Accepted: 16 August 2016
DOI: 10.1111/exd.13172

VIEWPOINT

WILEY Experimental Dermatology

Fullerene C_{60} with cytoprotective and cytotoxic potential: prospects as a novel treatment agent in Dermatology?

Angelique Rondags | Wing Yan Yuen | Marcel F. Jonkman | Barbara Horváth

Department of Dermatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

Correspondence
Angelique Rondags, Department of Dermatology, University Medical Center Groningen, Groningen, the Netherlands.
Email: a.l.v.rondags@umcg.nl

Abstract
It is known that an excess amount of (oxygen) radicals in the skin can lead to (local cellular) oxidative stress. From one side, oxidative stress can contribute to the existence of various (inflammatory) skin diseases such as acne vulgaris and alopecia, as well as to accelerated photo-ageing of the skin. From the other side, oxidative stress could also be a wanted process for curing particular skin diseases, such as skin cancer and microbial skin infections. Therefore, novel treatment agents with the ability to scavenge or generate radicals can potentially be meaningful in the treatment of various skin diseases, especially for those diseases that have limited effective treatment options. This viewpoint essay will discuss the potential of fullerene C_{60}, i.e. buckminsterfullerene, derivatives as novel treatment agents in dermatology. Fullerene C_{60} is an all carbon molecule with a unique dual ability; fullerene C_{60} can act as a radical scavenger or as an oxygen radical generator. Hence, fullerene C_{60} derivatives offers most interesting prospects as a therapeutic protective or therapeutic toxic agent. Because of their extraordinary physicochemical properties and numerous chemical functionalization possibilities, chemists can design derivatives with a wide scope of unique properties. The experimental data, mostly from in vitro and in vivo animal studies, on the safety and therapeutic potential of fullerene C_{60} derivatives, in the field of dermatology will be discussed.

KEYWORDS
anticancer, antimicrobial, antioxidant, dermatology, fullerene C_{60}, inflammatory skin diseases, photodynamic therapy, reactive oxygen species, topical drug, UVR protection

1 | INTRODUCTION

1.1 | Fullerene C_{60}: a nano-scale molecule with a dual property: radical scavenging and generating ability

Fullerenes, discovered in 1985, form the third major carbon allotrope after diamond and graphite.[1,2] They exclusively exist out of n three-coordinate carbon atoms that are arranged in exactly 12 pentagons and (n/2-10) hexagons, where n≥20, forming hollow spheres, tubes and other shapes.[2] In 1990, the synthesis of macroscopic (milligrams) amounts of fullerenes through resistive heating of graphite began to develop.[3] Because it appeared that fullerenes possessed many unique and attractive physiochemical properties, such as lightweight, high tensile strength, thermal/chemical stability and conductivity, the possibilities of these carbon molecules have been extensively explored in many fields of science. The discovery of fullerenes was considered of great importance and has strongly influenced the field of chemistry. In 1996, the discoverers of fullerenes were awarded the Nobel Prize in Chemistry.

The most known and most abundant type of fullerene is fullerene C_{60} (C_{60}). C_{60} consists of 60 carbon atoms forming a highly symmetrical truncated icosahedron, which in structure looks like the pattern of a soccerball and the geodetic dome designed by the architect R. Buckminster Fuller in 1967, hence its nicknames “buckminsterfullerene” and “buckyball” (Fig. 1).[1,4]

C_{60} is a remarkably stable molecule of ~0.7 nanometer, with a molecular mass of 720 gram/mol, and has a large surface area.[1] C_{60} possesses a unique dual property. First, it is known to act as a “radical sponge” or antioxidant because of its 30 carbon double bonds to which multiple radicals, including reactive oxygen species (ROS), can easily be added.[5,6] Importantly, fullerenes do not become a self-reactive free radical during this process unlike most other antioxidants, including α-tocopherol and ascorbic acid, making them one of the most potent radical scavengers.[7] Second, in the presence of molecular oxygen ($^3O_2$), C_{60} is able to generate ROS such as singlet oxygen ($^1O_2$) via a type II (energy transfer) photochemical pathway and superoxide anion (O_2^-) via a type I (electron transfer) pathway (Fig. 2).[6-9,s1-s3]

However, pristine C_{60} is biologically inert, because it is extremely hydrophobic, forms aggregates upon contact with water and is nearly insoluble in polar solvents, which limits its use in biomedicine.[s4-s7] Several methods to overcome this problem are incorporation of C_{60} in water-soluble compounds and addition of hydrophilic functional groups to the C_{60}-cage.[s7-s9] Fullerenes are chemically reactive with almost all known classes of chemical compounds, which may allow scientists to functionalize them and optimize desired properties.[s10]

Briefly, in biomedicine, C_{60} is potentially useful in anticancer, antibacterial, antifungal and antiviral therapy, for enzyme inhibition, cyto- and neuroprotective purposes, targeted controlled drug delivery and diagnostic purposes (e.g. contrast- or radioactivity-based imaging).[s19-s21]

In this essay, we focus on the current knowledge of C_{60} and its derivatives as potentially novel treatment agents in dermatology. Currently, functionalized C_{60} agents are already available for approximately a decade in cosmetics; however, the introduction in clinical dermatology has not occurred yet.

2 | SAFETY ASPECTS OF DERMAL EXPOSURE TO FULLERENE C_{60}

Major factors which influence the behaviour and potential toxicity of C_{60} and its derivatives are size, shape, charge, methods of production, chemical compositions, surface functionalization, tendency to aggregate, dose and exposure time.[s14-s16]

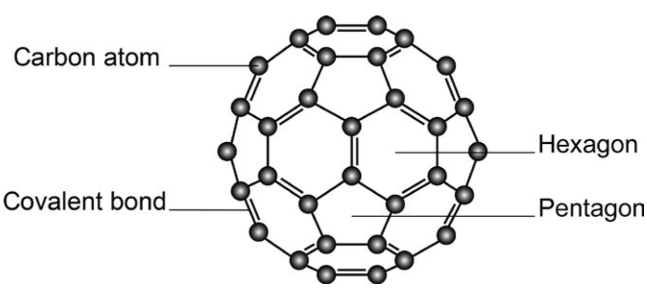

FIGURE 1 Schematic representation of a fullerene C_{60} molecule

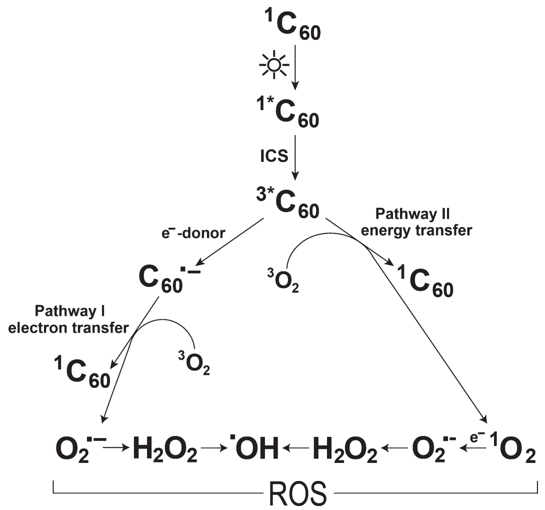

FIGURE 2 Schematic overview of photochemical pathways involved in ROS generation by fullerene C_{60}. When the ground state of C_{60} ($^1C_{60}$) gets excited by sun light or artificial light ($^{1*}C_{60}$, lifetime of a few nano-seconds), it undergoes intersystem crossing (ISC) to its relatively long-lived triplet excited state ($^{3*}C_{60}$, lifetime of tens–hundreds microseconds). In ICS, the singlet state non-radiatively passes to a triplet state in which the spin of the excited electron is reversed. In type I (electron transfer) pathway, $^{3*}C_{60}$, in the presence of an electron-donating compound (e^--donor, e.g. amines or antioxidants), can accept an electron which results in the radical anion of C_{60} (C_{60}^-) after which C_{60}^-donates an electron to molecular oxygen ($^3O_2$) to generate superoxide anion (O_2^-). In type II (energy transfer) pathway, the radical singlet oxygen ($^1O_2$), a highly reactive form of $^3O_2$, is generated by energy transfer from $^{3*}C_{60}$ to $^3O_2$ when $^{3*}C_{60}$ goes back to its ground state ($^1C_{60}$). In biological systems, O_2– and $^1O_2$ can also be converted to other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$)

2.1 | Skin penetration/absorption depends on C_{60} functionalization and skin condition

Very small nanoparticles (<10 nm) seem to be able to penetrate across the stratum corneum barrier into the dermis and can accumulate in hair follicles.[s17-s19] Results of an ex vivo (human) and a combined in vivo (porcine) and in vitro skin permeation experiments found a C_{60} penetration depth through the stratum corneum.[s20,s21] However, other research in which porcine skin was flexed, to resemble skin with a less intact condition, showed that a fullerene substituted peptide (~3.5 nm) could penetrate the dermis, suggesting the possibility of systemic absorption.[s19]

At this moment, it is still unclear what exactly happens to the C_{60} compound after topical application on human skin in vivo. Does it break down in parts? Can it be metabolized?

2.2 | C_{60} seems to locate near ROS-producing mitochondria

Cellular uptake studies of C_{60} (derivatives) demonstrate that C_{60} could pass the external cell membrane of human keratinocytes and fibroblasts, possibly through an endocytic cellular pathway.[s22-s25]

When internalized by cells, C_{60} appears to be preferentially located near mitochondria, which are ROS-producing organelles.[s23,s26,s27] It is therefore thought that C_{60} can scavenge ROS before the ROS can cause unwanted local cellular damage.

2.3 | On a cellular level, toxicity is dose- and time-dependent and functionalized C_{60} seems to possess less acute toxicity potential than pristine C_{60}

Several in vitro studies investigating the effect of C_{60} (derivatives) towards epidermal and dermal cells showed different reactions.[s15] One study found no effect of fullerenes on fibroblast proliferation.[s22] However, another did find a 50%–60% decrease in keratinocyte proliferation at the higher tested concentrations after 8 days.[s28] In vitro safety studies with fibroblast cells showed no acute toxicity of a tested C_{60} containing nano-emulsion.[s29] The overall in vitro results demonstrated that pristine C_{60}, more so than functionalized C_{60}, in a dose-dependent manner, has a potential cytotoxic effect, mainly because of ROS generation with its subsequent intra-cellular oxidative stress.[s28,s30-s33]

2.4 | No adverse skin reactions observed upon topical administration

In vivo studies (animal and human) suggest that single and repeated topical fullerene administration does not result in adverse skin reactions.[s34,s35] Furthermore, in vivo animal and human (with a history of various irritation and allergy susceptibilities) dermal patch tests showed no evidence for skin irritation (e.g. erythema and oedema) within the tested time.[s34-s36] Several research groups found no signs of irritation or a skin sensitizing effect of C_{60} determined in vivo (animal and human).[s29,s35,s37] Moreover, skin corrosion due to fullerenes was not found in current literature.

3 | CYTOPROTECTIVE THERAPEUTIC POTENTIAL OF FULLERENE C_{60}

Of fundamental interest is the role of free radicals in skin inflammation. In physiological systems, ROS are metabolic by-products, primarily generated in mitochondria, and are involved in both cellular signalling and cell damaging.[s26,s38] ROS have a wide range of biological targets including lipids, proteins and nucleic acid.[s26,s38] The skin has several enzymatic and non-enzymatic defense mechanisms, which interact with ROS to keep the skin homoeostasis balanced.[s39] When the physiological defense mechanisms fail, a state of oxidative stress occurs.[s39] In dermatology, oxidative stress is associated with inflammatory skin disorders including psoriasis, acne vulgaris, alopecia areata, bullous dermatoses, vitiligo, atopic dermatitis, rosacea, (photo)damage from sun and the various stages of carcinogenesis.[s39-s42] Controlling the inflammation, by tackling oxidative stress, with a novel agent, has a very wide-ranging potential in dermatology. Hence, ROS-scavenging fullerenes are possible novel therapeutic agents for these conditions.

3.1 | Ability to protect against detrimental effects of UVA and UVB irradiation?

Ultraviolet A (UVA), and to a lesser extent ultraviolet B (UVB) radiation, accelerates the skin's ageing process through a ROS-induced oxidative pathway, which can result in degenerative changes, for example wrinkles, pigmented spotsand both in melanoma and non-melanoma skin cancer.[s38,s43-s45] Experiments with C_{60} derivatives have shown a cytoprotective ability against UVB- and UVA-induced cellular damage.[s27,s46-s52] Moreover, also an anti-tanning effect was observed.[s50]

3.2 | Reducing inflammatory lesions of acne vulgaris?

Propionibacterium acnes has a significant role in the pathogenesis of acne vulgaris and seems to initiate the inflammatory process by producing neutrophil chemotactic factors.[s53] Once neutrophils reach the inflamed site, they release inflammatory mediators such as ROS causing a state of pathological oxidative stress.[s53] Therefore, it seems logical, and experimental evidence exists, that therapeutics with antioxidant activity can reduce inflammation symptoms in acne.[s53-s56] The topical antibiotics tetracycline, erythromycin and minocycline are favoured over other antibiotics because of their ability to suppress ROS generation from neutrophils.[s53,s55,s57] Benzoylperoxide has a dual effect: it induces ROS, which causes an antibacterial effect and it is directly cytotoxic towards leucocytes, which results in the inhibition of ROS generation by neutrophils.[s58,S59] C_{60} derivatives with strong radical scavenging ability are therefore possible new additions for epicutaneous acne vulgaris treatment.[s60,s61]

3.3 | Beneficial in after laser treatment care?

ROS generation, as a result of skin laser irradiation treatment, is crucial for the therapeutic effect, but could also lead to unwanted post-treatment side effects, such as erythema and pigmentation. ROS-scavenging fullerenes could ease the irritated skin after laser treatments and might prevent pigmentation.[s64]

3.4 | New therapeutic options for various hair loss conditions?

Oxidative stress might play a role in the pathogenesis of hair loss conditions.[s62] One study investigated the potential effect of several C_{60} in hair loss conditions in shaved mice and genetically hairless mice and also in human skin sections maintained in culture. An increased rate of hair growth and number of hair strands was observed.[s63]

3.5 | Helpful in successful wound healing?

Also for accelerated and successful wound healing, for example after injury and surgery, C_{60} derivatives might be beneficial.[s64] It is known that normal wound healing can be hindered by many

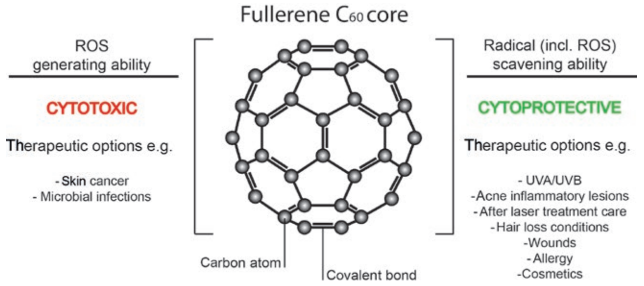

FIGURE 3 Summary fullerene C_{60} in dermatology

processes, including oxidative stress that inhibits cell migration and proliferation.[s64]

3.6 | Positive effects of C_{60} already demonstrated in cosmetics

Some C_{60} derivatives have already been available for circa a decade in topical applications for skin care, for example anti-ageing products, sunscreens and make-up bases, suggesting they should be safe to use for topical administration.[s15] Antioxidants, such as α-tocopherol and ascorbic acid, are known to efficiently scavenge and quench ROS and are thus being widely used in cosmetic formulations. Fullerenes are thought to be the most efficient antioxidant.[5,s65] Fullerenes used in cosmetics are commonly wrapped in polyvinylpyrrolidone or dissolved in squalane.

C_{60} derivatives seem to have the ability to improve fine wrinkles, increase collagen, improve skin hydration with decrease in transepidermal water loss and reduce the visibility of conspicuous pores.[s29,s51]

3.7 | Controlling ability of allergic response?

Several recent studies suggest that ROS, possibly generated by mast cells (MC), play a significant role in the pathogenesis of inflammatory and allergic skin conditions and diseases such as atopic dermatitis, urticaria and psoriasis.[s66] Findings from an in vitro investigation showed that C_{60} has anti-allergic properties by inhibiting type I hypersensitivity reactions to allergens.[s67]

4 | CYTOTOXIC THERAPEUTIC POTENTIAL OF FULLERENE C_{60}

Contradictory to the radical scavenging effect, C_{60} also has the ability to generate ROS. Because C_{60} can be photoexcited, where after ROS are generated, they have the ability to induce ROS-mediated cell death (Fig. 2). ROS are known to be able to cleave DNA.[s68] This led to investigations exploring the therapeutic ability of fullerenes as a photosensitizing drug (PS) for photodynamic therapy (PDT) for inactivating both malignant cancer cells as well as pathogenic microbial cells.[s69] The first in vitro studies were carried out with white light, visible light from a mercury lamp and with UVA and UVB light to demonstrate the (photo)cytotoxic effects of functionalized C_{60}.[s70-s72] The subsequent ROS generation could cause DNA cleavage and induce mutagenicity and cell membrane damage.[s69,s71]

4.1 | A new photosensitizer option for anticancer PDT?

C_{60}-glucose conjugates were investigated as a PS for anticancer PDT with positive results.[s73] Cancer cells endocytose glucose more effectively than normal cells because of an upregulation of glucose receptors.[s73] Another research group created a gadolinium chelated C_{60} to assess the efficiency of PDT in combination with magnetic resonance imagining (MRI) tumor imaging ability.[s74]

4.2 | Alternative for antibiotics?

Multiple (in vitro and in vivo (animal)) investigations also demonstrate the potential of C_{60} mediated antimicrobial PDT.[s75-s81] Due to the increasing worldwide problem of antibiotic resistance, fullerenes could be an important treatment option against pathogens in the future. Currently, topical use of a PS on superficial infected tissues and subsequent illumination seems to be the most prominent application of C_{60}-mediated antimicrobial PDT.

5 | CONCLUSION AND FUTURE PERSPECTIVES

To our knowledge, several C_{60} derivatives are used in commercially available skin care products with favourable results, indicating these derivatives are most likely safe and effective. However, they have not been implemented in clinical dermatology yet. Although the number of in vivo investigations is still minimal and sound data about the (long-term) safety of C_{60} derivatives is necessary, results of experiments regarding the potential of C_{60} in dermatology are positive. Due to extraordinary basic properties and numerous derivatization possibilities, the sky seems the limit for developing novel therapeutic and also diagnostic C_{60} agents for various dermatologic purposes in which oxidative stress plays either a pathogenic or therapeutic role (Fig. 3).

ACKNOWLEDGEMENTS

AR wrote this manuscript. BH provided the idea for this viewpoint. WY, MJ and BH critically read the drafts, contributed to revisions and gave final approval for submission.

FUNDING

None.

CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

ABBREVIATIONS

C_{60}, fullerene C_{60}; PDT, photodynamic therapy; PS, photosensitiser; ROS, reactive oxygen species; UVA, ultraviolet A radiation; UVB, ultraviolet B radiation.

REFERENCES

1. Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE. *Nature*. 1985;318:162–163.
2. Godly EW, Taylor R. *Fuller Nanotub Car N*. 1997;5:1667–1708.
3. Krätschmer W, Fostiropoulos K, Huffman DR. *Chem Phys Lett*. 1990;170:167–170.
4. Haddon R, Brus LE, Raghavachari K. *Chem Phys Lett*. 1986;125:459–464.
5. Krusic P, Wasserman E, Parkinson B, et al. *J Am Chem Soc*. 1991;113:6274–6275.
6. Krusic PJ, Wasserman E, Keizer PN, et al. *Science*. 1991;254:1183–1185.
7. Hirsch A, Bettreich M. *Radical Additions. Fullerenes: Chemistry and Reactions*. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA; 2005. 213–230.
8. Arbogast JW, Darmanyan AP, Foote CS, et al. *J Phys Chem*. 1991;95:11–12.
9. Yamakoshi Y, Sueyoshi S, Fukuhara K, et al. *J Am Chem Soc*. 1998;120:12363–12364.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Data S1. Supplementary References.