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Aspergillus flavus Mediated Extracellular One-pot Synthesis of Zirconium and Titanium Oxide Nanoparticles and their Antioxidant and Anti-inflammatory Efficacy Study

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Abstract

The present study reports the extracellular one-pot synthesis of zirconium and titanium oxide nanoparticles (Zr/TiO-NPs) mediated by Aspergillus flavus. This green synthesis approach leverages the bio-reductive capabilities of fungal metabolites, providing an environmentally friendly and efficient method for nanoparticle synthesis . The synthesized nanoparticles were evaluated for their antioxidant and anti-inflammatory activities. Antioxidant activity, assessed at varying concentrations (20–80 µg/ml), demonstrated a concentration-dependent manner , with the highest activity observed at 80 µg/ml. Similarly, the anti-inflammatory efficacy, determined using the albumin denaturation method, revealed maximum activity at 100 µg/ml and the lowest at 20 µg/ml, highlighting their potential to mitigate inflammatory responses. These findings underscore the potential of Aspergillus flavus Zr/TiO-NPs as promising agents for biomedical applications, particularly in combating oxidative stress and inflammation.

Keywords: *Anti-inflammatory Activity, Antioxidant Activity, Characterization, Eco-friendly, Fungus, Titanium Nanoparticles, Zirconium.*

Introduction

Nanotechnology has revolutionized the field of material science, providing innovative solutions across various domains, including medicine, energy, and environmental applications [1]. Among the plenty of nanomaterials, Zirconium oxide (ZrO_2) and Titanium oxide (TiO2) nanoparticles have received a lot of attention due to their exceptional physiochemical properties. Including high thermal stability, biocompatibility, and catalytic efficiency. These characteristics make them suitable for applications ranging from biomedicine to industrial analysis. The conventional methods for synthesizing zirconium and titanium oxide nanoparticles often involve the use of toxic chemicals and energy-intensive processes, raising environmental and health concerns [2]. To address these issues, green synthesis techniques have emerged as eco-friendly and sustainable alternatives. One such approach is the use of biological entities, such as fungi, bacteria, and plant extracts, as mediators for nanoparticle synthesis. In this context, *Aspergillus flavus*, a filamentous fungus, has shown remarkable potential as a biological nanofactory for the extracellular synthesis of nanoparticles. The fungus produces a variety of secondary metabolites, enzymes, and bioactive compounds that can reduce metal precursors and stabilize the resulting nanoparticles in a

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one-pot synthesis process [3]. This method not only minimizes the use of hazardous chemicals but also simplifies the synthesis by combining reduction and stabilization into a single step. Further more, the biomedical potential of zirconium and titanium oxide nanoparticles synthesized via green methods is of particular interest [4]. These nanoparticles exhibit antioxidant and anti-inflammatory properties, which are crucial for applications in therapeutic and pharmaceutical fields. Antioxidants play a vital role in scavenging free radicals and preventing oxidative stress, while anti-inflammatory agents are essential for managing inflammation-related diseases. This study focuses on the *Aspergillus flavus-mediated* extracellular one-pot synthesis of Zirconium oxide (ZrO_2) and titanium oxide (TiO_2) nanoparticles and evaluates their antioxidant and anti-inflammatory activities. The green synthesis approach not only aligns with sustainable practices but also enhances the biocompatibility of the synthesized nanoparticles, paving the way for their potential biomedical applications.

Materials and Methods

Aspergillus flavus fungus was obtained from the Department of Microbiology, Saveetha Medical College and Hospitals, Chennai. Saboroud Dextrose Broth (SDB) and Titanium (IV) dioxide (TiO_2) were purchased from Hi-media, Mumbai. Zirconyl Nitrate Hydrate (ZrO $(NO_3)_2.xH_2O$ was procured from Sisco Research Laboratories, (SRL) Chemicals, Maharashtra.

Sample Preparation and Synthesis

The fungus was cultured in Saboroud dextrose Broth and incubated for 48 hours. After incubation, visible fungal growth was observed. The culture was centrifuged, and the supernatant containing the fungal extract was collected whilethe pellet was discarded. For nanoparticle synthesis, 25mM of Zirconyl Nitrate Hydrate and 25mM of Titanium oxide were added drop wise into fungal extract using a titration (One-pot synthesis) method. After completion of the titration, the added solution was kept for 24 hours after incubation, The colour change was observed from light yellow to white precipitate, Thus, confirming the synthesis of Zirconium- Titanium oxide (Zr/TiO-NPs) nanoparticles. The Solution was then centrifuged, and the resulting pellet was collected. The pellet was dried in a hot air oven at 55-60^{o} C and stored for further characterization and future studies.

Characterization

UV-Vi's spectroscopy (Labman Double Beam UV-Vis Spectrophotometer LMSPUV1900S, India, 190–1100 nm) was utilized to characterize the synthesis of Zr/ TiO-NPs by analyzing samples at T0 and T24 within the wavelength range of 190–1100 nm. FTIR analysis (Bruker Alpha II, Germany) was performed at room temperature over a spectral range of 500–3500 cm^{-1} to investigate the bonding interactions between functional groups and metal ions. This analysis aimed to identify potential biomolecules responsible for capping the Zr/TiO-NPs and reducing the metal precursors. The morphological characteristics of the nanoparticles were examined using field-emission scanning electron microscopy (FE-SEM; JEOL-800S). Additionally, FE-SEM combined with energy-dispersive X-ray spectroscopy (EDX; OXFORD X-Plor-30/C-Swift) was used to determine the elemental composition of the nanoparticles.

Anti-inflammatory Activity

The anti-inflammatory effectiveness was assessed using the Albumin Denaturation Method, with a few modifications. Bovine serum albumin (BSA) in different concentrations of 20, 40, 60, 80, and 100 µg/mL as well as 80, 60, 40, and 20 µg/mL made up the reaction mixture. In the same volume, dimethyl sulfoxide (DMSO) served as a negative control. After 15 minutes of incubation at 37°C, the reaction mixture was heated for 20 minutes at 55°C. At 660 nm, absorbance was measured. The same concentrations of the conventional medication, diclofenac sodium (20, 40, 60, 80, and 100 μg/mL), were evaluated. Using the above formula, the % inhibition of protein denaturation was computed. Results were averaged in triplicate, and SPSS 21 was used to calculate the standard deviation.

Antioxidant Activity

The DPPH radical scavenging technique was used to measure the in-vitro antioxidant activity. Ascorbic acid and Zr/TiO-NPs were produced in different quantities. 2.96 mL of 0.1 mM DPPH solution was added to each solution, for a total volume of 3 mL. After giving the mixture a vigorous shake, it was incubated for 20 minutes in a dark environment. The Shimadzu UV-2450 spectrophotometer (Japan) was used to measure the absorbance of the reaction mixture at 517 nm. The control was 0.1 mM DPPH solution, and the standard was ascorbic acid. The mean values were computed after the findings were acquired in triplicate. SPSS software (version 21) was used to analyze the standard deviation.

Results and Discussion

The observed colour change from light yellow to a white precipitate during the synthesis of zirconium/titanium oxide nanoparticles (Zr/TiO-NPs) mediated by *Aspergillus flavus* provides a visual indication of the nanoparticle formation [Figure 1]. This transformation likely reflects the reduction and stabilization of metal precursors facilitated by the biomolecules produced by the fungus [5]. The fungus serves as a biocatalyst, releasing enzymes, proteins, and other metabolites that assist in reducing metal ions and capping the nanoparticles. These biomolecules stabilize the nanoparticles and influence their size, shape, and properties. While the colour change is a qualitative indicator of nanoparticle synthesis, it is essential to corroborate this observation with analytical techniques like UV-Vis, XRD, and SEM for definitive confirmation of nanoparticle formation and characterization.

Figure 1. *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

UV visible Spectra Analysis

The UV-analysis of the biosynthesized Zr/TiO-NPs showed a peak at 450 nm can be observed in [Figure 2].This peak corresponds to the Surface plasmon resonance (SPR) or the bandgap transition of the nanoparticles, showing that electrons are being excited from the valence band to the conduction band, causing an electrical transition. A similar study showed that the biosynthesized Zirconium oxide nanoparticles showed a peak at 403nm [6, 7] and Titanium oxide nanoparticles synthesized using *Cassia fistula* revealed a peak at 350nm wavelength. These differences highlight the influence of material composition, biosynthetic conditions, and the nature of surface modifications on the optical properties of the nanoparticles. The redshift of the absorption peak in Zr/TiO-NPs compared to pure zirconium oxide and titanium oxide nanoparticles suggests a possible interaction or synergistic effect between Zr and Ti in the composite, which may alter the electronic structure and reduce the bandgap energy. This result revealed the potential of Zr/TiO-NPs for applications requiring visible-light activity, such as photocatalysis or optoelectronics [8].

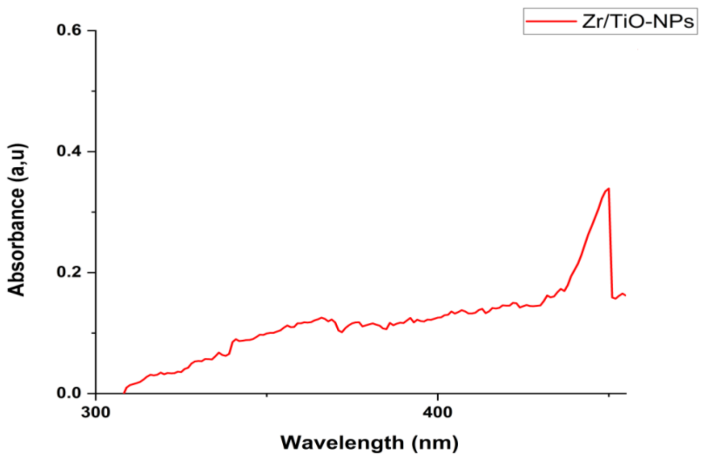


Figure 2. UV Analysis of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

Fourier Transform Infrared Spectroscopy

In Figure.3 The result of FTIR analysis of Zr/TiO-NPs exhibited different types of functional groups such as 555cm^{-1} (Aliphatic iodo compounds, C-I stretch) possibly introduced during the biosynthesis or from precursors used, 673 cm^{-1} (Primary amine, CN stretch) indicates the presence of primary amine groups which could be derived from biomolecules or organic stabilizers involved in the synthesis, 1027 cm^{-1} (ammonium ion) suggests the presence of ammonium groups possibly resulting from nitrogen-containing organic compounds, 1395cm^{-1} (aliphatic nitro compounds) indicates nitro functionality, which may arise from organic precursors or as a by-product of the synthesis process, 1544 cm^{-1} (Aromatic combination bands) suggests the incorporation of aromatic structures, possibly from fungal extracts or stabilizing agents used in the biosynthesis, 1626cm^{-1} (Organic nitrates) indicates the presence of organic nitrate groups, which might be associated with the surface modification of nanoparticles, 1982cm^{-1} (Aromatic combination bands) further confirms the contribution of aromatic compounds in the surface chemistry, 2876cm^{-1} (Methyl C-H asymmetric stretch) indicates the presence of aliphatic methyl groups, suggesting that organic molecules with methyl functionalities are absorbed on the nanoparticle surface [9].

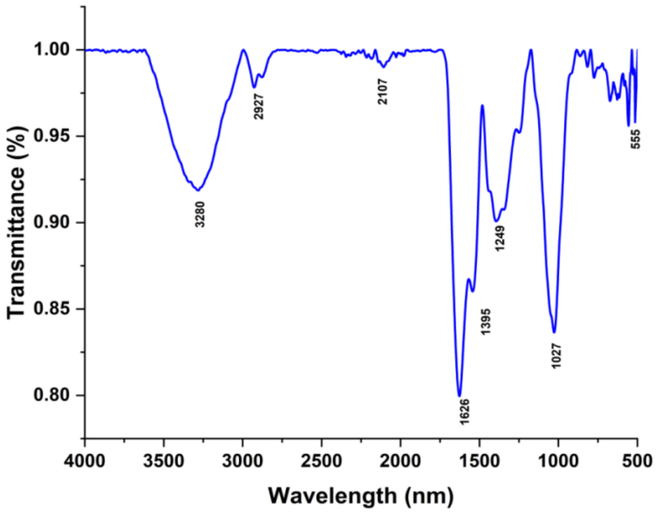
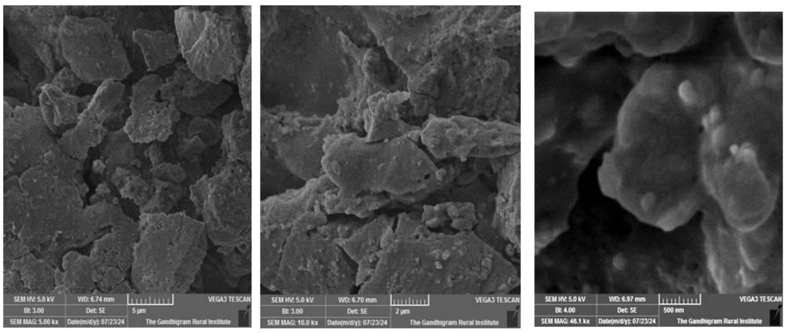


Figure3. FTIR Analysis of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

Scanning Electron Microscopy (SEM)

The surface morphology and size of the nanoparticles were analyzed using Scanning Electron Microscopy (SEM), which revealed that the Zr/TiO nanocomposite exhibited an irregular shape (90±10 nm size) with an agglomerated structure as seen in [Figure 4]. A similar study reported that zirconium nanoparticles demonstrate dense packing and uniform distribution, contributing to their stability [10]. The microstructure analysis of the synthesized nanoparticles showed that they exhibit spherical, triangular, and irregular shapes [11]. Notably, the nanoparticles showed no evidence of aggregation, likely due to the interactions among the various phytochemical molecules bound to their surfaces.



A B C

Figure 4. SEM Analysis of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs. A) 1µm scale. B) 2µm scale. C) 500nm scale **.**

EDX Analysis

The EDX analysis confirmed the successful synthesis of Zr/TiO-NPs, with signals corresponding to Zr (4.39%), Ti (4.72%), O (47.76%), and C (43.13%) [Figure 5]. The substantial proportion of oxygen (O) highlights the oxide nature of the nanocomposite material. The high carbon content (43.13%) suggests the presence of organic compounds or residues, likely originating from the biosynthesis process. These may include bioactive compounds from fungal extracts or stabilizing agents bound to the surface of the nanoparticle. The relatively balanced proportions of Zr and Ti indicate the incorporation of both elements in the composite structure, potentially leading to synergistic effects that enhance the material's properties [12].

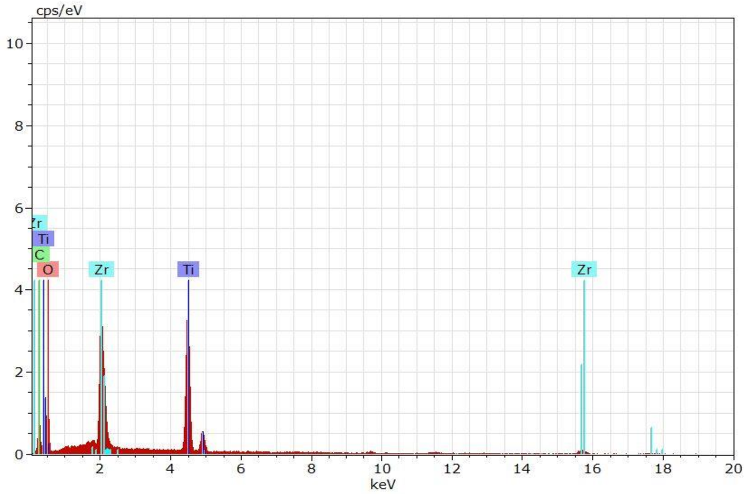


Figure 5. EDX Analysis of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

XRD Analysis

The XRD analysis of the synthesized Zr/TiO-NPs reveals the presence of both crystalline (31.7%) and amorphous (68.3%) phases [Figure 6]. The crystalline phase (31.7%) indicates regions of ordered atomic structure, while the amorphous phase (68.3%) reflects a disordered atomic arrangement. This composition suggests that the nanoparticles possess both structural stability and functional flexibility. The sharp peaks observed at 37.8°, 48.3°, 53.9°, 55.08°, 70.6°, and 75.08°correspond to specific crystallographic planes of the Zr/TiO nanocomposite. These peaks confirm the crystalline nature of the material and indicate the formation of distinct phases of zirconium and titanium oxides [13]. The presence of sharp peaks signifies the presence of well-formed crystalline domains within the nanoparticles. The positions of the diffraction peaks suggest the incorporation of Zr and Ti in the composite, forming a mixed oxide structure. The crystallinity and peak intensities reflect the degree of order within these domains.

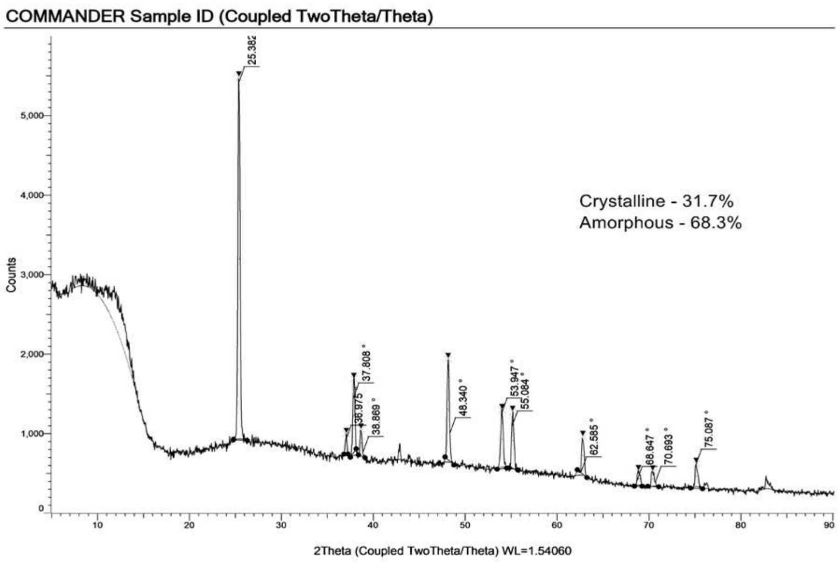


Figure 6. XRD Analysis of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

Antioxidant Activity

The results indicate that the antioxidant activity of *Aspergillus flavus* Zr/TiO-NPs is concentration-dependent [Table 1, Figure 7]. The nanoparticles exhibited their maximum antioxidant potential at the highest tested concentration of 80 µg/ml, whereas the activity significantly decreased at the lowest concentration of 20 µg/ml. This suggests that higher concentrations of these nanoparticles are more effective in counteracting oxidative stress. Since reactive oxygen species (ROS), generated through biological processes [14], can cause oxidative damage to cellular components and lead to cell death, the strong antioxidant potential observed at higher nanoparticle concentrations highlights their potential as effective agents for mitigating ROS-induced cellular damage [15].

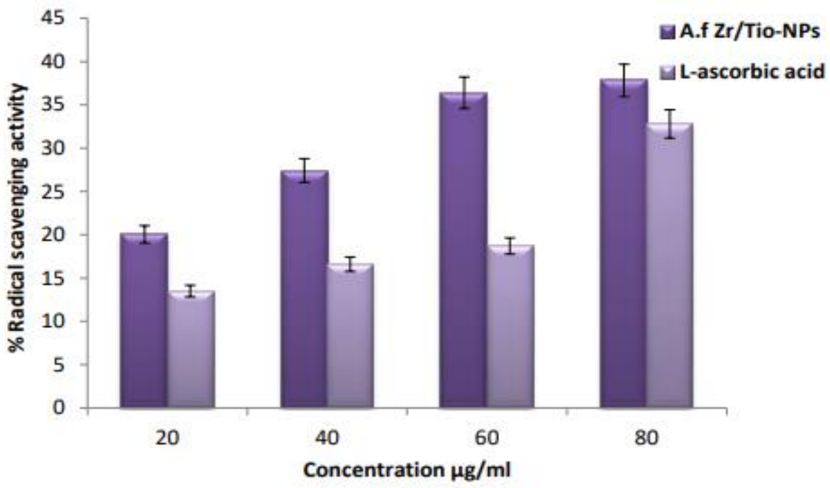


Figure 7. Antioxidant Activity of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

Table 1. Antioxidant Activity of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

A.F Zr/TiO-NPs	**L- ascorbic acid (Standard)**
20.05±0.21	13.5±0.01
27.4±0.23	16.6±0.12
36.4±0.12	18.7±0.20
37.8±0.18	32.8±0.13

Anti-inflammatory Activity

The results indicate that the anti-inflammatory activity of *Aspergillus flavus* Zr/TiO-NPs, evaluated using the albumin denaturation method, is concentration-dependent [Table 2, Figure 8]. The nanoparticles exhibited the highest anti-inflammatory activity at the maximum tested concentration of 100 µg/ml, while the activity was significantly reduced at the lowest concentration of 20 µg/ml. This suggests that higher concentrations of these nanoparticles are more effective in preventing protein denaturation, a key marker of anti-inflammatory potential [16].

Anti-inflammatory Activity

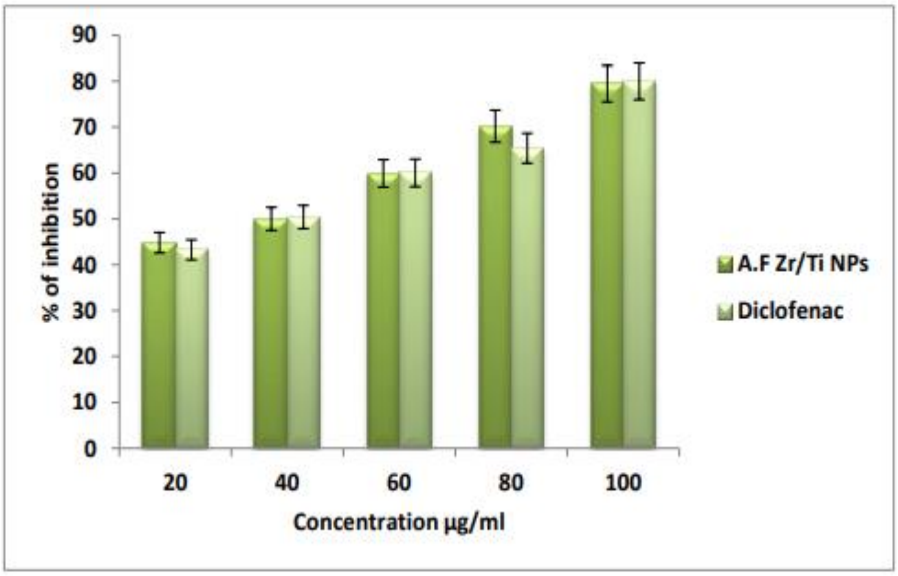


Figure 8. Anti-inflammatory Activity of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

Table 2. Anti-inflammatory Activity of *Aspergillus flavus* Mediated Synthesis of Zr/TiO-NPs

A.F Zr/TiO-NPs	Diclofenac (Standard)
45.6±0.1	42.8±0.3
49.1±0.2	49.6±0.2
58.3±0.2	57.5±0.1
69.01±0.04	78.3±0.2

Conclusion

This study successfully demonstrated the extracellular one-pot synthesis of zirconium and titanium oxide nanoparticles (Zr/TiO-NPs) using *Aspergillus flavus*, providing an eco-friendly and efficient method for nanoparticle production. The synthesized nanoparticles exhibited significant antioxidant and anti-inflammatory activities in a concentration-dependent manner. The highest antioxidant activity was observed at 80 µg/ml, while the maximum anti-inflammatory activity, evaluated via the albumin denaturation method, was noted at 100 µg/ml. These results highlight the potential of *Aspergillus flavus* Zr/TiO-NPs as promising agents for therapeutic applications, particularly in managing oxidative stress and inflammation. Further studies are recommended to explore their mechanisms of action and potential applications in biomedicine.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

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