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Biotin-Minoxidil Conjugated Copper Oxide Nanoparticles for Antimicrobial Protection and Hair Regeneration

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Author`s Contribution

[AM] Conception and design, [LB] Collection and assembly of data, [TF, KF] Analysis and interpretation of the data, Statistical expertise, [MF] Final approval and guarantor of the article

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ABSTRACT

Background: Nanobiotechnology has greatly improved targeted drug delivery by facilitating localized therapeutic effects with customized nanocomposites.

Objectives: This research focused on synthesizing and characterizing a biotin-minoxidil-linked copper oxide (CuO) nanocomposite while assessing its dual capabilities for hair regeneration and antimicrobial effects.

Methodology: CuO nanoparticles were synthesized by chemical co-precipitation. Afterward, biotin and minoxidil were coupled to nanoparticles using PEG, glutaraldehyde, and EDC. The obtained nanocomposite was extensively characterized using UV-Vis spectrophotometry, and Fourier Transform Infrared (FTIR) spectroscopy. The well-diffusion method and culture-based assays were used to evaluate antimicrobial efficacy. The *in vivo* promotion of hair growth was assessed in albino mice with shaved dorsal areas.

Results: The results indicated that the biotin-minoxidil-linked CuO nanocomposite demonstrated strong antimicrobial activity against the evaluated microbial strains and markedly improved hair regrowth rate in the mouse model compared with control groups. Hair regrowth was observed after topical application of 100 µL of a nanocomposite containing 5% minoxidil for approximately 21 days.

Conclusion: The addition of biotin and minoxidil provided specific therapeutic advantages for hair growth while also enhancing the copper oxide core's natural biological characteristics. This nanocomposite shows great promise as a multifunctional tool for managing scalp health, integrating antimicrobial properties with effective hair growth promotion.

Key words: Nanobiotechnology, Copper Oxide Nanoparticles, Biotin, Minoxidil, Hair Regeneration, Antimicrobial Activity.

Introduction

Nanotechnology anticipated to revolutionize innovation in medicine, offering exceptional solutions to health-care related issues. Nanomaterials such as silver oxide (Ag_2O), copper oxide (CuO), zinc oxide (ZnO), and carbon nanotubes (CNTs) have acquired significant recognition because of their long-term effectiveness for many diseases lacking effective treatments, including cancer, microbial infections (bacterial and fungal), and immunological and cardiovascular conditions.[1] Copper and its alloys have been recognized by United States Environmental Protection Agency (EPA) as effective anti-microbial compounds.[2] Copper (I) oxide and copper (II) oxide, particularly in their nanoscale form (<100 nm), possess potent anti-microbial activity against wide range of pathogenic microorganisms.[3]

Copper oxide nanoparticles due to their unique physiochemical nature are extensively applied in the fields of physics, electronics and biomedical sciences. These nanoparticles contribute to significant advancements in drug delivery and diagnostic methodologies. Although several methods have been reported for the synthesis of copper oxide nanoparticles, the chemical precipitation method using copper acetate as precursor is among the most widely preferred route for synthesizing CuO nanoparticles. The chemical precipitation method is favored most suitable because of its short processing time and eco-friendly nature. This approach enables production of nanoparticles with well-controlled size and retained physiochemical properties.[4]

Hair constitutes a vital anatomical structure, contributing to numerous physiological, protective and sensory roles. Beyond its cosmetics importance, hair serves as critical functions in

thermoregulation, protective mechanisms and sensory perceptions. Furthermore, the hair covering scalp safeguards head from harmful ultraviolet radiations reducing the risks of mechanical damage.[5] The incorporation of copper oxide, biotin and minoxidil into one bio-composite offers a synergistic approach to enhance hair regeneration and promoting hair health. The therapeutic effects of this combination arise from minoxidil-induced enhancement of follicular blood flow; biotin mediated keratin synthesis facilitate targeted drug delivery while exhibiting antimicrobial activity and reducing inflammation. Collectively, these composites provide a multiple faceted therapeutic approach to target various aspects of hair health. The principle therapeutic application of this formulation is intended to manage hair loss including androgenic alopecia, alopecia areata, and other forms of hair thinning.[6]

To advance the development of nanotechnology-based therapeutic system for scalp hair regrowth, this study presents preliminary assessment of copper oxide nanoparticles functionalized with biotin and minoxidil. The findings of this study lay the groundwork for designing more effective therapeutic alternative to conventional topical therapies in pharmaceutical sciences for alopecia.

Materials and Methods

Synthesis of Copper Oxide Nanoparticles

The standard co-precipitation method was successfully employed for the preparation of copper oxide nanoparticles. A 0.02 M copper acetate solution was prepared using distilled water and subjected to continuous stirring at 500 rpm while heating at 70°C on a hot plate to prevent particle agglomeration. Following 05 minutes of continuous stirring, 01 mL of glacial acetic acid was introduced into the reaction mixture, followed by further stirring for 30 minutes. To achieve a pH of 13, a total of 1.0 g NaOH pallets were added to the solution. The addition of NaOH pallets caused the solution to change the color instantly from light blue to black, resulting in precipitate formation. The resulting nanoparticles were subsequently collected by centrifugation at 8000 rpm for 05 minutes. [7] the synthesized copper oxide nanoparticles were washed three to four times with deionized water to remove impurities. The purified nanoparticles were then suspended in phosphate-buffer saline and stored for subsequent use. [8]

Synthesis of PEG-Conjugated CuO NPs

The synthesis was initiated by preparing 0.02 M aqueous solution of copper acetate in which 01 mL of glacial acetic acid was subsequently introduced. The hot plate temperature was maintained at 70 °C before being transferred into 40% polyethylene glycol (PEG) solution. While maintaining the same temperature the solution was stirred in a shaking incubator for approximately 30 minutes. The reaction pH was adjusted to 13 by addition of NaOH pallets, accompanied by rapid change in color from blue to brownish black, followed by the formation of precipitates. Washing cycles were followed three- to four times for washing the nanoparticles using deionized water to remove residual impurities. After drying at 45 °C, nanoparticle pallets were transferred to phosphate-buffer saline (PBS) and stored.[9]

Synthesis of Minoxidil Conjugated NPs

A 10 mg portion of PEG conjugated CuO nanoparticles was weighed and suspended into 25 mL of 0.01 M pyridine solution. Subsequently, 5% of glutaraldehyde was added to the mixture and followed by incubation of 3 hours. A total of 25 mg minoxidil was introduced to the solution, which was then retained for pre-coupling step. Pre-coupling was monitored using UV-Visible spectrophotometry at a wavelength of 285 nm. Upon completion of 24 hours of incubation, centrifugation of reaction mixture was carried out at 7000 rpm for 10 minutes. The supernatant was separated for analysis of post-coupling performed at 285 nm. A volume of 1 mL of 01 M glycine was added to the solution following reaction mixture to centrifuge. The supernatant was carefully discarded, and pallet was washed repeatedly with wash buffer.[10]

Synthesis of Biotin Conjugated NPs

A measured amount of 10 mg of minoxidil-conjugated nanoparticles was weighed and dispersed in 25 mL of pyridine solution. Furthermore, a volume of 2 mL EDC was also added allowing the reaction mixture to complete incubation of 3 hours. A carefully weighed 5 mg of biotin was added into the solution, and pre-coupling was evaluated at a wavelength of 204 nm using UV-Visible spectrophotometry. Subsequently, the incubated solution was centrifuged, and its supernatant was subjected for post-coupling at the same wavelength. The reaction mixture was treated with 01 M glycine and incubated for 30 minutes. The sample was centrifuged, washed and subjected to store in phosphate-buffer saline at 4 °C.[10]

Ultraviolet-Visible Spectroscopy

UV-Visible spectroscopy was performed over the spectrum of 200-800 nm range equipped with quartz cuvette employing UV-Visible spectrophotometry using distilled water as a baseline reference for analysis.[11] Nanoparticle suspensions were diluted to maintain absorbance within the linear range of 0.1-1.0, consistent with the Beer-Lambert Law. The characteristic peak of Surface Plasmon Resonance (SPR) corresponding to CuO NPs was observed in the range of 300-380 nm, confirming successful nanoparticle formation.

Fourier-Transform Infrared Spectroscopy

FTIR spectra were recorded using Bruker's Alpha spectrophotometerat a spectral resolution of 400-4000 cm^{-1}. 1-2 mg of dried CuO NPs biocomposites was mixed with 100 mg KBr. Additional peaks corresponding to functional groups of biotins and minoxidil were identified, confirming successful surface conjugation of the biocomposite. [11]

Antibacterial Properties of CuO NPs Biocomposite

Agar Well Diffusion Method

Antimicrobial activity of the CuO nanocomposite was accessed via agar well diffusion assay. Inocula of the two bacterial speices *E. coli* and *Staphylococcus aureus* were successfully prepared by inoculating a colony into Luria-Bertani (LB) broth, followed by overnight incubation at 37 °C. Agar plates were prepared, and 20 µL of bacterial suspension was applied; sterile wells were aseptically punched into the agar surface with the aid a cork borer. Four wells per plate were prepared and labeled S1-S4, loaded with the CuO NPs biocomposite at varying concentrations of20-80 µg/mL and maintained at 37 °C for incubation. Control wells included *minoxidil* and biotin. The diameter of clear zones of inhibition was recorded in millimeters (mm).[3]

Antibacterial Activity Assessment by Broth Method

A volume of 100 µL of prepared inoculum of *E. coli* was transferred to each test tube containing LB broth. Nanoparticle samples were then introduced at varying final concentration adjusted to 10, 20, 30 and 40 µg/mL, while a control sample tube without nanoparticles was maintained. The initial optical density (OD) values of all the samples were determined at 600 nm. Following incubation, bacterial growth inhibition was assessed by recording the OD at 600 nm. A decrease in optical density compared to the control indicated antibacterial activity of the biocomposite against *E. coli*.[12]

Hair Growth Analysis

Hair growth analysis was performed by shaving the dorsal region of albino mice with an electric trimmer. The nanocomposite formulation was applied to mice, while the control mice were left untreated and allowed to regrow hair naturally. A 100 µL volume of the test formulation containing 5% minoxidil was applied once daily to the shaved area of each treated mouse.

Statistical Analysis

Each experiment was carried out three times independently (n=3), and results are reported as mean values ± standard deviation (SD). Statistical evaluations were performed using SPSS software.

Results

Synthesis of Copper Oxide Nanoparticles

The successful synthesis of copper oxide nanoparticles was achieved using the co-precipitation method. The formation of black precipitates during the reaction indicated the conversion of copper ions into CuO NPs (Fig 1). The precipitates were collected, washed, and dried for further characterization and analysis.

Figure 1: Synthesis of copper oxide nanoparticles via co-precipitation method.

The FTIR spectrum was acquired, which allowed identification of the bonds present in the samples analyzed (Figure 02). According to the results, it was possible to define the functional groups of each component on the basis of their characteristic ranges. First, in the first sample, a wide range around 3400 cm-1 indicates the presence of alcohol, while bands below 1000 cm -1 suggest the existence of Cu–O, thereby proving the presence of copper oxide. Further, the second sample, PEG, reveals a band around 1100-1200 cm-1 that corresponds to C–O–C stretching, which is typical for ether-containing polymers. Simultaneously, the peak at 2800-2900 cm-1 is consistent with C–H stretching, thus confirming the structure of PEG. Third, the third sample, minoxidil, was revealed to have amine groups, which can be seen from the peaks at 3300-3400 cm-1 corresponding to N–H stretching. Furthermore, there was a peak at 1300-1400 cm -1 that corresponds to aromatic amine, which can be explained by the presence of a pyrimidine ring. Fourth, biotin has several characteristic bondings, including the urea ring, amide, carboxyl group, and methylene groups. First, the band around 1240-1260 cm-1 is consistent with the stretching of the urea ring. Second, amide bonds show a peak at approximately 1660 cm-1. Third, the carboxyl group is evident from the band at 1725 cm-1. Fourth, the C–H stretching of methylene rings shows a peak at 2800-2900 cm -1. Hence,

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the presence of the aforementioned functional groups proves the successful isolation of CuO, PEG, minoxidil, and biotin.

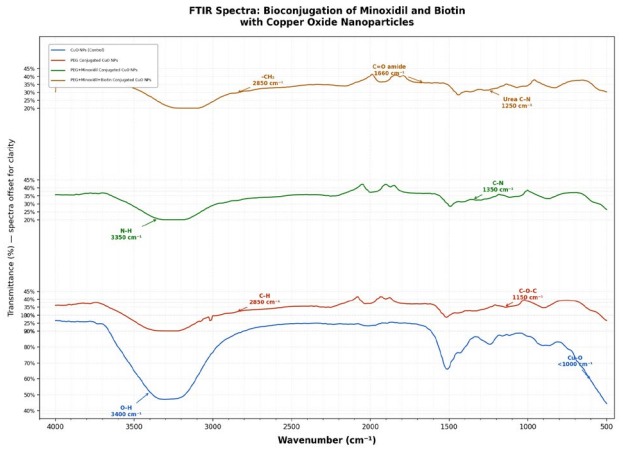

Figure 2: FTIR analysis of the CuO NP control (blue), with the remaining peaks indicating conjugation with PEG (red), PEG + Minoxidil (green), and PEG + Minoxidil + Biotin (brown), respectively.

Agar-well diffusion method

The antimicrobial potential of synthesized CuO NPs was employed via agar well diffusion assay. Results revealed that the zones of inhibition increased proportionally with the increased sample concentration against *E. coli* and S. *aureus.* The size of the inhibition zones increased progressively with increasing nanoparticle concentration (Fig 3, Table 1). Higher nanoparticle concentrations produced larger inhibition zones, confirming the effectiveness of the nanoparticles as antimicrobial agents.

Table 01: Comparison of zones of inhibition between two bacterial strains with different sample concentration.

Escherichia coli		***Staphylococcus aureus***
Vol. (µL)	**Zone of Inhibition (mm)**	**Zone of Inhibition (mm)**
20	16.0 ± 1.4	14.0 ± 0.5
40	17.0 ± 0.8	17.0 ± 0.2
60	20.0 ± 1.08	18.0 ± 1.04
80	22.0 ± 1.25	21.0 ± 1

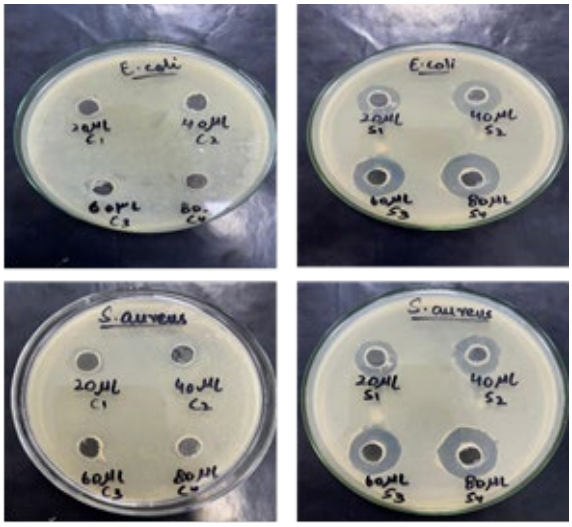

Figure 3: Control samples containing minoxidil and biotin showing no antimicrobial activity against two bacterial strains. Test group containing minoxidil and biotin conjugated copper oxide nanoparticles exhibited potent antimicrobial activity against both of the strains.

Antibacterial Activity Assessment by Broth Dilution Method

This negative correlation indicates that our nanocomposite exhibits strong dose-dependent antimicrobial activity, effectively suppressing bacterial proliferation or inducing cell death at higher concentrations. Consequently, the resulting LB broth clearance reduces light scattering, providing a reliable quantitative measure of the material's efficacy for determining its Minimum Inhibitory Concentration (MIC).

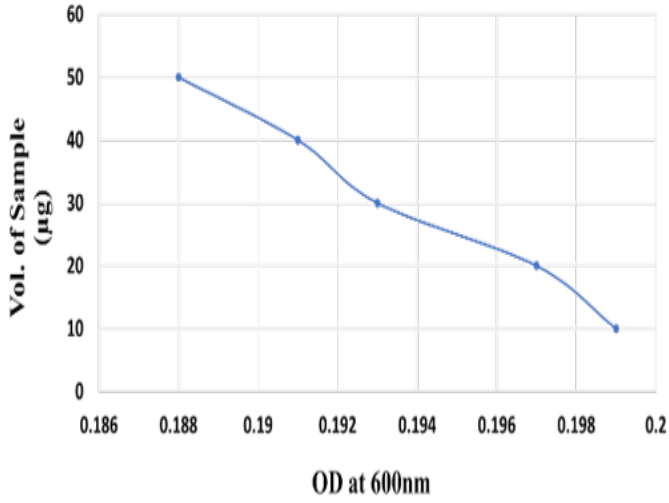

Figure 4: Graph showing a negative correlation between the volume of copper oxide nanoparticles conjugated with biotin and minoxidil and optical density (OD). Increasing sample volume led to reduced OD, suggesting improved inhibitory activity against *E. coli.*

Hair growth analysis in mice

This enhanced hair growth suggests that the nanocomposite may actively stimulate hair follicles, possibly by accelerating the shift from telogen (resting phase) to the anagen (growing phase) of the hair cycle. Furthermore, this localized response highlights the material's potential as a biocompatible therapeutic agent for tissue regeneration and the treatment of hair loss disorders.

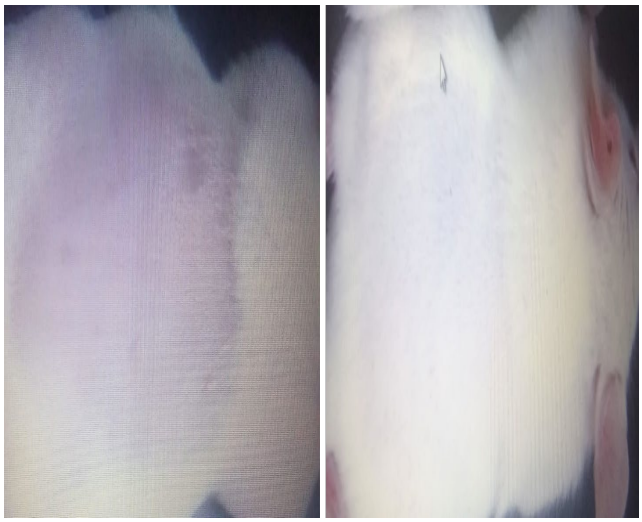

Figure 5 (a): Control mice exhibited persistent hair loss in the shaved regions after three weeks of observation. **5 (b)** Test mice completely exhibited hair regrowth with complete coverage of shaved regions in three weeks of observation.

Discussion

The development of biocompatible and biodegradative formulations with integration of biotin and minoxidil may provide an advanced therapeutic platform by improving drug delivery, enhancing follicular targeting and reducing treatment related side-effects.[13] Copper oxide nanoparticles beyond promoting hair regrowth also exhibit potential antimicrobial properties that may support a healthier scalp environment during hair loss therapy.[5]

FTIR spectrum (Fig. 2) confirms successful incorporation of CuO, PEG, minoxidil, and biotin in the nanocomposite. Characteristic bands include ~3400 cm^{-1} (–OH) and Cu–O vibrations (<1000 cm^{-1}) for CuO[14], C–O–C (1100–1200 cm^{-1}) and C–H (2800–2900 cm^{-1}) for PEG, N–H (3300–3400 cm^{-1}) and pyrimidine signals (1300–1400 cm^{-1}) for minoxidil (Chao, 1988), and urea (1240–1260 cm^{-1}), amide (~1660 cm^{-1}), plus C–H bands for biotin (Lapin, 2009). These match literature reports, indicating preserved functional groups and structural integrity of the formulation. The ability to deliver effective therapies via topical applications is particularly appealing to patients who prefer non-invasive options to surgical interventions such as hair transplants. The topical regimen (100 µL daily for 21 days) provides a non-invasive alternative to hair transplants with strong translational potential[15], though murine-to-human extrapolation requires further safety studies due to skin differences. However, human skin differs significantly from murine skin in thickness, hair follicle density, and barrier function. Therefore, extrapolating these results to human application will require additional safety and efficacy studies.[16]

In the future, it could become possible to create more eco-friendly products that would appeal to the growing number of consumers who are interested in sustainable beauty and healthy lifestyles. Due to the increased awareness and recognition of the problem of alopecia, the demand for new hair growth stimulating treatments and products will also grow. Moreover, these agents could be included in other cosmeceutical preparations to create multi-tasking hair care products. The joint work of cosmetic surgeons, dermatologists, and biotech companies could lead to the creation of unique product lines that would combine the merits of all participants. In addition, individual approaches to combating alopecia in patients will be developed, which would take into account their lifestyle, the nature of their hair loss, and even genetic predispositions.[17,18,19,20]

Conclusion

Nanoparticles were successfully prepared and characterized by utilizing UV-Vis spectroscopy, microscopy, and FTIR to establish effective binding of the incorporated components to the nanoparticles while evaluating their antimicrobial activity and hair regenerative potential analysis showed that these nanoparticles have potent antimicrobial properties as well as hair regeneration capabilities. All the experiments and antimicrobial assays were performed under controlled conditions. Successful conjugation of CuONPs with minoxidil indicates efficient hair regrowth using mouse models. Consequently, these outcomes suggest that the developed nanotherapeutic system overcomes the limitations of existing topical formulations used for managing hair loss.

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