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Evaluation of Fullerene Derivatives Functionalized with Methionine and Cysteine for Dermal Antioxidant Applications

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Abstract*– Fullerene C_{60}, a nanomaterial, has been shown to demonstrate high antioxidant potential. The conjugated spherical structure of the compound enables its ability to accept multiple electrons and neutralize reactive oxygen species (ROS). However, its application in dermal applications is limited by its insolubility in water and low bioavailability. To enhance the efficacy of these materials, fullerene derivatives functionalized with polar amino acids, such as methionine and cysteine, were developed to improve their solubility, biocompatibility, and antioxidant capacity in dermal applications. The chemical functionalization of fullerene with amino acid functional groups was confirmed using Fourier transform infrared (FTIR) spectroscopy. The functionalized fullerene samples exhibited a substantial enhancement in solubility within aqueous media, thereby overcoming the aggregation characteristic of pure C_{60}. In cell assays, the functionalized derivatives exhibited adequate biocompatibility at low concentrations, in contrast to pure C_{60}, which demonstrated significant toxicity after 24 hours. In terms of antioxidant capacity, the formulations with the functionalized product exhibited a significant reduction in ROS production, with the 3% C60-Methionine formulation demonstrating the highest efficacy. In comparison to a commercial dermal product, the experimental formulations exhibited satisfactory antioxidant performance in the initial phase; however, they demonstrated diminished cellular stability after a 24-hour period. This study underscores the potential of methionine and cysteine modifications to enhance the properties of C60, presenting promising alternatives for safer and more functional antioxidant dermal formulations.

Keywords*-- Fullerene C60 Functionalized with Amino Acids, Polar Fullerene, Reactive oxygen species ROS, Antioxidant capacity, Oxidative stress.

I. Introduction

Fullerenes represent a particularly intriguing allotropic form of carbon. The structure of C_{60} consists of 60 carbon atoms arranged in a spherical geometry that is reminiscent of a soccer ball, resulting in the presence of exceptional electronic, optical, and physicochemical properties. These include their capacity to accept multiple electrons, their high chemical stability, and their ability to interact with reactive species. Notably, their antioxidant capacity allows them to neutralize reactive oxygen species (ROS), making them highly effective in reducing oxidative stress. These characteristics have fostered the study of these materials in diverse fields, including medicine, pharmacology, nanotechnology, and dermal applications.

In the field of skin science, research on fullerene C_{60} is driven by its potential to neutralize reactive oxygen species (ROS) at the dermal level. These species are the primary mediators of photoaging and damage cutaneous macromolecules. Photoaging is a multifactorial process in which exposure to ultraviolet (UV) radiation increases the production of reactive oxygen species, triggering damage to lipids, proteins, and nucleic acids, as well as alterations in the extracellular matrix. This process causes damage to collagen and elastin in the skin, which manifests as premature wrinkles, pigmentation, and loss of firmness [3,4,5].

A. Oxidative Stress in skin

Skin aging is a multifaceted and incremental process influenced by a combination of intrinsic and extrinsic factors, including various environmental stressors. These factors, which include ultraviolet (UV) radiation, pollution, smoking, and an inadequate diet, contribute to the formation and accumulation of reactive oxygen species (ROS). The overproduction of ROS instigates oxidative stress, which in turn damages skin cells by affecting lipids, proteins, and DNA, thereby compromising their structure and function over time. To mitigate these effects and maintain healthy skin and its appearance, it is imperative to

protect the skin by using antioxidants, maintaining a balanced diet, and avoiding environmental factors that promote oxidative stress.

This oxidative stress is attributable to an imbalance between the production of ROS and the body's capacity to neutralize or repair them with antioxidants. The molecular mechanisms of reactive oxygen species (ROS) contribute to skin aging is in Figure 1.

Fig. 1 The molecular mechanisms of ROS contribute to skin aging. [Edited from 6].

The skin's antioxidant mechanisms for combating reactive oxygen species (ROS) include several enzymes and molecules that work in concert to protect cells from oxidative damage. Key enzymes include superoxide dismutase (SOD), catalase and glutathione peroxidase. Furthermore, molecules such as vitamins C and E, as well as coenzyme Q10, play a crucial role in neutralizing free radicals and regenerating other antioxidants in the process. These antioxidant systems collaborate closely to maintain the integrity and function of skin cells against oxidative stress [6,7,8].

B. The New Generation of Antioxidants

As previously stated, the antioxidant defense mechanism plays a crucial role in protecting the skin against oxidative damage. As the mechanisms by which reactive oxygen species (ROS) affect skin cells are better understood, it becomes crucial to innovate in product formulations that neutralize these free radicals more efficiently. The development of new antioxidant systems for the skin is crucial for enhancing the efficacy of protection against oxidative damage.

However, while traditional antioxidant systems are generally effective to a certain extent, they are often insufficient to counteract the damage caused by continuous exposure to adverse environmental factors such as UV radiation, pollution, and stress. In recent years, there has been a surge of interest in leveraging the potential of novel antioxidant systems, encompassing both those derived from natural compounds and plant extracts, which constitute a significant source of bioactive compounds that confer anti-aging benefits, and high-tech antioxidants, some of which exhibit synergy with other systems and facilitate high penetration [9,10].

New antioxidant systems may incorporate combinations of ingredients that work synergistically, offer sustained release of the active ingredient, or even incorporate advanced technologies such as nanoparticles for better penetration and effectiveness in the deeper layers of the skin.

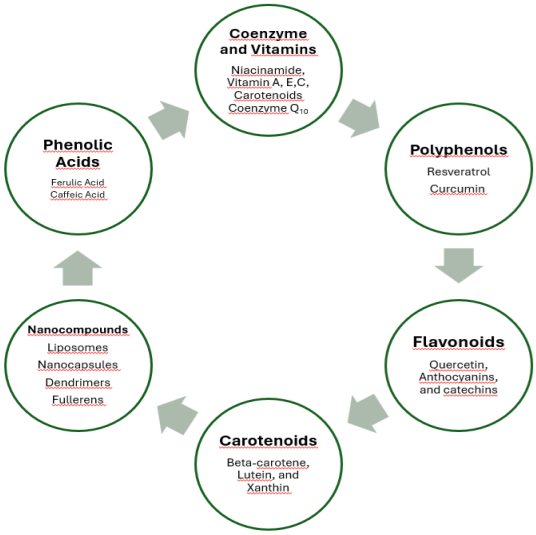

Fig. 2 Some antioxidants with anti-aging activity. [12,13].

Figure 2 presents a selection of new-generation antioxidants employed in dermo-cosmetics. It is important to acknowledge that, while certain vitamins have been used for an extended period, the development of new, stable forms offers distinct advantages, including enhanced stability and increased antioxidant capacity. This multifaceted approach not only prevents premature aging but also promotes skin repair and regeneration, thereby enhancing its appearance and long-term health.

The potential benefits of nanotechnology and nanocomposites in the domain of dermo-cosmetics stem from their ability to ensure the delivery of active ingredients with high levels of penetration. By reducing ingredient size to the nanoscale, products can penetrate deeper into the skin's layers, enabling more effective, concentrated absorption. This approach enhances the efficacy of skincare products, ensuring optimal delivery of active ingredients, such as antioxidants and moisturizers, that contribute to both immediate and sustained benefits. Additionally, nanotechnology facilitates the regulated release of ingredients, thereby enhancing their efficacy and reducing the potential for adverse effects. Among these advancements, fullerenes are emerging as a key active ingredient, leveraging their nanostructure to offer potent

antioxidant and regenerative benefits, thus improving the long-term health and appearance of the skin.

However, a central problem exists: the fullerene C60 is practically insoluble in water and tends to aggregate, which reduces its bioavailability and efficacy. The hydrophobic nature and poor aqueous solubility of fullerene are the primary factors contributing to its low bioavailability. This hinders the body's ability to absorb and distribute the compound efficiently.

To overcome these limitations in potential biomedical or dermo-cosmetic applications, researchers employ various strategies to make fullerenes more water-soluble and improve their bioavailability. These methods frequently entail the functionalization process, which involves the chemical modification of the fullerene structure with polar groups. The incorporation of polar functional groups onto the surface of C60 is intended to enhance its dispersion in aqueous media and augment its biocompatibility. As illustrated in Figure X, the primary functionalization employed to enhance the polarity, solubility, and bioavailability of fullerenes are demonstrated [14,15,16,17].

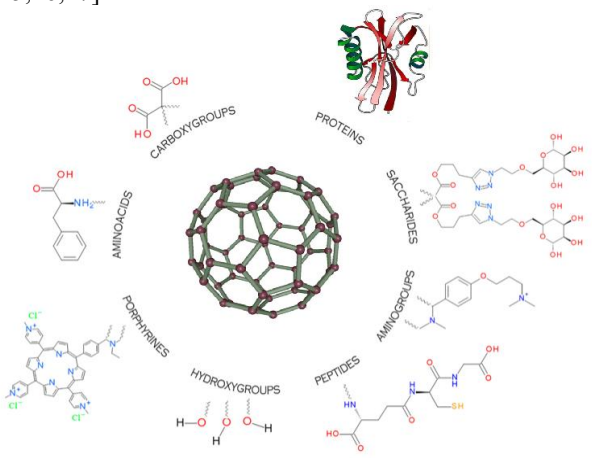

Fig. 3 Functional groups of polar fullerenes [Edited from 17].

These modifications enable fullerenes to be utilized more effectively in biological environments, thereby expanding their potential for therapeutic and diagnostic applications. This work aims to develop and evaluate the antioxidant capacity of C60 fullerene derivatives that have been functionalized with the polar aminoacids methionine and cysteine, both individually and in combination. The objective is to synthesize compounds that exhibit both high water solubility and low cytotoxicity to human skin fibroblasts. The research question this study aims to answer is: Can fullerene derivatives functionalized with cysteine and methionine exhibit high water solubility, low cytotoxicity, and antioxidant capacity, thereby allowing their application in cosmetic formulations?

II. Methodology

The proposed methodology involves the functionalization of fullerene derivatives, a process carried out in two main phases.

A. *Functionalization of C_{60} Fullerene*

The synthesis of amino acid-functionalized C_{60} was carried out following the methodology reported by Jiang et al. [18] The fullerene is uniformly dispersed in a solution containing one of two selected solvents, toluene or ethyl lactate, with 3% potassium polysorbate added as an emulsifier. Simultaneously, an aqueous phase is prepared with 10% w/w sodium hydroxide (NaOH) and ethanol, to which the amino acids cysteine and methionine are added for 24 h with constant stirring.

After the completion of the reaction, the solvents are subjected to evaporation through the utilization of a rotary evaporator under conditions of reduced pressure of 100 mbar. The sample is then subjected to a controlled drying process in an oven maintained at 30-50 °C. This drying process yields the functionalized compound in solid or viscous form.

B. *Characterization of functionalized fullerene*

Fourier Transform Infrared Spectroscopy (FT-IR) was used to evaluate the functionalization of the fullerene by identifying the formation and attachment of amino and sulfur groups from the amino acids linked to the C60 structure. This task was performed using a PerkinElmer Frontier spectrometer with 16 scans. This provided relevant information about the structure of the protein backbone and amino acid side chains, as well as how they bond with the fullerene [19]. The IR spectrum was acquired within the desired range, from 4000 to 400 cm^{-1}. The results were then analyzed to identify the characteristic bands of the functional groups present in the fullerene [19,20]. Additionally, the thickness (width and height) of C60(NH)n·mH2N was determined using a Molecular Vista PiFM Vista One device.

C. *Preparation of the cosmetic formulation*

The study used the active ingredient C60 functionalized with methionine and cysteine, incorporated at concentrations of 3% and 5%. The cosmetic formulation contained carbopol as a rheological agent in the aqueous phase; cetyl alcohol, stearic acid, and isopropyl myristate in the oil phase; and additives and preservatives such as propylene glycol, methyl paraben, propyl paraben, and potassium polysorbate 20 in an additional phase. Triethanolamine was used as a pH adjuster.

D. *Cell culture*

Human primary dermal fibroblast (HDFa) cells cultured in Dulbecco's Modified Eagle's Medium (DMEM) F12 supplemented with 5% FBS and 1% ampicillin/streptomycin, incubated for 24 h at 37 °C and 5% humidified CO_2 atmosphere.

E. Determination of cell viability

The CellTiter 96® Aqueous One Solution Cell Proliferation Assay is a colorimetric method used to determine cell viability. The assay utilizes a reactive solution that

produces a color when metabolized by viable cells, which can then be measured spectrophotometrically.

To determine the viability of HDFa cells, they were seeded at 5×10^5 cells/mL in a 96-well plate, and a cell viability assay was performed using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium)]-based CellTiter 96 Aqueous One Solution Cell Proliferation assay. Cells were treated with different concentrations of samples diluted in Dulbecco's Modified Eagle's Medium (DMEM) F12 supplemented with 5% FBS and 1% ampicillin/streptomycin. After the 24-h treatment period, the MTS reagent was added to each well, and the plate was incubated for 45 min at 37 °C. Following this incubation, absorbance was recorded at 490 nm using a 96-well microplate reader (Synergy HT, Bio-Tek, Winooski, VT, USA. Cell viability was expressed as a percentage relative to the untreated control cells. The absorbance value is directly proportional to the number of live cells in culture, providing a quantitative measure of cell proliferation or cytotoxicity under specific experimental conditions [23].

F. *Measurement of compounds with antioxidant activity*

The reactive oxygen species (ROS) measurement assay is a rapid, homogeneous, fluorescent method that quantifies ROS production by cells. This assay enables the evaluation of conditions or compounds that alter ROS levels as inducers or inhibitors. This assay uses APPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) as the pro-oxidant agent and DCFH (dichlorodihydrofluorescein diacetate) as the fluorescent reagent required for the spectrophotometric measurement. The measurement methodology is based on that reported by Wan et al. and López-Barrios et al. [21,22].

Human primary dermal fibroblast (HDFa) cells were seeded at 5×10^5 cells/mL and incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h to allow adhesion. The cells were subsequently rinsed with phosphate-buffered saline (PBS, pH 7.4) and treated with different concentrations of the samples, each containing 2',7'- dichlorofluorescin diacetate (DCFH-DA, 60 µM). After incubation (20 min or 24 h), the treatment solutions were discarded, and the cells were washed twice with PBS. Later, 100 µL of a 500 µM solution of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) was added to each well. Blank wells contained cells, but neither DCFH-DA nor AAPH was added. Positive control wells contained DCFH-DA and AAPH, but no samples. Negative control wells contained DCFH-DA without AAPH or samples. Fluorescence measurements were recorded using a microplate reader (Synergy HT, BioTek, Winooski, VT, USA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm every 2 min over 90 min at 37°C.

II. Results

A. *Results of C_{60} Fullerene functionalization and solubility measurement*

As presented in Table I, after the drying process both C_{60} + L-methionine and C_{60} + L-methionine + L-cysteine were obtained as dark brown colored materials; however, their final physical state could be attributed to the solvent used, for example, samples synthesized in toluene resulted in a powder after drying which is explained by the non-polar nature and high volatility of toluene, which favor efficient solvent evaporation and limit strong solvent of the solute interactions, thereby reducing intermolecular cohesion within the dried material [24].

TABLE I. RESULTS OF FULLERENE FUNCTIONALIZED AFTER THE DRYING PROCESS

	Toluene	Ethyl Lactate
C_{60}+ L-methionine		
C_{60}+ L-methionine and L-cysteine		

In contrast, samples prepared in ethyl lactate exhibited a viscous texture following the same drying procedure. This observation may be attributed to the higher polarity and hydrogen-bonding capacity of ethyl lactate, which can promote stronger interactions with the amino acid functional groups present on the fullerene surface. Such interactions, together with the lower volatility of ethyl lactate compared to toluene, may facilitate residual solvent retention or enhanced intermolecular association, resulting in a more compact and viscous material after drying [25]

B. Solubility measurement

The solubility behavior of the dried samples revealed a significant difference between unmodified C_{60} and its functionalized derivatives, as shown in Tables II and III.

TABLE II. SOLUBILITY RESULTS OF FULLERENE FUNCTIONALIZED WITH METHIONINE

System	Solvent	Mass Added (mg)	Volume of water (ml)	Tested concentration (mg/ml)	Observation
Fullerene C_{60}	-	0.2	2	0.1	Completely insoluble
C_{60}+ L-methionine	Toluene	0.2	2	0.1	Completely soluble
C_{60}+ L-methionine	Ethyl lactate	0.2	2	0.1	Completely soluble

For the aqueous solubility assessment, 0.2 mg of each dried sample was dispersed in 2 mL of distilled water, corresponding to a final concentration of 0.1 mg/mL.

Under these conditions, C_{60} alone did not dissolve, showing visible aggregation and sedimentation, consistent with its hydrophobic nature and its extremely low intrinsic solubility in water.

TABLE III. SOLUBILITY RESULTS OF FULLERENE FUNCTIONALIZED WITH METHIONINE AND CYSTEINE

System	Solvent	Mass Added (mg)	Volume of water (ml)	Tested concentration (mg/ml)	Observation
Fullerene C_{60}	-	0.2	2	0.1	Completely insoluble
C_{60}+ L-methionine and L-cysteine	Toluene	0.2	2	0.1	Completely soluble
C_{60}+ L-methionine and L-cysteine	Ethyl lactate	0.2	2	0.1	Completely soluble

On the other hand, all functionalized samples obtained from both toluene and ethyl lactate systems were completely soluble at the same concentration, forming homogeneous solutions without visible particulates.

The increased aqueous solubility can be attributed to the incorporation of polar amino acids (L-methionine and L-cysteine) onto the fullerene surface, which enhances overall polarity and enables favorable interactions, such as hydrogen bonding and dipole–dipole interactions, with water molecules. These results confirm that chemical functionalization effectively modifies the hydrophobicity of C60, significantly improving its compatibility with aqueous environments, independent of the solvent used during synthesis [26].

C. *Results of Characterization of functionalized fullerene*

For a fullerene functionalized with methionine and one functionalized with cysteine, the main infrared (IR) spectroscopy signals include characteristic bands in the region of approximately 1420 cm^{-1} and 1180 cm^{-1}, corresponding to the vibrations of the C=C and C-C bonds of the fullerene skeleton.

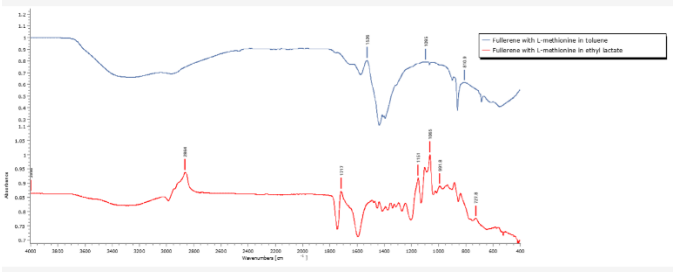

Fig. 4 Methionine- and cysteine-functionalized fullerene

Figure 4 shows the main signals of functionalized fullerene.

Additionally, bands corresponding to the methionine functional groups would be observed, such as a band at approximately 3300 cm^{-1} for the amine group (-NH_2) and a band around 1600 cm^{-1} for the carbonyl group (C=O) stretching. Similarly, for the cysteine functional groups, a band at approximately 2550 cm^{-1} would be seen for the thiol group (-SH) and another at 1600 cm^{-1} for the carboxyl group (C=O), like methionine.

D. *Determination of cell viability*

At 0.5 mg/mL (Fig. 5), the base formulation exhibited the highest cell viability (120%), forming a statistically distinct group (a) and suggesting a possible proliferative or protective effect on the cells.

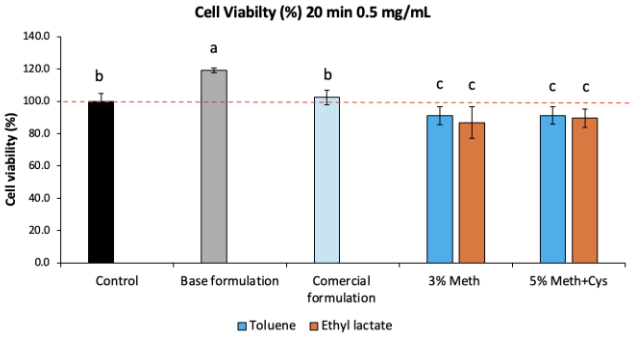

Fig. 5. Cell viability exhibited by human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.5 mg/mL.

Both the control and commercial formulation were grouped as b, suggesting no significant difference between them and confirming that the commercial product-maintained viability close to baseline levels.

Differently, the formulations containing amino acids (3% Meth and 5% Meth+Cys) showed slightly lower viabilities (85–92%), demonstrating a statistically significant reduction compared to the base formulation. However, viability remained well above the cytotoxicity threshold, indicating that methionine alone or in combination with cysteine does not induce severe cellular damage at this concentration. The similar response observed in both solvents further suggests that the

amino acids may modulate cell response without causing major solvent-dependent effects [30].

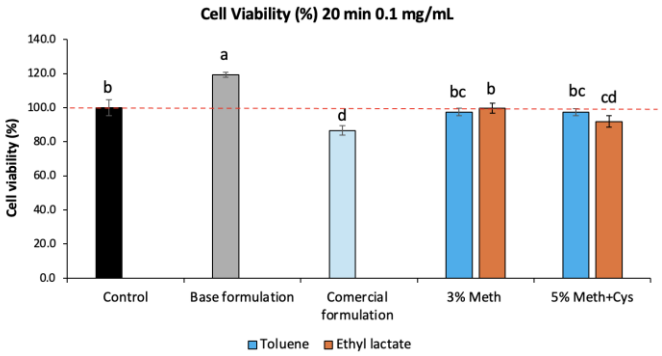

Fig. 6. Cell viability exhibited by human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.1 mg/mL.

At the lower concentration of 0.1 mg/mL, the base formulation again produced the highest viability, as shown at 0.5 mg/mL, remaining statistically superior to all other treatments and reinforcing its biocompatibility.

The commercial formulation, however, showed a marked decrease in viability, indicating a significant negative effect relative to the control. Notably, the amino acid formulations demonstrated improved cellular responses compared to the commercial product. The 3% Meth treatment reached viability values near those of the control (98–101%) and fell within the groups. In comparison, the 5% Meth+Cys formulation showed intermediate behavior, suggesting that cysteine addition may slightly reduce the protective effect observed with methionine alone [31].

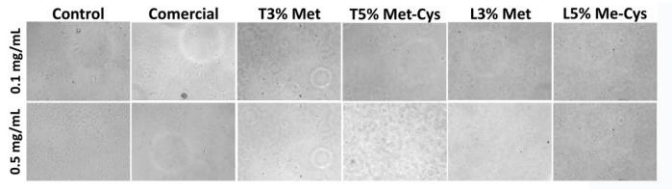

Fig.7. Representative images of human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.1 and 0.5 mg/mL.

Figure 7 shows control cells with typical morphology and high confluence. Methionine-based treatments preserved cellular structure, whereas the commercial formulation induced slight morphological alterations, and Methionine–Cysteine formulations exhibited intermediate effects without clear signs of cytotoxicity. Morphological evaluation showed that control cells maintained normal shape, adherence, and confluence at both concentrations. Treatments containing methionine (T3% Met and L3% Met) exhibited a cellular architecture comparable to the control, indicating good cytocompatibility. The commercial formulation presented lower confluence and mild structural irregularities, consistent with reduced viability.

Meanwhile, the Met–Cys formulations displayed moderate heterogeneity but no clear evidence of severe membrane damage or detachment.

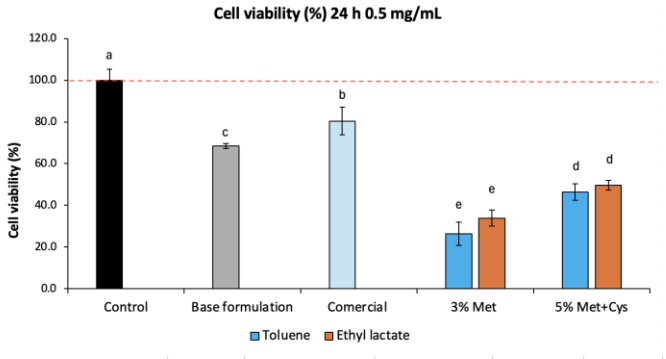

Fig 8. Cell viability exhibited by human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.5 mg/mL.

At 0.5 mg/mL, prolonged exposure intensified the cytotoxic response. The control again remained near 100% viability (a), while the commercial formulation dropped to 80%, indicating moderate but significant cellular stress. The base formulation, at around 68%, showed a similar decline to that observed at the lower concentration. Notably, amino acid formulations produced the greatest reduction in viability: 3% Met reached 25–35%, representing the strongest cytotoxic effect, whereas 5% Met+Cys improved slightly (45–50%) but remained significantly lower than the other treatments. These findings suggest a clear concentration and time-dependent decrease in viability, where the addition of cysteine may attend but not fully counteract the toxic effects associated with methionine during extended incubation [32].

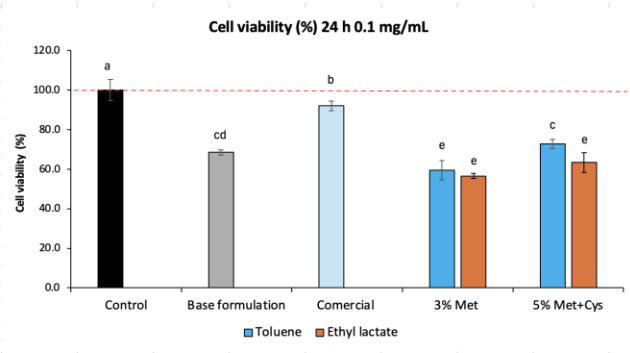

Fig 9. Cell viability exhibited by human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.1 mg/mL.

After 24 h exposure at 0.1 mg/mL, the control maintained the highest viability (100%) and was statistically different from all treatments, confirming optimal cell conditions. The

commercial formulation showed relatively high viability (90%), but remained significantly lower than that of the control. In contrast, the base formulation decreased to 68%, indicating a moderate cytotoxic effect over prolonged exposure. Amino acid–functionalized samples exhibited a stronger reduction in viability, with 3% Met presenting the lowest values (55–60%) and forming a distinct statistical group. The 5% Met+Cys formulation showed slightly improved viability (63–73%), suggesting that cysteine may provide partial protection but not enough to prevent long-term toxicity [33].

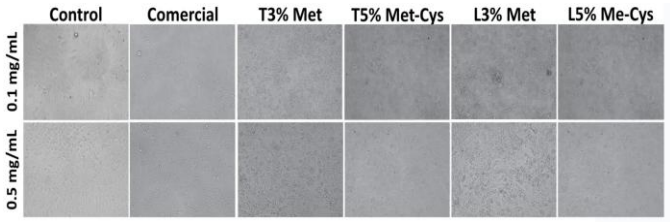

Fig 10. Representative images of human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.1 and 0.5 mg/mL.

Methionine-based treatments preserved cellular structure, whereas the commercial formulation induced slight morphological alterations, and Methionine–Cysteine formulations exhibited intermediate effects without clear signs of cytotoxicity.

E. *Measurement of compounds with antioxidant activity*

As shown in Figures 11 and 12, ROS production/cell viability were evaluated after 20 minutes of exposure at 0.1 mg/mL and 0.5 mg/mL.

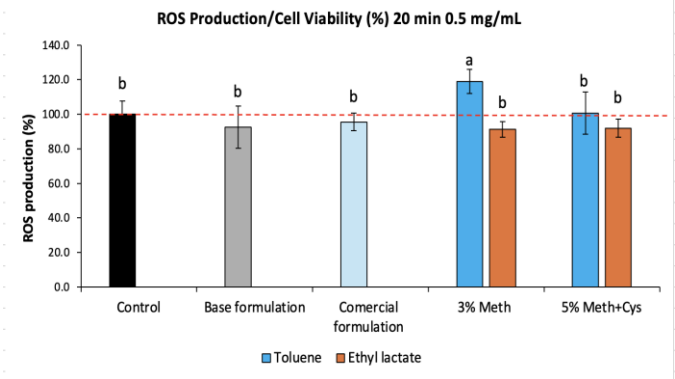

Fig 11. Reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.5 mg/mL.

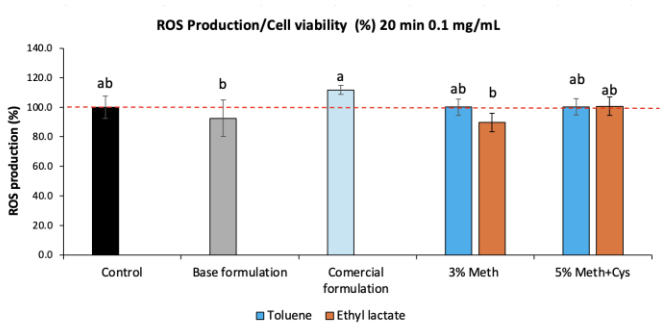

Fig. 12. Reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.1 mg/mL.

Statistical analysis indicates that treatments sharing the same letter are not significantly different ($p < 0.05$). As observed, at 0.1 mg/mL, the commercial formulation showed statistically higher ROS production than the base formulation, while the control and the amino acid–functionalized systems (3% Meth and 5% Meth+Cys) were classified within overlapping groups, indicating no significant difference among them. This suggests that at the lower concentration, methionine and methionine with cysteine incorporation did not induce a significant increase in oxidative stress relative to the control, but at 0.5 mg/mL, a concentration effect was observed.

The 3% Meth sample in toluene exhibited significantly higher ROS levels compared to the other treatments, indicating a potential pro-oxidant effect at increased concentration [27]

In contrast, the 5% Meth+Cys system remained statistically similar to the control, suggesting a more stable oxidative profile, it is known that the presence of cysteine, which contains a thiol group known for its antioxidant properties, which means that the thiol group may contribute to modulating ROS levels, whereas methionine, although sulfur-containing, may exhibit different redox behavior depending on concentration and formulation matrix [28].

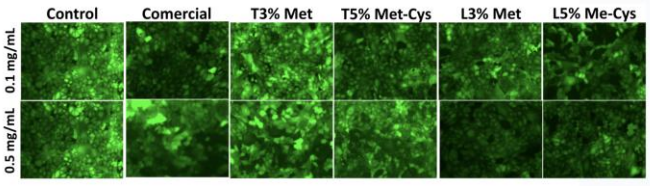

Fig 13. Representative fluorescence microscopy images of intracellular reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 20 minutes of exposure to commercial and experimental skin care formulations at a concentration of 0.1 and 0.5 mg/mL.

As shown in Figure 13, intracellular ROS production was qualitatively assessed based on green fluorescence intensity after 20 minutes of exposure at 0.1 mg/mL and 0.5 mg/mL. In this essay, higher fluorescence intensity indicates increased ROS generation. As shown, at 0.1 mg/mL, fluorescence levels were comparable to the control in most treatments, suggesting minimal oxidative stress.

However, at 0.5 mg/mL, certain treatments, particularly those with methionine 3% in toluene, exhibited stronger fluorescence, indicating elevated ROS production. In contrast, formulations containing methionine combined with cysteine showed comparatively lower fluorescence intensity, suggesting a more controlled oxidative response at the higher concentration as mentioned before.

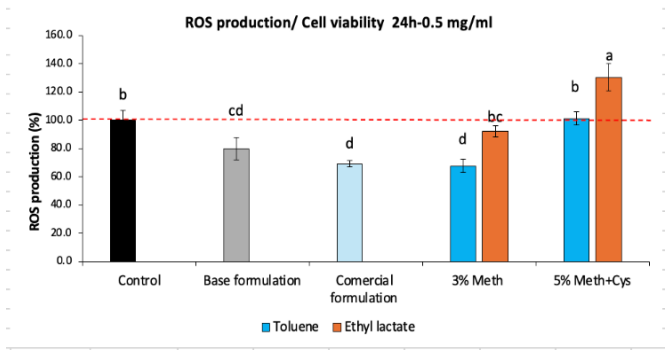

Fig. 14. Reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.5 mg/mL.

As presented in Figure 14, ROS production after 24 hours of exposure showed statistically significant differences among treatments ($p < 0.05$), as indicated by the distinct letter groupings. At 0.5 mg/mL, 5% Meth+Cys in ethyl lactate exhibited the highest ROS production, significantly exceeding that of the control and the other formulations. The control was grouped separately, while the base and commercial formulations showed significantly lower ROS levels, indicating a reduction in oxidative stress. The 3% Meth formulation presented intermediate values, particularly in ethyl lactate, suggesting that methionine alone may modulate ROS production depending on the solvent [29].

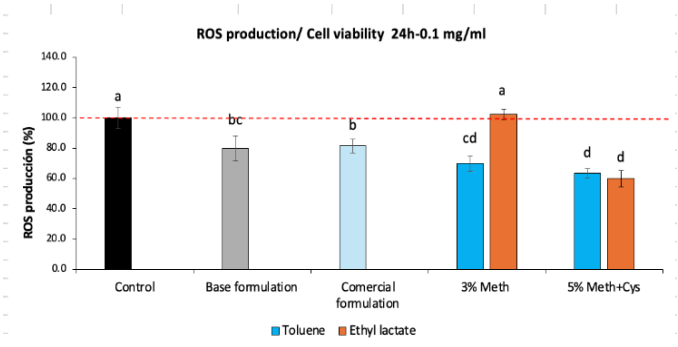

Fig. 15. Reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.1 mg/mL.

At 0.1 mg/mL (Fig. 15), most treatments were statistically comparable to or lower than the control. The 3% Meth sample in ethyl lactate was assigned to the highest statistical category, remaining close to baseline levels, whereas the 5% Meth+Cys samples in both solvents were assigned to the lowest statistical category, showing a significant reduction in ROS production. This decrease suggests a potential antioxidant effect associated with the combined presence of methionine and cysteine, likely related to their sulfur-containing functional groups, which are known to participate in redox regulation and free radical scavenging mechanisms [30].

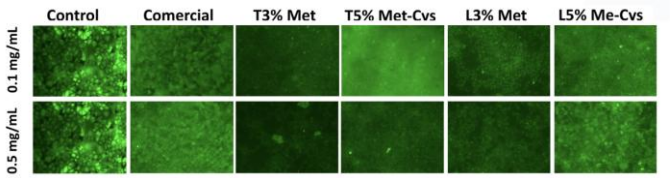

Fig 16. Representative fluorescence microscopy images of intracellular reactive oxygen species (ROS) production by human primary dermal fibroblast (HDFa) cells after 24 h of exposure to commercial and experimental skin care formulations at a concentration of 0.1 and 0.5 mg/mL.

Figure 16 shows representative fluorescence microscopy images of intracellular ROS production after 24 hours of exposure at 0.1 mg/mL and 0.5 mg/mL. Green fluorescence intensity is directly proportional to ROS generation, where higher fluorescence indicates increased oxidative stress.

At 0.5 mg/mL, some treatments exhibit visibly stronger fluorescence than the control, suggesting elevated oxidative stress at this concentration. At 0.1 mg/mL, fluorescence intensity appears generally lower and more homogeneous across treatments.

IV. CONCLUSIONS

The study demonstrates that fullerene derivatives functionalized with polar amino acids, such as methionine and cysteine, significantly improve the solubility and biocompatibility of fullerene C60 in dermal applications. Fourier confirmed the chemical functionalization of fullerenes with amino acid groups, as shown by transform infrared spectroscopy (FTIR), which showed a significant increase in solubility in aqueous media compared to unmodified C60.

In cell assays, the functionalized derivatives exhibited adequate biocompatibility at low concentrations, whereas pure C60 showed significant toxicity after 24 hours. Regarding antioxidant capacity, the formulations containing the functionalized product showed a significant reduction in reactive oxygen species (ROS) production, with the 3% C60-Methionine formulation proving the most effective.

While the experimental formulations exhibited satisfactory antioxidant performance in the initial phase compared with a commercial dermal product, they showed a decline in cellular stability after 24 hours, which requires future studies to stabilize the active ingredient. This study underscores the potential of methionine and cysteine modifications to enhance the properties of C60, thereby presenting promising alternatives for safer and more functional antioxidant dermal formulations.

The encouraging preliminary findings on antioxidant capacity and biocompatibility suggest new avenues for developing more efficacious and secure dermal products. As these techniques are refined and further studies are conducted, these modifications could lead to the creation of innovative antioxidant treatments to combat photoaging and other skin damage, thereby improving long-term skin health and appearance.

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