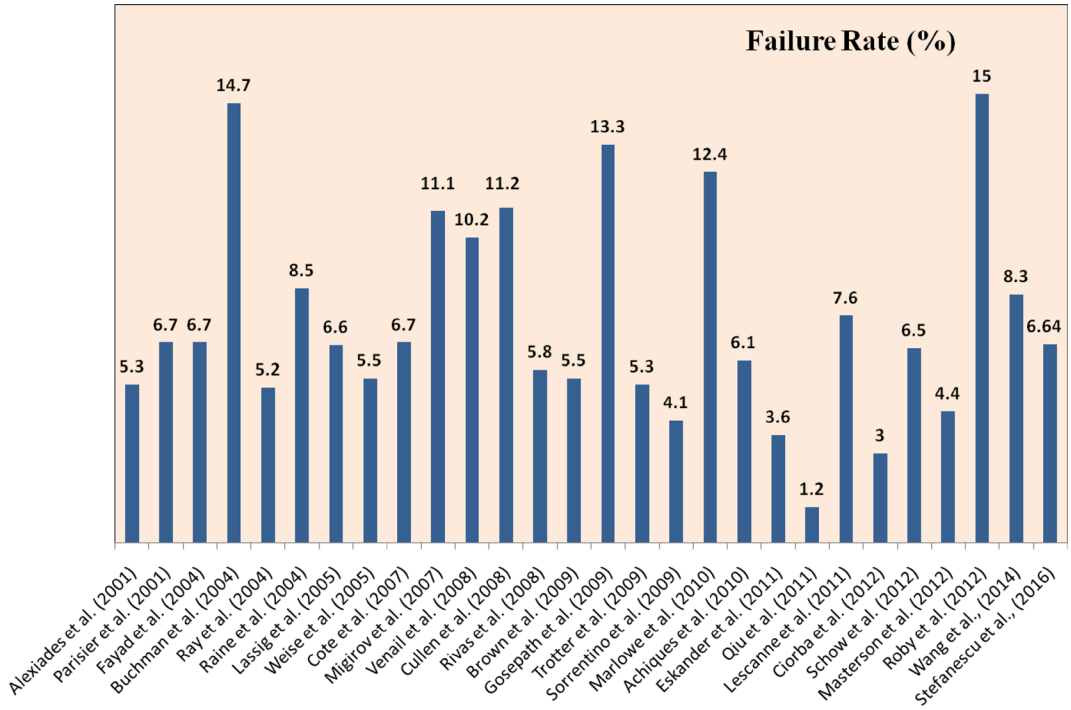

Figure 1: Statistical data of failure of cochlear implants from 2001 to 2016[23].

patients and severe loss in 35.8% (24/67) patients after 1 year of implantation. In one patient, a complete loss of residual hearing was reported immediately after cochlear fixation.

4.3 Reactive Oxygen Species, Oxidative Stress, Natural Antioxidant Functions and ROS-Induced Damages on Internal Organs

Reactive oxygen species (ROS) are normally unstable and highly reactive because of having an unpaired electron in their outer orbit, which are produced during normal biological process. The most common ROS are superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). At physiological concentrations, ROS is helpful in several cell signaling and regulation of cell activity. However, the excess level of ROS is very harmful as it is very reactive and if not controlled can react with proteins, lipids, amino acids, DNA, etc., causing cell death, mutations and/or aging of cells. A condition in which the ROS generation is more than the body can neutralize is termed as oxidative stress, which is responsible for a number of diseases[25]. Many major diseases have been confirmed to be associated with oxidative stress and it is shown in Fig. 3[26].

Under healthy conditions, the concentration of reactive oxygen species is controlled by the antioxidant enzyme system. Body produces two types of antioxidant enzyme, enzymatic and non-enzymatic. Enzymatic antioxidant directly removes ROS by converting them into more stable form whereas non-enzymatic antioxidants such as vitamin C, vitamin E, and carotenoids work by interrupting the free radical chain reactions[27]. Two of the primary endogenous enzymatic antioxidants that directly remove ROS are superoxide dismutase (SOD) and catalase. The SOD reacts with superoxide radical (O_2^-) and converts it into hydrogen peroxide (H_2O_2) and oxygen (O_2)[28].

The SOD reactions to neutralize superoxide (Eqs. 1, 2) are suggested by Xu and Qu[11], which are given below:

$$M^{(n+1)+} \text{ - SOD} + O_2^{\bullet -} \rightarrow M^{n+} \text{ - SOD} + O_2, \quad (1)$$

$$M^{n+} \text{ - SOD} + O_2^{\bullet -} \rightarrow M^{(n+1)+} \text{ - SOD} + H_2O_2. \quad (2)$$

Catalase neutralizes hydrogen peroxide and converts it into oxygen and water and the corresponding reactions are given below (Eqs. 3, 4):

$$H_2O_2 + \text{Fe(III)} - \text{E} \rightarrow H_2O + \text{O} = \text{Fe(IV)} - \text{E}^{\bullet +}, \quad (3)$$

$$H_2O_2 + \text{O} = \text{Fe(IV)} - \text{E}^{\bullet +} \rightarrow H_2O + \text{Fe(III)} - \text{E} + O_2. \quad (4)$$

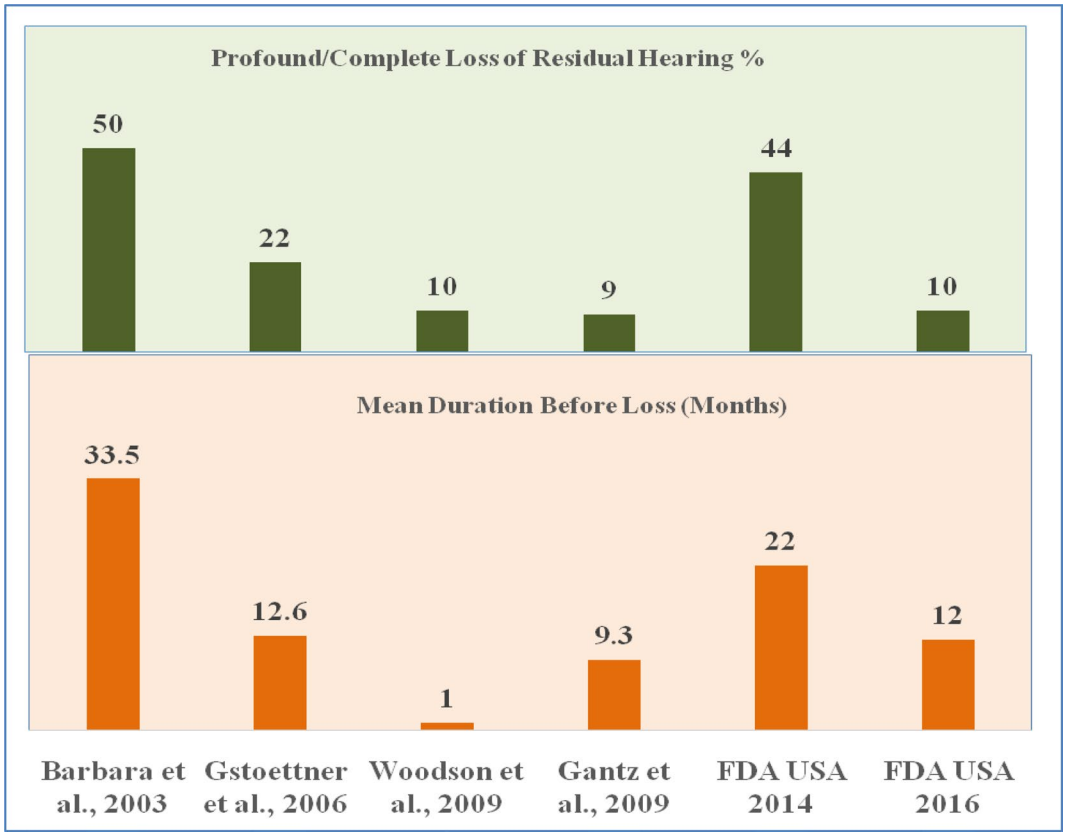

Figure 2: Statistical data on failure of implants due to loss of residual hearing.

4.3.1 Relation Among Electrode Insertion, Damage to Surrounding Structures and Loss of Residual Hearing Due to Reactive Oxygen Species

Bas et al.[7] have confirmed initiation of inflammatory cytokine such as TNFα and oxidative stress conditions due to increased level of ROS at the trauma site following cochlear implantation. O'Leary et al.[8] carried out in vivo study on 73 guinea pigs and have confirmed the inflammation and injury after cochlear implantation which resulted into tissue reaction causing progressive hearing loss after 4 weeks. Bas et al.[9] in their animal model study have also confirmed significant reduction in number of outer hair cells throughout cochlea length following insertion trauma. At the basal region, reduction was highest with a total loss of 73% and the same was reduced to 54 and 48% at middle and apex, respectively. They have also reported 23% loss of inner hair cells, which was limited only in the apex region. Few drug therapy studies in animal models such as delivery of corticosteroids, anti-inflammatory agents and antioxidants have been carried out in an attempt to preserve the residual hearing following electrode fixation. Protective effect of antioxidant NAC (*N*-acetylcysteine) on hearing has been reported after 4 weeks of cochlear implantation. The protective effect was believed to be due to reduced level of free radicals. However, the said benefit was limited only in the high-frequency region[10]. Optimum concentration of dexamethasone (DXMb) required to effectively protect hair cells and neural elements against electrode insertion damages were studied on guinea pig (350 g) model using DXMb eluting electrode. After 90 days of implantation, it was found that auditory brainstem response (ABR) thresholds that elevated immediately after implantation reduced with time and approached to pre-implantation level in 10% and 1% DXMb eluted electrode samples for all the frequencies (0.5–16 kHz), however, only partial recovery was achieved in case of 0.1% DXMb. Effective protection of outer hair cells in all regions was also achieved for 10% and 1% DXMb eluted electrode[9]. These studies supported that the underlying mechanism in loss of low-frequency residual hearing is associated with excess generation of ROS, which has been schematically explained in Fig. 4.

4.3.2 Conventional Method Using Natural Antioxidants to Treat ROS-Induced Damages and Their Limitations

If the production of ROS is more than the antioxidant defense system of a body, antioxidants should be delivered to prevent the damage by excess ROS. Conventional antioxidants such as vitamin E, vitamin C, amifostine, and trolox have been tested to study their ability to scavenge ROS. However, such antioxidants have not been found to be 100% effective. The limited success by these antioxidants was due to poor uptake by cells resulting into unsatisfactory levels at the injury site. Therefore, the dose requirement for effective protection is very high. Furthermore, they fail to provide protection for longer duration due to short degradation time. In addition, they are sometimes unable to penetrate to the radical production sites. In addition, the scavenging capability of many conventional antioxidants is limited to only one type of reactive oxygen species[11].

4.3.3 Novel Approach Using Synthesized Antioxidants/Radical Scavengers to Treat ROS-Induced Damages (In Vitro and In Vivo Studies)

After testing nanoceria in several in vitro and in vivo models[30–35], it has been confirmed that nanoceria is biocompatible and can effectively bind different reactive oxygen species such as superoxide, hydroxyl and hydrogen peroxide. The studies have also shown effective protection against the cell damages due to the excess level of reactive oxygen species. A comparative study on the protection against ROS-induced cell death by nanoceria and amifostine (the most effective free radical scavenger) showed a better ROS scavenging and protection by nanoceria compared to amifostine[33]. The antioxidant activity of oleic acid-coated nanoceria with a particle size of 3.8 nm and coating thickness of 2.5 nm was found to be 9 times higher than trolox (a water-soluble derivative of vitamin E)[36]. Furthermore, ceria nanoparticles are found to be more durable compared to conventional antioxidants, and are believed to provide long-term protection. In addition, nanoceria can scavenge more than one ROS species as well.

Several studies have been performed to measure the effect of nanoceria on ROS scavenging and protection against ROS-induced damage. Chen et al.[30] studied the protective effects of nanoceria (5 nm size and 1, 3, 5, 10 and 20 nM concentrations) against ROS-induced damage, which was induced by exposure to H_2O_2 (1 μM, for 30 min) in cell cultures study and exposure to

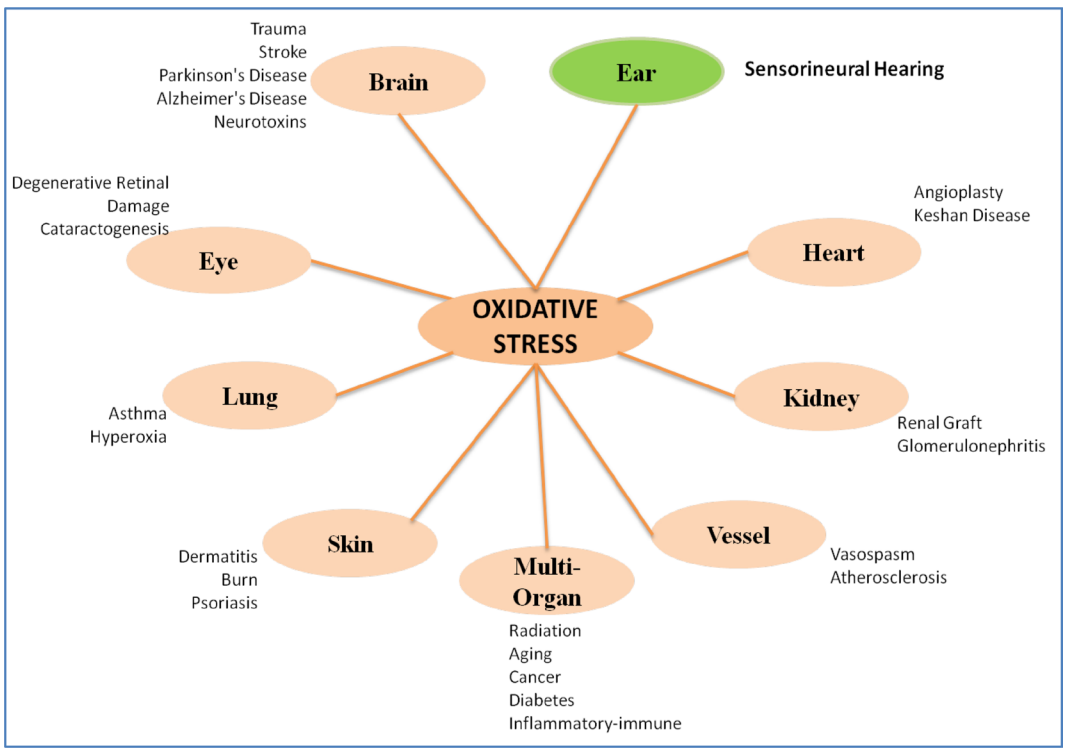

Figure 3: Diseases associated with oxidative stress conditions[26]—Reproduced by permission of The Royal Society of Chemistry.

a high intensity of light in an albino rat model. It is reported that the nanoceria solution having 5 nM showed best protection by reducing the ROS intensity by threefold (in vitro). Injection of 0.1, 0.3 and 1 μM concentration of nanoceria in both the eyes of rat model prior to the exposure of high-intensity light showed nearly 100% protection at 1 μM concentration under in vivo condition. Tsai et al.[32] studied the free radical scavenging ability of nanoceria by treating the test cells with CeO_2 (2–6 nm, 0, 200 and 100 μM, for 8 h) followed by exposure to hydroquinone (a free radical generator). Generation of free radicals was suppressed by 39% in those cells that were treated with nanoceria at 100 μM prior to exposure to hydroquinone. The presence of nanoceria (2–5 nm in 10 nM doses) ensured long-term survival of spinal cord neurons of adult rat[31]. Protection against H_2O_2 insult by nanoceria and B27 that contains five antioxidants: vitamin E, vitamin E acetate, superoxide dismutase, catalase, and glutathione were also compared. Cultures that were pretreated with nanoceria had significantly higher number of live cells compared to control culture having B27, where number of live cells was less than half. Radiation-induced cell death is also associated with excess generation of ROS and its scavenging by nanoceria (3–5 nm) was performed by exposing the lung fibroblast cells to radiation with and without pre-treating with nanoceria (10 nM or 0.0017 μg/mL) and measuring the cell viability under in vitro conditions after 48 h of irradiation. Significant protection against radiation-induced damage was found when the cells were pretreated with nanoceria prior to their exposure. Similar protection was also observed during in vivo study[33]. During radiation therapy for cure of cancer, there is inadvertent radiation damage to surrounding healthy cells due to the formation of excess ROS. Colon et al.[37] investigated the protective effects of nanoceria (3–5 nm) on normal human colon cells against radiation-induced damage. First, they confirmed that pretreatment of cells with nanoceria (1, 10 and 100 nM) did not allow excess generation of ROS upon exposure to radiation using ROS detection kit in combination with fluorescent microscopy. Cell survival study showed 15% reduction in the untreated cells whereas near 100% protection for cells treated with nanoceria for all the three concentrations. In vivo study performed on mice model (25 g) showed 50% less cell death, when nanoceria was injected (60 nM, 0.00004 mg/kg) prior to radiation exposure. Similar protection by nanoceria against radiation-induced damage on cells was also reported by Zholobak et al.[38]. While ceria nanoparticles have shown to be promising antioxidants in several in vitro and in vivo studies, the design parameters to develop nanoceria with optimum ROS scavenging are yet not defined. This is due to the lack of techniques to quantitatively measure the antioxidant characteristics. The oxygen radical absorbance capacity assay is the standard method to quantify antioxidant properties of nanoscale antioxidants. In this method, the intensity of a fluorescent indicator is measured in the presence of free radical initiator alone and in the presence of free radical initiator along with antioxidant. The free radical reacts with fluorescent indicator and the intensity goes down. In the presence of antioxidants, the free radical reacts with them and the intensity of the fluorescent indicator is preserved.

A UV–Vis spectrum of methyl violet showed a maximum absorbance at about 582 nm. Methyl violet reacts with hydroxyl radicals, and thus the intensity of methyl violet is decreased in the presence of hydroxyl radicals. This concept was utilized by Xue et al.[35] to study the hydroxyl scavenging characteristics of nanoceria. Hydroxyl radicals were generated by adding H_2O_2 to $FeSO_4$ and UV–Vis spectra were obtained for MV, $MV + FeSO_4 + H_2O$ and $MV + CeO_2 + FeSO_4 + H_2O_2$ after different incubation time (5, 10, 15, 20 and 25 min). The effectiveness of the scavenging ability was measured in terms of change in absorbance. The protection against hydroxyl radical by nanoceria (5–10 nm) was 25%, 67% and 80% for 1, 10 and 100 nM concentrations, respectively. The protection was reduced by 17%, when the size of nanoceria was increased to 15–20 nm from 5 to 10 nm for 10 nM and 100 nM concentrations.

Estevez et al.[34] measured ROS for both the tests cells treated with and without nanoceria after ischemic insults. Test cells treated with

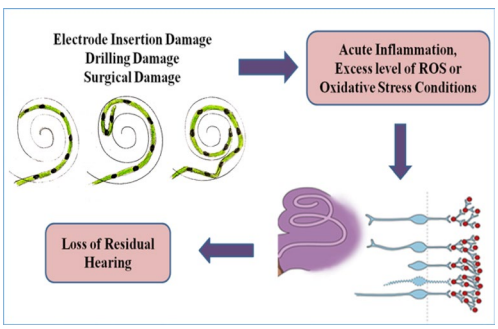

Figure 4: Possible mechanism of loss of progressive residual hearing[18, 29]. (Reprinted by permission of American Scientist, magazine of Sigma Xi, The Scientific Research Honor Society).

nanoceria (10 nm) showed a reduction of 30% in total ROS (peroxyl ROO·, HO· − radicals and ONOO − radicals) and 15% reduction in NO radicals and superoxide radicals in comparison to cells without nanoceria treatment.

4.3.4 Effect of Dose/Concentration on Protection by Nanoceria in ROS-Induced Models

ROS scavenging characteristics of nanoceria are dose-dependent. Higher concentration may induce toxicity while low concentration might not give optimum protection. Several studies have been performed to test the maximum concentration that can be well tolerated in the biological systems and optimum concentration of nanoceria that provides effective protection. Tsai et al.[32] have reported the detrimental effects of large concentrations of nanoceria (>200 μM) to the test cells and 54% reduction in number of cells after being exposed to 200 μM CeO_2 for 48 h. Colon et al.[33] reported that the nanoceria (3–5 nm) concentration of up to 135 mg/kg in mice was well tolerated. MPTP is a pro-drug which causes permanent symptoms of Parkinson's disease by destroying dopaminergic neurons in the brain. Protection against MPTP-induced damage in a mice model by nanoceria (10 nm) with 0.05, 0.5, 5 and 50 μg/g concentrations showed the highest protection (70%) at 0.5 μg/g concentration[39]. Estevez et al.[34] have also reported optimum protection (50%) at 1 μg/ml concentration of nanoceria (10 nm) whereas reduced effectiveness at higher concentration (2 μg/ml) due to sedimentation of particles. Kim et al.[40] studied neuroprotective effects of nanoceria as well as the optimal concentration for the neuroprotection against ischemic stroke. They found that the low concentration (0.1 and 0.5 mg/kg) and high concentration (1.0 and 1.5 mg/kg) failed to show considerable protection whereas the intermediate concentration (0.5 and 0.7 mg/kg) showed about 50% protection compared to control group ($p<0.05$). D'Angelo et al.[41] tested four different concentrations of nanoceria (50, 100, 150, 200 μg/ml, 6–16 nm) against Aβ-induced neurotoxicity, which has been associated with excess generation of ROS and found best protection (100%) at 100 μg/ml. DeCoteau et al.[42] observed best results at 20 mg/kg concentration using 3.3 nm nanoceria in their animal model.

5 Synthesis of Nanoceria

Factors that need to be considered while synthesizing nanoceria for biological applications include nontoxicity of the solvents, surfactants and chemical reagents. In addition, the produced particles should be free of sharp edges to avoid any damage during cell interactions. A large number of articles in the field of synthesis of ceria nanoparticles for biomedical applications and parameters controlling its redox behavior have been published in the past decade. Das et al.[43] reviewed extensively on different traditional synthesis methods of ceria nanoparticles such as precipitation, microemulsion, hydrothermal, and sol–gel and effects of physiochemical properties on catalytic activity and biological response. The most common method of synthesis via precipitation method is shown in Fig. 5.

For biomedical applications, nanoceria has also been synthesized using green methods which minimize the use of toxic substances and utilize safer precursors such as egg white and plant extracts[45–47].

6 Biological Characteristics of Nanoceria

6.1 Antioxidant Characteristics

Factors that play significant role in determining the redox and catalytic properties of nanoceria include particle size, phase modification, structural defects and chemical nonstoichiometry[48, 49]. The defective sites are considered as the sites for catalytic reactions. When the size of the nanoparticle is decreased, the surface area and the density of defects at the surface are increased leading to improvement of the catalytic activity and radical scavenging abilities of these materials. Hydroxyl scavenging ability of nanoceria having size range of 5–10 nm was found to be better than the 15–20 nm size particles. This was due to higher concentration of Ce^{3+} at the surface of smaller particles, i.e., 30.4% for size range 5–10 nm in comparison to larger particles, 20.9% for size range 15–20 nm[35]. Korsvik et al.[50] studied the effect of particle size on SOD activity of nanoceria and found that the particle size in the range of 3–5 nm with a 40% Ce^{3+} concentration showed better SOD activity in comparison to powder that was in the range of 5–8 nm size with 22% of Ce^{3+} concentration[51].

6.2 Superoxide Dismutase (SOD) and Catalase Activity

The ability of ceria to oscillate between Ce^{3+} and Ce^{4+} state makes them to behave like SOD enzyme. Korsvik et al.[50] have confirmed the SOD activity of nanoceria by measuring the produced H_2O_2 using horse-red peroxidase assay, when ceria was added in a solution containing superoxide. Based on the reaction mechanism available

for known SOD enzymes like Fe-SOD or Mn-SOD, they have also proposed the dismutation of superoxide by nanoceria (Eqs. 5, 6):

$$Ce^{4+} + O_2^{\bullet -} \rightarrow Ce^{3+} + O_2, \quad (5)$$

$$Ce^{3+} + O_2^{\bullet -} + 2H^+ \rightarrow Ce^{4+} + H_2O_2. \quad (6)$$

Figure 6 shows a more detailed mechanism of reduction of superoxide by nanoceria[26, 30]. Oxygen vacancies or defective sites are metastable states that convert radicals such as superoxide and peroxides into oxygen ions which migrate into ceria lattice. The superoxide radical binds itself at oxygen vacancy site around two Ce^{3+} cation (4) and is released as H_2O_2 by taking two protons from the solution oxidizing one of the Ce^{3+} to Ce^{4+} (5). Binding of another superoxide results in release of another H_2O_2 and conversion of 2nd Ce^{3+} to Ce^{4+} (6). The released H_2O_2 binds itself at oxygen vacancy around two Ce^{4+} (1) and is released as oxygen molecule reducing both the Ce^{4+} to back to its initial state Ce^{3+} (3). Therefore, the complete reduction of two superoxides involves oxidation of 2 Ce^{3+} to 2 Ce^{4+} and back to its initial state Ce^{3+} by generating one molecule of unreacted H_2O_2 and oxygen. From the explained mechanism, it is clear that a high concentration of Ce^{3+} is desired for improved SOD activity.

The second molecule of unreacted H_2O_2 may further bind itself at the oxygen vacancy around the reduced $2Ce^{3+}$ (4) and oxidize it to $2Ce^{4+}$, reducing itself to water (5). This reaction resembles like catalase activity and is given in Fig. 7.

The overall reduction of two superoxides into oxygen and water can be seen as conversion of $2Ce^{3+}$ to $2Ce^{4+}$ through a SOD activity followed by a catalase activity.

From the above mechanisms, it can be inferred that H_2O_2 can bind itself at the oxygen vacancy around Ce^{3+} and Ce^{4+} both. However, it has been confirmed that rate of reaction of H_2O_2 with Ce^{4+} is high compared to the reaction rate of H_2O_2 with Ce^{3+}. Reaction rate of nanoceria having 6.69% of Ce^{3+} with H_2O_2 has been reported to be 9.08×10^{-4} and 2.71 nmol/min from Red Amplex and UV spectroscopic study, respectively. However, this rate was found to be five times lower than that of the nanoceria having 28.83% Ce^{3+} concentration[51].

6.3 Regenerative Characteristics

The regenerative characteristics of nanoceria have been attributed to the inter-conversion of the two oxidation states Ce^{3+} and Ce^{4+}. As per Chen et al.[30], the reactions involved in regeneration have been given in the following equations:

$$Ce^{3+} \rightleftharpoons Ce^{4+} + e^-, \quad (7)$$

$$Ce^{3+} + OH^{\bullet} \rightarrow Ce^{4+} + OH^-, \quad (8)$$

$$Ce^{4+} + O_2^{\bullet -} \rightarrow Ce^{3+} + O_2. \quad (9)$$

The regenerative or autocatalytic characteristics of nanoceria (3–5 nm) have also been demonstrated in a UV–Vis spectroscopic study. Figure 8 shows UV spectrum of nanoceria solution with and without the addition of H_2O_2 over time. A shift in the spectrum at day 0 can be seen upon addition of H_2O_2, which reverse back to spectrum of nanoceria without H_2O_2 on days 15 and 30. The same shift can be observed again when H_2O_2 was added at day 30 and reversal on day 45[31].

The regenerative ability of nanoceria is believed to eliminate the need for repetitive doses as in the case with conventional antioxidants like vitamin C and E[30]. However, the regenerative activity has not been tested in biological systems.

7 Limitations of Nanoceria as Antioxidant

Section 4.3.3 indicates that the radical scavenging of nanoceria is inefficient even at optimum concentration. This is believed to be associated with less defects present in the nanoparticles, which has been discussed in Sects. 6.1 and 6.2.

Another drawback that limits the usefulness of ceria in clinical trials is the difficulty to dissolve or disperse in biological fluids. Being a ceramic material, nanoceria is insoluble in water or saline, which is commonly used as a carrier liquid/medium during drug delivery. The homogeneity of the nanoceria solution is essential for biological applications to ensure homogenous delivery and uniform CeO_2 concentration in the vicinity of cells. This limits its potential clinical application.

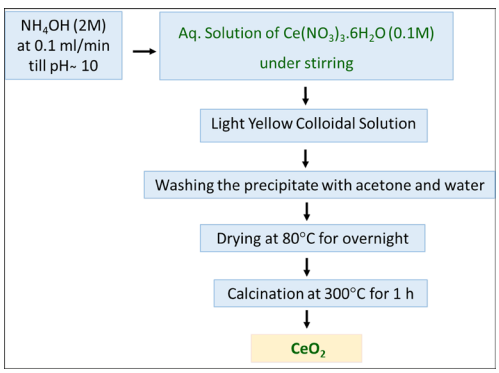

Figure 5: Synthesis of nanoceria via precipitation method [44].

8 Methods to Overcome Limitations of Nanoceria

8.1 Increasing the Lattice Defects by Introducing Zirconium

As the protective effect of nanoceria is believed to be due to the presence of defects in ceria lattice, the radical scavenging effect of the same could be improved by increasing the defects in the lattice by introducing zirconium in it.

Since ionic radius of Zr (IV) (0.084 nm) and room temperature crystal structure of zirconia (monoclinic and tetragonal) differs from that of ceria (ionic radius of Ce^{4+} is 0.097 nm and crystal structure is cubic), the incorporation of zirconium into ceria lattice brings its structural distortion, which controls the catalytic characteristics of ceria–zirconia solid solutions. In pure stoichiometry CeO_2, all the oxygen anions are at a distance of 0.2312 nm from each cerium cation. Replacement of some of the cerium cations by zirconium cations results into significant disorder in the structure, which also brings disturbance to the oxygen sublattice altering the distance between cation and oxygen. In case of bulk $Ce_{0.5}Zr_{0.5}O_2$, the length of

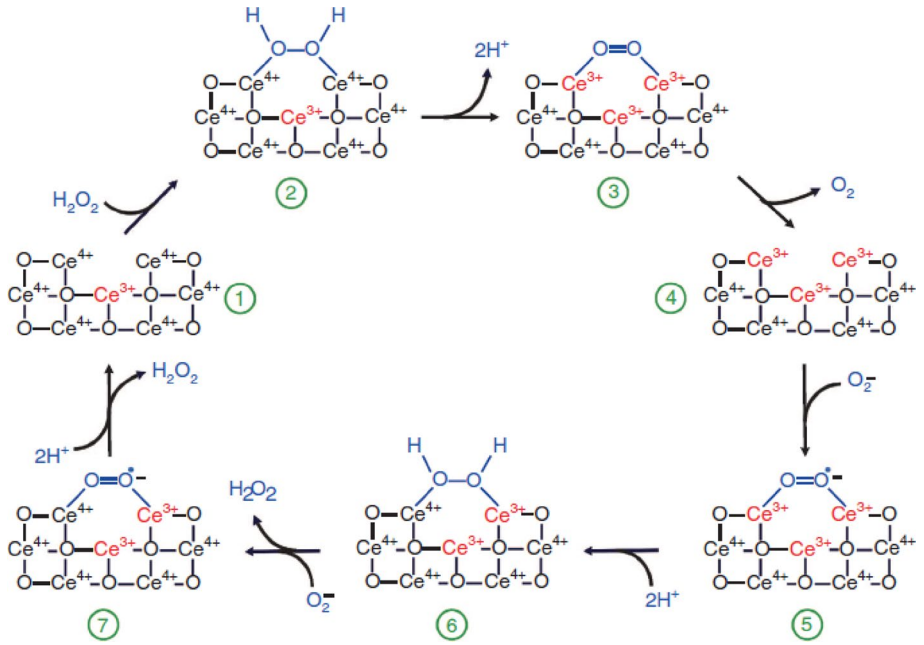

Figure 6: Mechanism of reduction of superoxide by Nanoceria (SOD activity)[26]—Reproduced by permission of The Royal Society of Chemistry.

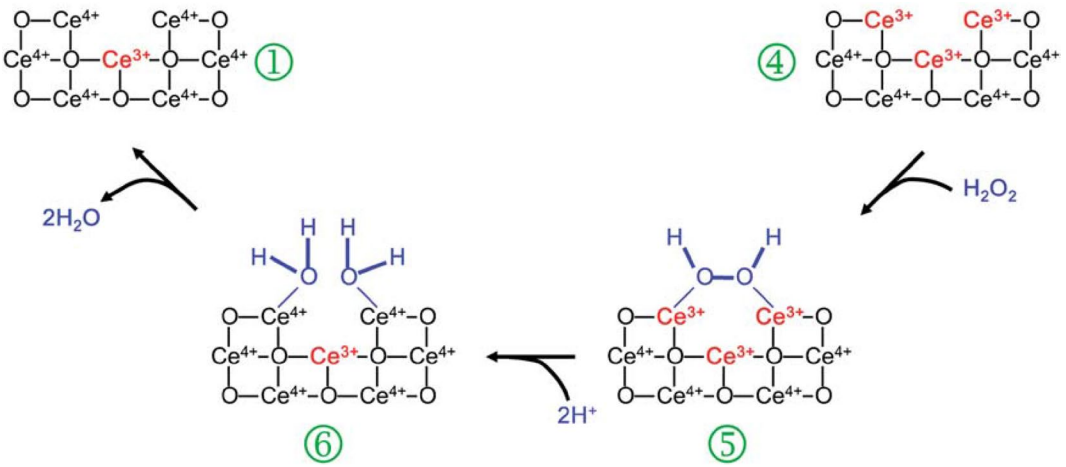

Figure 7: Mechanism of reduction of H_2O_2 by nanoceria (catalase activity)[26]—Reproduced by permission of The Royal Society of Chemistry.

shortest and longest Zr–O bond is observed to be 0.2115 nm and 0.26 nm, respectively. The longer Zr–O distance is responsible for lower energy for oxygen removal leading to improved catalytic activity[52]. Andriopoulou et al.[53] studied the evolution of structure and oxygen vacancies in ceria–zirconia solid solutions using Raman spectroscopy and have confirmed the presence of more oxygen vacancies with an increase of Zr concentration. Apart from oxygen vacancies in nanoceria and nanoceria–zirconia solid solutions, Frenkel-type defects also exist due to the mobility of oxygen atom from the tetrahedral site to otherwise empty octahedral sites[54].

Oxygen storage capacity (OSC) that measures the catalytic ability of ceria and its solid solutions is found to improve upon incorporation of zirconium into ceria lattice compared to pure ceria due to structural distortions, which reduce the energy for oxygen removal. In addition, introduction of zirconium in ceria lattice increases the oxygen vacancies. Madier et al.[55] studied the effect of concentration of zirconium in ceria lattice on oxygen storage capacity, redox properties and oxygen exchange process in $Ce_xZr_{1-x}O_2$ ($x=1$, 0.68, 0.63, 0.5, 0.15 and 0). The highest OSC was found for $Ce_{0.63}Zr_{0.37}O_2$ which was 2.7 times more than the pure ceria. Reddy et al.[56] have reported OSC of $Ce_{0.75}Zr_{0.25}O_2$ (4.7 nm) to be four times better in comparison to pure ceria. Priya et al.[57] studied five different ceria–zirconia compounds and reported highest OSC value for $Ce_{0.6}Zr_{0.4}O_2$ (0.147 $\mu molO_2/g$ CeO_2). Montini et al.[52] have also reported optimum value of oxygen storage capacity for 20–40% of Zr in ceria lattice, i.e., for $Ce_xZr_{1-x}O_2$ when $0.6<x<0.8$.

8.2 Dispersion of Nanoceria in Liquid

To obtain homogenous and better dispersed nanoceria solution, few researchers have synthesized coated nanoceria. The coating should be thin enough to allow the surface reactions and stable for longer duration. Perez et al.[58] synthesized hydrophilic dextran-coated nanoceria of average size of 4 nm using precipitation method and assessed the effect of the coating on its autocatalytic characteristics at physiological and acidic pH. The dextran-coated nanoceria revealed excellent stability in phosphate-buffered saline (PBS) at the concentrations of 40 mM or more for months. Even upon centrifuging at 8000 rpm for 30 min, there was no sedimentation. Further, effect of coating on the autocatalytic characteristics was

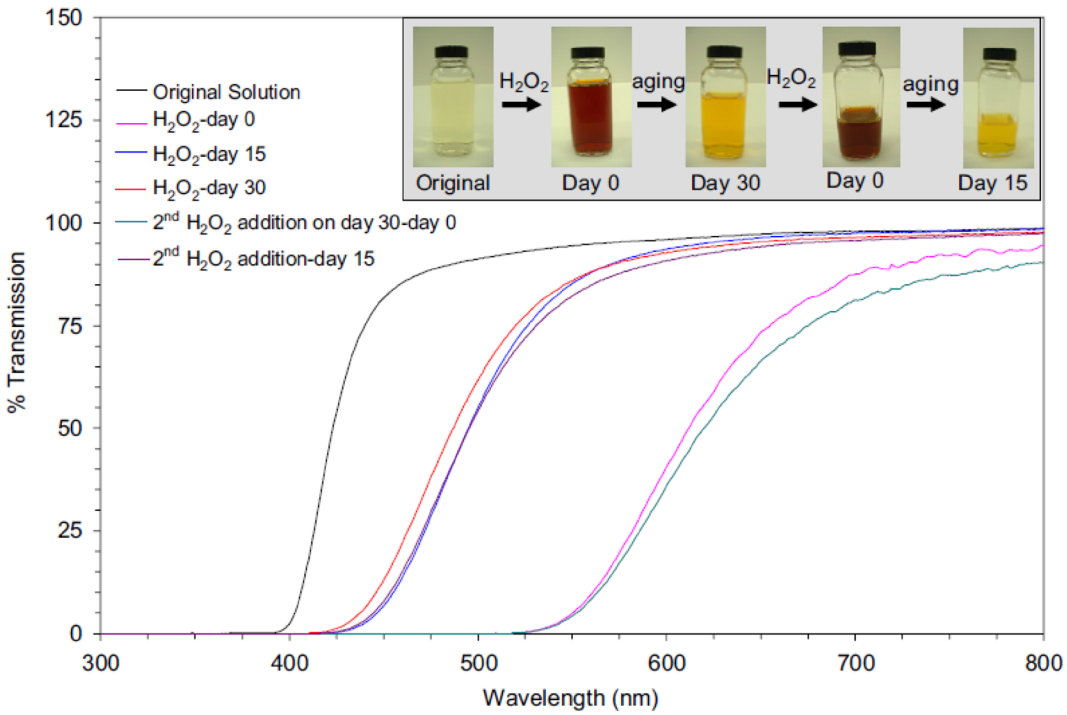

Figure 8: UV spectra of nanoceria with time after addition of H_2O_2[31]. (Reprinted from Biomaterials, 28/10, M. Das, S. Patil, N. Bhargava, J. F. Kang, L. M. Riedel, S. Seal, and J. J. Hickman, Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons, Pages No. 1918-1925, Copyright (2019), with permission from Elsevier).

studied at physiological and acidic pH using UV spectrometer and the results are shown in Fig. 9. It was found that coating did not alter the autocatalytic characteristics. Upon addition of H_2O_2 to dextran-coated nanoceria (2.54 mM) solution in PBS having the pH value of 7.4, the concentration of Ce^{4+} was increased and then changed back to its initial value after 7 days, whereas the reverse mechanism did not occur at the pH value of 4. In contrast, Karakoti et al.[59] believed that acidic conditions (pH 2.5–3.5) favor Ce^{3+} oxidation state of nanoceria and have reported the conversion from Ce^{3+} to Ce^{4+} immediately upon addition of H_2O_2 to PEG-coated nanoceria in PEG solution and reversion to Ce^{+3} state after 7 days of aging. In addition, these coated nanoparticles did not show any cytotoxicity and showed over 80% protection against H_2O_2-induced damage performed using MTT assay. However, it is also reported that dextran is not very commonly used as drug delivery medium.

As dextran or chitogen are not commonly used as a drug delivery medium, Karakoti et al.[59] synthesized PEG-coated nanoceria (3–5 nm) and studied the effect of different volume fraction of PEG during synthesis on cell viability, SOD and redox activity. Cell viability studies using MTT assay showed that exposure to PEG-coated nanoceria in 100 μM concentration and less did not show any toxicity for any volume fraction of PEG up to 80% for 72 h. The SOD activity using ferricytochrome C and redox property using H_2O_2 also showed that coating and different volume fraction of PEG did not affect its SOD activity. However, these concentrations have influence on its regenerative property and the regeneration is achieved faster in case of 5–40% of PEG.

Water-soluble chitosan-coated nanoceria (3–5 nm) synthesized using wet chemical method have also shown good hydroxyl scavenging characteristics when the thickness of coating was not very high or the concentration of chitosan added during synthesis was below 24.65 mM. The stability and antioxidant characteristics of coated powder at physiological pH remained unaffected even after 7 months for nanopowder synthesized at 1:1 molar ratio of glucose units to cerium(III) ion[60].

Lee et al.[36] prepared oleic acid-coated nanoceria solutions having two different sizes of 3.8 and 8.2 nm. They studied the stability of the solutions over time, and the effect of coating and its size on the ROS scavenging ability of solution. The colloidal solutions of the coated nanoceria having 3.8 nm were found to be stable for over 6 months. Colorimetric tests carried out by adding H_2O_2 in ceria solution and measuring shift in wavelength showed that the oleic acid-coated 3.8-nm size ceria particle did not affect the free radical scavenging ability of nanoceria. However, the similar results were not observed in case of 8.2-nm size particle.

Caputo et al.[61] synthesized biocompatible nanoceria (10 nm) using ethylene glycol precipitation method followed by surface modification using silane molecules, (6-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-hexyl) triethoxysilane (MEEETES) to improve its dispersibility in biological fluids. Excellent hydroxyl scavenging was observed in their electron spin resonance (ESR) measurements and effective protection against H_2O_2-induced damage in cell studies at concentration 200 μg/mL.

9 Conclusion

Retaining the residual low-frequency hearing after cochlear implantation is very challenging considering the fact that the exact mechanism of the loss is not yet well understood. Several attempts in the past have been made to prevent the loss such as modification in the design of electrodes, improvement in surgical procedures and use of protective drugs to bring down the level of trauma and its adverse effects. However, the residual hearing has been preserved in less than 50% patients only. Confirmation of the presence of inflammatory cytokines, progressive decrease in the number of hair cells after cochlear implant fixation and protective effect of locally delivered NAC (*N*-acetylcysteine) on hearing preservation, strongly supports that the principle mechanism of the gradual loss of residual hearing after cochlear implantation is associated with excess generation of reactive oxygen species which is induced due to inevitable damage during implantation. Based on the above hypothesis, it has been proposed that the delivery of effective radical scavengers at the electrode insertion site will minimize the level of ROS and, therefore, may provide an effective solution in preserving the residual hearing. Conventional natural antioxidants such as vitamin E, vitamin C, amifostine, and trolox fail to provide 100% protection against ROS-induced damages because of their short degradation time, high-dose requirement and inability to regenerate. Overcoming these limitations, nanoceria and its solid solutions present an excellent choice as an antioxidant cum radical scavenger. However, further research in this direction is required to improve their characteristics in biological environments.

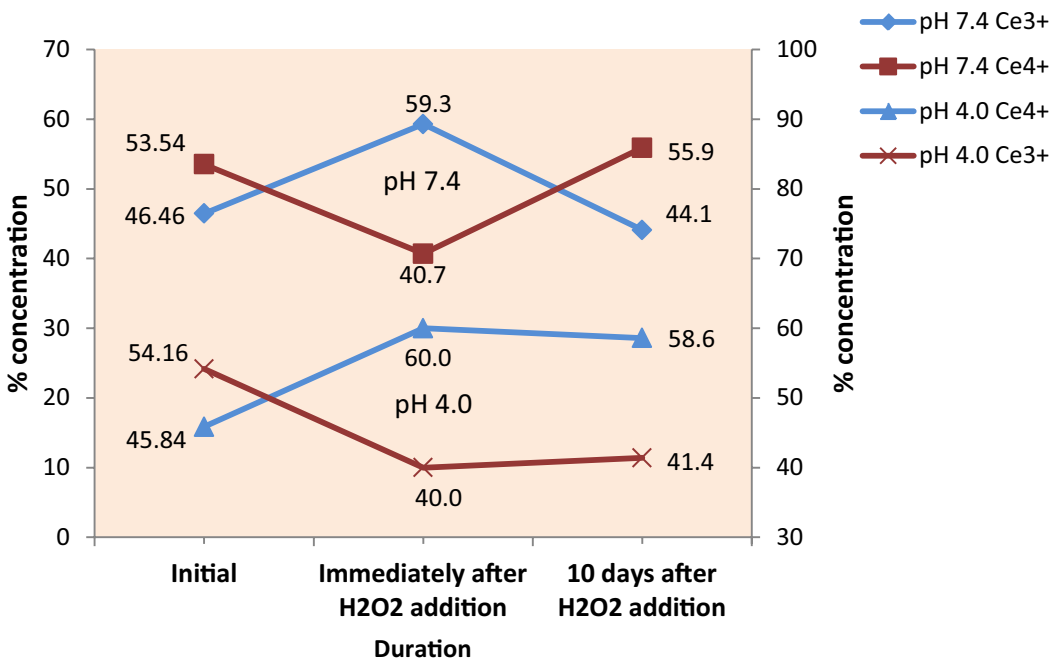

Figure 9: Change in concentration of Ce^{3+} and Ce^{4+} (determined by XPS) with time after H_2O_2 addition to dextran-coated nanoceria solution at pH 7.4 and 4.0[58].

10 Future Work

This review highlighted the relation among surgical and mechanical trauma, excess level of reactive oxygen species and progressive loss of residual hearing and accordingly it has been proposed that the delivery of an effective antioxidant cum radical scavenger will present an effective solution in retaining the residual hearing. With this regard, the effectiveness of nanoceria in preventing the hair cells from the damage needs to be studied. Furthermore, design parameters to optimise ROS scavenging of nanoceria and its solid solutions need to be assessed and defined. Additionally, conditions that overcome the drawbacks that limit the clinical application of nanoceria should also be established.

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