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A Retrospective Investigation of Novel Catenated Multi-shelled Fullerene-like Material for Biocompatibility after Prolonged Inhalation, Dermal, and Ingestion Exposure in Human Subjects. Part 3 in a series: Will Nanocarbon Onion-Like Fullerenes (NOLFs) Play a Decisive Role in the Future of Molecular Medicine?

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Abstract

While there have been several favorable *in vitro* studies on different cell types and components along with *in vivo* animal studies; there have been no published studies regarding the effect of nanocarbon fullerene material in humans that are readily known. Although fullerenes have been generally accepted as biocompatible and are being increasingly used in medical research, studies in human models has been mostly absent. Following the publication of the 2012 study by Baati., *et* al., which reported on the apparent antioxidant and life-extending effects of C_{60} fullerenes, a vigorous cottage industry of self-experimenters and bloggers developed; using fullerenes in olive oil or other lipid suspensions, participants have been reporting beneficial effects of using such. Herein, is presented a retrospective study on a small number of individuals that have experienced extensive inhalation, dermal, and oral exposure to a novel multi-walled nanocarbon fullerene material which has been demonstrating biocompatibility and an absence of induced pathology after prolonged exposure.

Keywords: *Allotrope; Aromatic Ring; C_{60}; Cyclic Native Aggregation; Dielectric Property; Fullerene; Hormesis; Mitochondria; Mitochondrial QED; Nanocarbon; Oxphos; Pharmacophore; Pi Clouds; Raman Band; Shungite; Thermodynamic; Quantum Electrodynamic*

Abbreviations

IRB: Institutional Review Board; GNO: Graphitic Nano Onions LLC; HIPPA: Health Insurance Portability and Accountability Act; NOLF: Nano Onion-Like Fullerene; PDT: Photodynamic Therapy; USAT: University of Science, Arts and Technology

Preface

The following preface is from the first and second in a series of articles by Bourassa and Kerna on nanocarbon onion-like fullerenes, titled: *Will Nanocarbon Onion-Like Fullerenes (NOLFs) Play a Decisive Role in the Future of Molecular Medicine? Part 1. Foundation in Fullerenes: Theoretical Application of NOLFs in the Quantum Cell* and *Part 2. Pristine Nanocarbon-based Fullerene-like Material Toxicity*

and Biocompatibility. The preface is provided verbatim, herein, so the concepts in this Part 3 may be readily grasped and the discussion proceed without having to refer to or review Part 1 and or Part 2; however, a review of Part 1 and Part 2 is favored:

The biology underlying medicine is undergoing a subtle but radical paradigm shift. Investigations into the quantum cell have been gaining acceptance. At some point, there must be a paradigm shift in medicine to reconcile the emerging realization that life's cellular machinery teeters on quantum criticality between order and chaos. Thus, an understanding of the specific mechanisms behind this apparent self-organized criticality [1] becomes necessary in the prevention and treatment of disease.

Biology, based on classical physics, has fallen short in explaining how the highly organized molecular machinery (taking care of myriads of complex processes such as DNA replication, protein synthesis, cell division, and metabolism) can operate with certain timing and precision in a healthy cell. The electrodynamics of the quantum cell provides the perpetual and precise transfer of charges throughout the system for exacting execution of biochemical tasks [2]. To more fully fathom the following exploration, it is helpful to be acquainted with concepts of the quantum cell, quantum electrodynamics, and electromagnetic fields in biologic systems as well as protein conformational change, structured cell water, and alternative cell physiology [3,4].

Introduction

Research into the more complex multi-shelled nano onion fullerene material has been limited due to its availability and cost. However, these nanomaterials are being recognized for their potential as collagen scaffolds, drug and gene carriers, and biosensors [5-7]. Most reported observations and anecdotal reports of favorable clinical properties of C_{60} and other single-walled fullerenes are often observed in multi-shelled nano onion-like material research.

The "discovery" of the C_{60} fullerene in 1986 as a "new" allotrope of carbon revolutionized the chemical world. Also, C_{60} and other spherical multi-layered carbon fullerene-like materials were confirmed in shungite, known for its healing "powers" and health benefits [8,9].

The results of the Baati study (2012), which evaluated C_{60} fullerene toxicity [10], spurred interest in the scientific community as well as the public; in particular, in anti-aging and alternative medicine circles. The Baati study (2012) not only demonstrated an absence of toxicity of C_{60} in laboratory rats but also showed the potential of C_{60} in free radical antioxidant protection. Also, increased vitality, longevity, and reduced tumorigenicity were observed in the subject animals. As a media consequence, several referenced blogs investigating the benefits of C_{60} in olive oil soon emerged. One such site was Anti-Aging Fire Walls [11]. These sites spurred interest, and the online availability of C_{60} in various edible oil suspensions have spawned a host of self-experimenters, including cancer patients and pet owners. Subsequently reported, there has been a notable absence of adverse effects from fullerene self-experimentation.

Similar beneficial effects, the absence of toxicity, and positive biocompatibility have been observed with the use of a catenated, multi-shelled nano onion-like fullerene (NOLF) material in experimental animal research. Hence, initially, a small number of people began self-experimentation by consuming NOLF orally in various plant oils. These first self-directed experiments into oral NOLF consumption included humans and pets—a few with severe or terminal illnesses including cancer. Among approximately one hundred known self-experimenters, to date, there have been no adverse events reported with its use; while the reported benefits are frequent and varied.

It is crucial to note that the authors are not advocating self-experimentation with fullerenes, and consider it potentially dangerous to consume substances of untested and questionable quality or safety. The reader is strongly advised to avoid self-experimentation with NOLFs or similar substances until any potential health benefits are scientifically confirmed as well as any severe adverse effects discovered and disclosed. However, self-experimentation it is being done by certain individuals (for a variety of reasons) and the reported results and anecdotal evidence offer a glimpse into the effects of fullerenes in human subjects. The first author of this paper had the opportunity

to interview and review the records of a limited number of individuals (with long-term exposure to a novel, high-quality and pure NOLF material) for evidence of adverse biological effects. All the subjects interviewed and or cases reviewed were either closely associated with carbon nanomaterial production or highly familiar with the carbon nanomaterial used, and were known to two of the authors.

Materials and Methods

Graphonyx (commercial name: Grafex) is a catenated, multi-shelled NOLF material that is available from Graphitic Nano Onions LLC (GNO), and was investigated by reviewing the effects of identified exposure in humans. This nano onion-like material consisted of catalyst-free, catenated, spherical and quasi-spherical, carbon nano onions (with a diameter of 20 - 60 nm) which required no further processing to approach a carbon purity level approaching 99.9%.

Exemption waiver was obtained from USAT IRB. HIPPA and informed consent were observed and performed regarding the retrospective review of medical records and interview comments of five human male self-experimenters (ages 61, 69, 71, 72 and 75 years) for the effects of human exposure to the NOLF material, Grafex. All subjects admitted to consuming between 1-2 tablespoons of 2-4.5 mg/ml (30-135 mg) GNO-produced material on a frequent to daily basis. Records and interviews were evaluated for exposure-related health problems, including liver toxicity, inflammation, respiratory problems, and physical impairments. Serum-derived lab values (drawn between 2015 and 2016) for all subjects were determined by Health Diagnostics Laboratory/True Health Diagnostics (head office: Frisco, Texas USA).

Three of the individuals studied had varying, intermittent dermal and inhalation exposure to Grafex material for 4-10 years working in the development, characterization, and production of the same Grafex material. One subject had intermittent, and significant, inhalation and dermal exposure, but reported no related respiratory disruptions or problems.

During the interviews, subjects reported that they felt it was safe and prudent to consume Graphex (based on the available research performed by GNO) and noted there were now several other self-experimenters consuming Grafex with compelling positive responses. They also indicated they were not aware of a single adverse response to its use. All reported they were intrigued by the results of the Desantis (2013) study [5]; especially Mouse #9 with the apparent resolution of PCa tumors. The self-experimenters noted, along with the cancer resolution, all of the Desantis mice maintained young adult appearance and vigor well into senescence. They also noted their extensive research into characterization and toxicity of Grafex that demonstrated its biocompatibility and potential for health benefits. This investigative review was limited due to the small population size and limited data available.

Results

All subjects reported favorable results associated with their use of Grafex. Subjective benefits varied with the individual and included feelings of increased energy and endurance, improved mental clarity and cognition, noticeable reduction in previous joint pain and inflammation, and overall good health. One subject, with prior occupationally-acquired lung pathology related to black lung disease, reported that his respiratory function improved enough (after inhalation and oral exposure to the material) that he was able to resume "singing in his church choir again". Another subject reported pain reduction when he applied Grafex oil emulsion to minor cuts and burns. There was a notable lack of adverse symptoms or health problems reported with its use up to and over 200mg per day. There was some anecdotal evidence that purported that the magnitude of positive effects may correlate with an increase in material consumption.

Of particular note, was the 72 y.o. subject with advanced osteoarthritis of both knees (verified by imaging), who reported relief from knee pain and resumed regular running after consuming two tablespoons per day of 4mg/ml in olive/coconut oil mixture containing Grafex. This individual also had extensive respiratory exposure to NOLF over a more than thirteen-year period. He reported a preexisting, mild, dry cough which was military service-related. He stated that this dry cough began in 1981 after arctic cold weather military training, and the dry cough had been occurring intermittently ever since. He noted his cough temporarily increased in frequency after The Deepwater Horizon Oil Spill Cleanup in 2010 in which he participated. He has had no other respiratory issues or findings. The subject agreed to a current chest x-ray, which was negative for lung pathology (Figure 1 and 2).

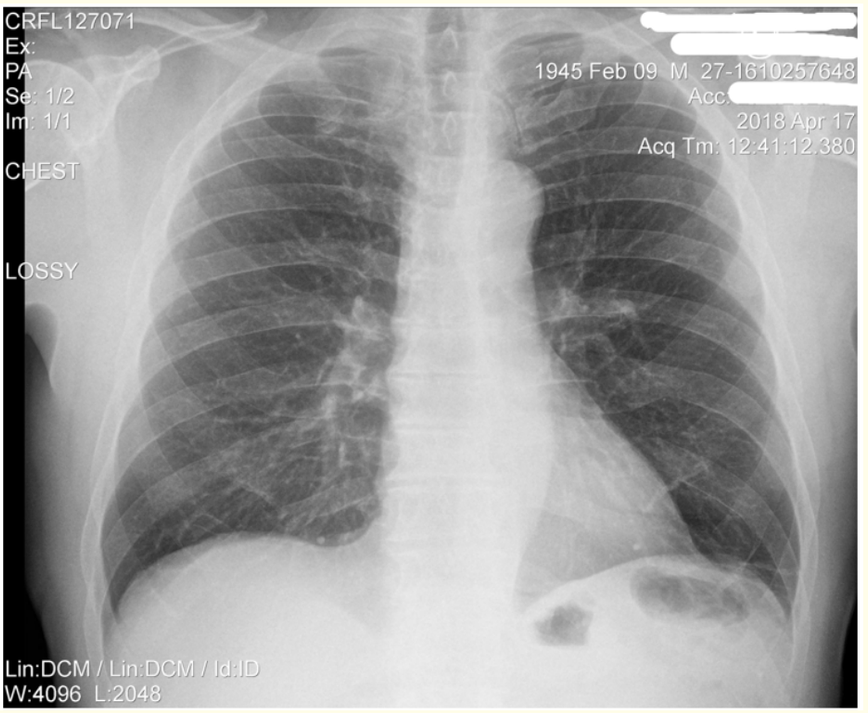

Figure 1: *Chest x-ray of 72 y.o. subject showing no apparent lung pathology or abnormality after regular exposure to NOLF over more than a thirteen-year period.*

Note: *The negative x-ray findings in a long-term self-experimenter may indicate, contrary to certain in vitro findings and animal studies, that NOLF has no adverse effects or inflammatory effects on the human lungs.*

PATIENT: [redacted]
DOB: 02/09/1945
MRN:
PHYSICIAN: [redacted]
EXAM DATE: 04/17/2018

EXAM: XR-Chest 2 views

REASON FOR EXAM: Contact with and (suspected) exposure to environmental pollution and hazards in the physical environment; Encounter for general adult medical examination without abnormal findings

ADDITIONAL HISTORY: Cough onset 2010, progressed over the past several years; nonsmoker.

TECHNIQUE: PA and lateral views of the chest.

FINDINGS: Heart and lungs are unremarkable. Mild bony enthesopathy away from the canal broadly through the thoracic region; osteoporosis of the age group; chest and upper abdominal patterns are otherwise unremarkable.

IMPRESSION:
1. Negative heart and lungs.
2. Bony enthesopathy through the thoracic region away from the canal.
3. Suspect osteoporosis.

Steven Sukstorf, MD

SJS/arf
DD: 04/18/2018 09:55 AM CST
DT: 04/18/2018 10:15 AM CST
Accession#: 27-1212763 VS#: 121585688 CS#: 2613358
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Electronically Signed and Reviewed by Steven Sukstorf, MD 04/18/2018 11:55 AM CST

Figure 2: *Radiology report corresponding to the chest x-ray (above).*

Note: *The negative x-ray findings in a long-term self-experimenter may indicate, contrary to certain in vitro findings and animal studies, that NOLF has no adverse effects or inflammatory effects on the human lungs.*

All subjects' lab results were indicative of general good health and above average health for their ages. There was no indication of liver or kidney toxicity related to their consumption of NOLF. Inflammatory markers were also generally low (Figure 3).

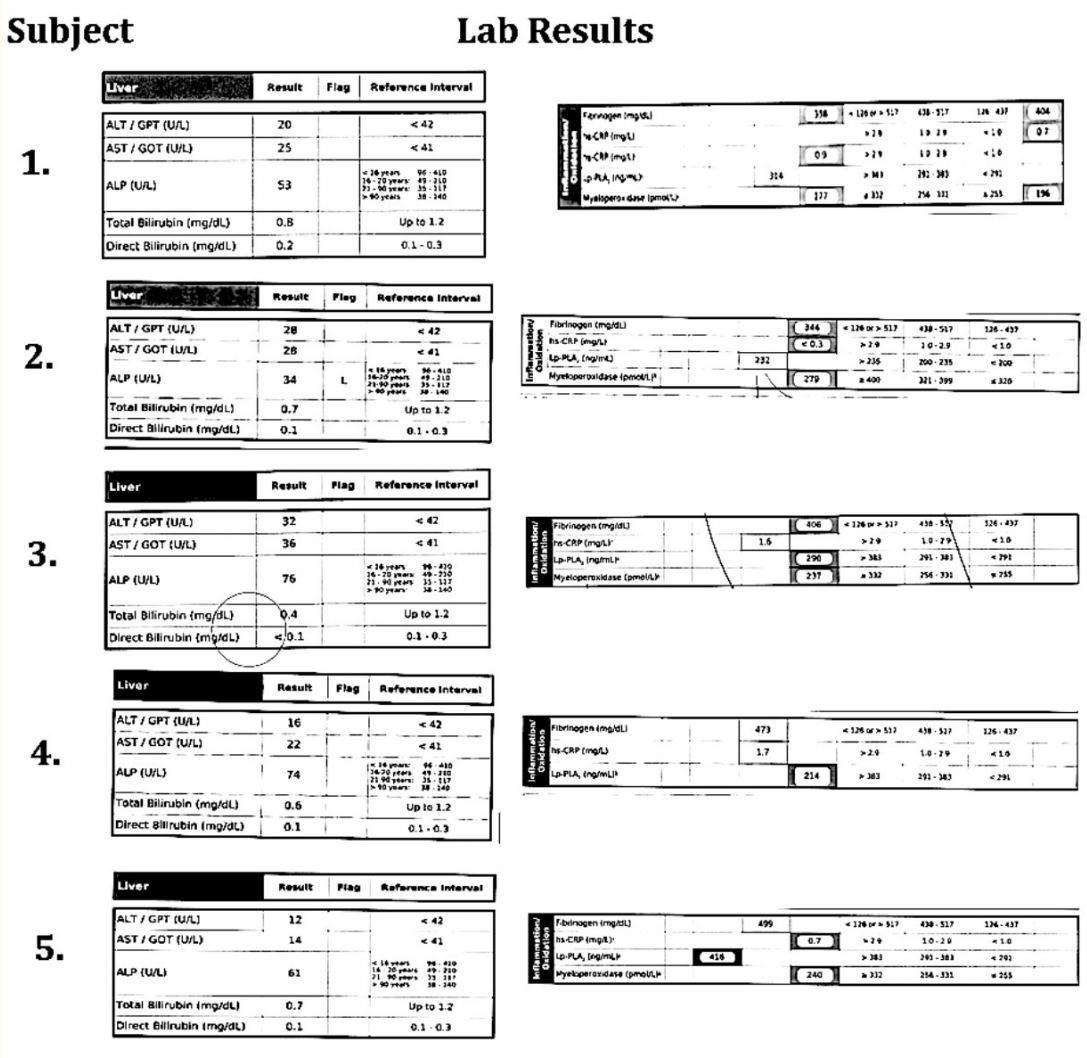

Figure 3: *Lab results for the five subjects taken after an average of three years near daily exposure to and or ingestion of NOLF.*

Note: *The normal blood laboratory findings of Subjects 1-5 indicate that the subjects are not experiencing any adverse effects from their long-term (3-5 years on average) exposure to NOLF (such as toxicity, inflammation, or infection) which some researchers posits will occur in humans, based on in vitro and or animal studies in exposure to NOLF.*

Discussion

Specific research on the breakdown of NOLF is lacking. However, the structural properties of NOLF are such that lysosomal and enzyme degradation of outer and subsequent inner shells would be expected to reach a C_{60} core. C_{60} and graphitic-based carbon materials are metabolized and eliminated within hours to weeks. Fullerene materials are pure carbon atoms. Given the biological conservation and

reuse of other carbon structures (such as the carbon skeletons of proteins), it seems likely that some of the fullerene-sourced carbon atoms are retained. There is no evidence of biopersistence in biological systems over years. Fullerenes are ubiquitous in nature, and evolutionary adaption to their presence would likely have occurred over time.

Regarding *in vitro* and *in vivo* animal models, the NOLF material–Grafex–has never exhibited toxicity. It has been shown to have biocompatibility in all testing, to date. In addition to biocompatibility, *in vivo* testing has revealed significant enhancement of qualitative and quantitative longevity benefits, anti-inflammatory properties, and reduced tumorigenicity in experimental animals. The proprietary production process used by GNO to produce Grafex results in a consistently ultra-pure, spherical multi-layered carbon fullerene-like material with a uniform catenated structure that does not require "cleaning or harvesting" and, thereby, avoids potential contaminants previously described in Part 1 and Part 2. The company supports these claims with documentation by several universities and US federally certified laboratory evaluations; testing was performed due to the material's high potential value in medicine and medical research. These studies were reported as being highly influential in the five subjects' decision to begin self-experimentation using Grafex.

DeSantis (2013) mentioned that there are a significant number of individuals who are self-experimenting with fullerene materials from shungite and C_{60} to complex NOLF, such as Grafex. The high number of unverified but overwhelmingly positive anecdotal reporting of benefits—coupled with the notable absence of negative experiences or toxicity (across the spectrum of pristine fullerene materials)—indicates the importance of further investigation and greater scientific scrutiny of these unique molecules. Furthermore, the effects of NOLF material due to inhalation, transdermal, or oral long-term exposure in humans have never been reported in the literature or is not readily attainable.

The uptake of fullerenes through transdermal, lung, or oral routes is poorly researched and limited. It is expected, however, that like many medications and supplements, the beneficial effects of fullerenes will exhibit a biologic biphasic response. Oral consumption up to 1g/kg of C_{60} in corn oil for 29 days showed little toxicity [12].

As mentioned previously, there is no evidence of biopersistence in biological systems over years. The lab work results of the self-experimenters reported on herein did not reveal liver toxicity despite, in some individuals, an up to 120+mg/day oral consumption on a long-term daily basis. Regarding an important question as to whether fullerene derivatives interfere with the immune response relative to autoimmune disease and blood viscosity (haemostasis and coagulation): cells of the innate immune system respond to fullerene exposure, but there is no readily available data demonstrating that pristine fullerenes impair the immune response or adversely affect autoimmune disease. Anecdotally, there have been some positive immune-related reports to the ingestion of the NOLF, Grafex. Although there have been studies regarding the affect of fullerene material and its interaction with hemoglobin, there is a lack of research on how fullerene material may affect blood viscosity, haemostasis, and coagulation.

Highly-pure, pristine fullerenes have been found to be biocompatible, in most studies. Toxicity testing should avoid the trap of merely attempting to assess general toxicity. The individual assessment—as to whether or not the tested fullerenes contain a contaminant or have had their outer shell modified by a previous contaminant or solvent—should always be determined. These aberrations and contaminants can result from manufacturing, purification, cleaning, or functionalization processes, and can adversely affect fullerene's biological interaction.

Pristine fullerenes have a strong affinity for electrons, and functionalization can modify their reactivity. As free radical scavengers, they are not reducing agents but rather oxidizing molecules that reduce or neutralize free radicals by addition reactions. As such, they remain biologically stable if an electron shell deficiency is not present in their outer shell orbital. Fullerenes absorb photon energy, especially in the UV spectrum, causing excitation energy from singlet to triplet state resulting in increased ROS activity; and may be desirable when used to target cells or destroy tumors via photodynamic therapy (PDT). However, any changes to their stable pristine condition—

that exceeds the cellular oxidative stress management capabilities—may render them damaging to biological systems. Therefore, each fullerene production process should be analyzed for induced-toxicity potential and assessed for biocompatibility in research.

Conclusion

The small population and other limitations of this retrospective investigation are recognized, but the length and the significant degree of exposure in the subjects present evidence suggesting a measure of the relative safety of this pristine, catenated, multi-shelled, nanocarbon fullerene material. These results, reported herein, add to the evidence of pristine, high-quality NOLF biocompatibility. However, any biological potential which indicates protective health and aging-related benefits must await greater scientific scrutiny for explanation and confirmation of such. While research often describes antioxidant free-radical scavenging as the primary biological mechanism of these fullerene materials, there may be other mechanisms benefitting cellular function that need to be explored.

Accordingly, there is a fundamental and compelling need for future medical research into multi-shelled fullerene material which—given the greater potential in their structure—may vastly exceed that of C_{60}. The mechanism of fullerene absorption, tissue concentrations, metabolism, elimination, and interaction in human cell biology remains widely unknown. In this retrospective investigation, limited but supportive evidence was revealed which indicates that fullerene research in humans may be approached with a blend of confidence and caution using pristine, high-quality fullerenes; and future research may well demonstrate further biocompatibility and lack of or low-toxicity.

The potential promise of fullerenes for humans may someday be realized or denied through scientific investigation. Currently, their potential benefits, combined with their apparent biocompatibility, seem so significant and far-reaching that in-depth research is warranted and should be forthcoming.

Conflict of Interest

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Acknowledgment

The authors wish to thank Graphitic Nano Onions, LLC for providing access to their existing research, data, and medical records collection.

Supplementary Note 1

Although the fullerene material, Grafex, was used primarily by the self-experimenters in this retrospective investigation, and Graphitic Nano Onions, LLC and its products and production standards were referred to at times within this paper; such occurrence should be viewed not as promotion but as coincidence, and due to the relatively few quality suppliers of pristine, biological-grade nano onion-like fullerene materials available to the general public.

Supplementary Note 2

The authors of this research advise against self-experimentation with any fullerene material until its biocompatibility and safety is supported by more scientific scrutiny and research. For now, testing of fullerene materials in biological systems, and in particular in humans, should be left to the research community, not the general public or untrained individuals; to do so is at one's own risk, and any

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