A Review of the State of the Art towards Biological Applications of Graphene-based Nanomaterials

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VKS, MY and MS designed the study, collected the data, performed the statistical analysis, wrote the protocol for first draft. Authors PK, Sheeba, Nafisa, VcW, SAK, NHAi and Sadiya helped in managing the literature data and helped in writing the MS and managed the graphical presentation. All authors read and approved the final manuscript.

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ABSTRACT

The field of nanoscience has evolved into a wide variety of successes over the past two decades and the emphasis on nanotechnology is to revolve around various dynamic fields, such as sensor, biomedical, and many useful applications. Advances in related fields are certainly due to the ability to synthesize nanoparticles from a variety of materials, structures, and to convert samples into

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complex nanoarchitectures. The promises of nanomedicine are broad. Graphene (Gr), the first 2-dimensional material to stand alone, is a type of new nanomaterial that leads to the excitement of natural biological applications. Number of researches has been conducted on applicability of GBNs in the area of environment, biomedical, and healthcare sectors. As compared to other nanomaterials, extraordinary properties of graphene-based nanomaterials (GBNs) like high surface area, multilayers, multifunctional and excellent biocompatibility make them capable to play great role of highly-tailored multifunctional delivery vehicles for drugs delivery, gene delivery, phototherapy and bioimaging. However, research communities performed plenty of research works on GBNs synthesis and biological activity evaluation, but there is limited comprehensive reviews published so far biological applications. So, we have studied a large number of scientific reports and investigations, presented in this review describing recent progress and modern perspectives with respect to graphene and related nanomaterials for biological applications.

Graph 1: Graphical Abstract

Keywords: Graphene; drug delivery; tissue engineering; nanomedicine; biomedical applications.

1. INTRODUCTION

In the 21st century, nanotechnology exhibit unfathomable influences on the entire world economy and society. R&D works in nanotechnology have gained promising breakthroughs in wide applicable areas such as materials and manufacturing, agriculture, bioengineering, healthcare, energy etc [1-3]. Nanomaterials have high surface to volume area, easy surface functionalization and the possibility offered by their multimodal conjugation with different functional groups, drugs, antibodies, contrast agents and selective ligands by covalent or not covalent interactions, make them ideal platforms for their application in biological and biomedical sector [4-6]. Although, traditional techniques like chemotherapy, radiotherapy and surgery [7] have many achievements. But there are so many drawbacks are present in clinical uses like resistant effect for multi-drugs, poor biological availability and non-specific bio-distribution in human body [8]. The biomedical applications have been found due to their
specialized and improved biophysical and biochemical properties and so, greatly influence biomedical synergistic effects [9].

Initially, graphene came into existence in the year 1859 discovered by British Chemist Benjamin Collins Brodie and theoretically further explained later Wallace. In recent times, graphene was considered to be a hidden gem and recognized as booming material in 2021 global revolution in all applied fields of science and technology. Graphene and its derivatives, due to a wide range of unique properties that they possess, can be used as starting material for the synthesis of useful nanocomplexes for innovative therapeutic strategies and biodiagnostics owing to its large theoretical specifications such as Young's modulus of approximately 1.0 TPa, great intrinsic mobility (~200,000 cm²/v·s) and surface area (~2630 m²/g) [4-7]. The strong available free π electrons, carbon-carbon bonding in the plane, and reactive sites for surface reactions boost graphene molecule as a brilliant as well as unique material with extraordinary, electronic, mechanical, physicochemical, optical thermal and biomedical properties [5-8]. Among GBNs, graphene oxide (GO) is one of the most potential materials for biomedical applications. GBNs, compared to the other carbon-based materials, have the large surface area, easily modified by different functional groups and better solubility that makes them an excellent choice for biomedical use. This review encompasses the recent trends and opportunities of graphene-based advanced materials indicate new potential applications of GBNs to anticipate more emphasis on the research exploration.

2. STRUCTURAL CONSIDERATIONS

In nature, pure carbon occurs in many allotropic forms made by the allotropic modifications of carbon like, graphite, diamond, lonsdaleite and chaoite etc. are some. Carbon is available in many forms such as graphene, graphyne, graphdiyne etc. Graphene is nanosized allotrope of carbon atom having very small thick layer approximately one atom. It has double sided surface available for organic reactions. There are several methods to synthesize graphene from graphite such as mechanical cleavage, graphite oxide/fluoride reduction, liquid phase exfoliation, intercalation, compound exfoliation, chemical vapor deposition, bottom-up approach of chemical synthesis and epitaxial silicon carbide decomposition. Preparation of graphene from graphite exfoliation is the most popular technique. This technique is done in the presence of various dispersants. Graphene has huge potential for technical applications by having light weight, high mechanical strength, stiffness, superior elasticity and thermal properties, electrical properties, high electron mobility. Graphene also possesses quantum hall effect, Dirac fermions transportation and good optical transparency.

Graphene has several structures and there should be deep knowledge of structure of particular material under consideration. Structure of graphene, graphene oxide and reduced graphene oxide revealed that they possess two-dimensional and has six-Carbon atoms in hexagonal array like honeycomb (Fig. 1). Graphene is a zero defective single plane of graphite with four sp²-hybridized bonds. In four bonds one bond is σ bonded with three neighbors and one is π-bonded which is oriented out of the plane. Metals and functional groups are employed in fabrication process of GBNs due to which some impurity is found in graphene and some surface area of graphene is reduced. So before applying GBNs some covalent and non-covalent modification is required to increase the efficiency of graphene [10]. Exfoliation is good technique in large scale syntheses of graphene. Graphene and GO can be differentiated on the bases of oxygen atoms bounded to carbon. Go was produced by Brodie during his study of chemical reactivity of graphite in 1859 [8-11]. GO is prepared by treating with acid and base and then sonication is done. Number of functional groups are found present on the surface of GO like oxygen, epoxide groups, carbonyl groups, hydroxyl groups and phenolic groups. Modified carboxylic group by covalent functionalization is helpful for studies of biological systems and applications. Due to Non-covalent functionalization of graphene, new chemical groups are not allowed to change structure or electronic properties.

GO can be synthesized using H₂SO₄/KMnO₄ by Hummer’s method through oxidative exfoliation. Composition and non-stoichiometric structure of GO are strictly depend upon production details [12]. Number of researchers studied on the GO structures. Some of them are named as Hofmann, Nakajima-Matsuo, Ruess, Lerf–Klinowski Scholz–Boehm and Szabo models [11]. There are plethora of evidences in the favor of Lerf–Klinowski structure to describe GO [13-15].
Table 1. Difference between normal and cancer cell

<table>
<thead>
<tr>
<th>Structural Parameters</th>
<th>Normal Cell</th>
<th>Cancer Cell</th>
</tr>
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<tbody>
<tr>
<td>Cell Shape</td>
<td>Uniform</td>
<td>Irregular</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Spherical</td>
<td>Irregular</td>
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<tr>
<td>Cytoplasmic Volume</td>
<td>Large</td>
<td>Small</td>
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<tr>
<td>Growth</td>
<td>Controlled</td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Location</td>
<td>Remain in their intended Location</td>
<td>Can spread to different Locations</td>
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<tr>
<td>Chromatin</td>
<td>Fine Chromatin</td>
<td>Coarse Chromatin</td>
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Fig. 1. Schematic formation of graphene, graphene oxide (GO), reduced graphene oxide (rGO)

rGO is prepared by chemical or thermal reduction of graphene oxide or graphite oxide. Structure of rGO is an intermediate form of graphene sheet and GO which should be highly-oxidized. Various reducing agents are employed during production of rGO such as L-ascorbic acid hydrazine, hydrazine hydrate, and sodium borohydride. Poh and co-workers have better described the properties of reduced rGO [15].

For better understanding of structure and properties of any material, characterization of that material is required. There are various techniques available for investigating the quality of graphene and number of atoms of oxygen present on the surface of GO and rGO. Several Spectroscopic techniques such as nuclear magnetic resonance, Raman spectroscopy; transmission electron microscopy spectroscopy, solid-state Fourier transform and atomic force microscopy are helpful to understand the structural properties of GBNs. Raman spectroscopy is a technique employed to evaluate the lattice defects of the graphene and it has non-destructive nature. UV–visible spectroscopy is a powerful tool to determined non-defective graphene layers. In these radiations are hindered by graphene layers and hindrance increases by layer to layer increase from bi-layer to six-layer at the same wavelength. Composition, electronic and chemical state of material can be studied by X-ray photoelectron spectroscopy [16]. This spectroscopic technique is a sensitive to surface and its nature is quantitative which provide information about different types of elements present in the sample.
and their bonding [17,18]. Morphological characterization of graphene can also be known by Energy dispersive X-ray spectra. It helps in finding metal catalyst which are trapped in lattice structure [19] and distinguishing functional groups on the surface of GO. Advanced Forced Microscopic Technique is necessary for getting information about characterization and its composites. Investigation of few-layer of graphene flakes can be done by using atomic force microscopy. AFM technique should be avoided for large areas and regular scanning. To study graphene nanosheets different modes are employed to such as electrical, magnetic, elastic, frictional and mechanical properties [20].

Morphological characterization can be achieved by Scanning Electron Microscope. Surface of sample is focused by beam of electrons and various signals are observed providing information for composition and surface topography. Fine microstructure characterization can be achieved by Transmission Electron Microscopy. This technique employs a beam of electrons to the specimen and image is produced. Using electron diffraction techniques in situ, atomic scale resolution is achieved with various crystallographic and imaging modes. [21]. Resolving power of TEM is found nearly 20 times more than the SEM. The minimum distance between two distinguishable points can be about 0.2 nm. Before TEM characterization, sample is prepared by long and sophisticated techniques [22,23]. Thermal stability of element and fractions of volatile components can be found by Thermogravimetric analysis or thermal gravimetric. It analysis the change in weight of specimen under observation on heating [24,25]. The weight is normally measured in presence of air or in presence of inert atmosphere, Air used is helium or argon and change in weight is dependent on increase in temperature. TGA and Infrared spectrophotometer both are jointly used to analyze the gases which are released during thermal decomposition. TGA technique has major roll to know about the difference in the thermal degradation among GO and rGO.

3. BIOLOGICAL APPLICATIONS

Emergence of GBNs lead to different application in multiple fields like drug and gene delivery, cancer treatment, cancer cells are different in size and shape with abnormal growth- Table 1 tissue engineering, antibacterial materials development, biosensing etc. Biocompatibility is key point for the selection of GBNs for desired application. Go and rGO are found better aqueous and have colloidal stability as compared to carbon based materials and hence GO and rGO are suitable for drug delivery and therapeutic applications. Number researches are going on graphene for regenerative medicine and tissue engineering applications. Graphene helped the researchers to get innovative ideas to deliver antitumor drugs to tumor cells. Graphene based nanocarriers are studded enormously from last few years and used as therapeutic agents such as, antibodies, DNA, RNA, chemotherapeutic drugs and genes etc. GO and rGO are biocompatible and easily modifiable surface area. GO and rGO can be modified for generating opportunity for linking with carboxyl, epoxide, hydroxy groups and hydroxyl. These groups can provide attachment sites to various molecules like protein, DNA and RNA [26,27].

3.1 Drug Delivery

GO is relevant for utilizing in many biological applications due to its properties of large surface area, multiple layers, surface chemistry, lateral dimension and purity. Graphene has every atom available on the surface of a layer due to which it posses higher drug loading capacity. GO based drug delivery systems have particle 1-100 nanometer size ,1-2 nanometer thickness and 1-3 layers. Graphene is suitable as nanocarrier for drug delivery by its amazing properties like large specific surface area, small size, low cost, intrinsic optical properties and non-covalent interactions with drug molecules. Physisorption properties make GBNs capable to use in loading hydrophobic drugs with antibodies for selectively killing of cancer cells such as doxorubicin and docetaxel.

Chemical modification, electrostatic interaction and non-covalent bonding are employed in drug delivery techniques of GBNs [28,29]. Hydrophobic interactions assist drug loading with high influencing power and high efficiency for less soluble drugs also. GO provides both sides surfaces for conducting and loading drug. Reactive sites are provided by hydrophilic groups to connect functional molecules to improve the anticancer efficiency such as carboxyl, hydroxyl, and epoxy. These functional groups present on surface are able to provide facility for conjugation with various systems and biomedical imaging [22,26].
Anticancer drugs such as CPT, PTX, and DOX have been widely used in clinical methods. There is interaction of cells membrane with nanocarriers by endocytosis. It is a necessary requirement for targeted drug delivery to the cell nucleus that drug carrier should escape endosomal compartment and release loaded drug into the cytosolic compartments. This proposed a new idea of loading DOX on GO surface to reverse the cancer drug resistance in DOX resistant MCF-7/ADR cells. This technique result higher cytotoxicity in comparison to free DOX. On the bases of tumor characteristics, the microenvironment Stimuli-responsive nanomaterials are designed and technique is used in tumor curing by assisting for penetration, diffusion and release of drugs. In the cancer treatment pH should be acidic in cancer micro environment, intracellular lyso-somes and endosomes and this help in active drug release in the tumor tissue/cells. DOX is loaded on nGO-PEG by simply mixing at pH 8 environment to form nGO-PEG-DOX [30]. DOX loaded graphene oxide has tumor growth inhibition about 66–91% cell death. GO loaded Paclitaxel and Methotrexate via π–π stacking and with amide bonds have found effective results in inhibition of tumor growth about 66–90%. It has effective cancerous effect on lung and breast cancer. GO with folic acid in drug loading system. Both DOX and CPT loaded on functionalized nGO have higher cytotoxicity to cancer cells as compared to GO-loaded with DOX or CPT alone for same drug delivery system. Higher cytotoxicity was observed on the composite rather than raw GO self loading. Some of specific targets are included such as HA receptor, FA receptor, Tf receptor, CAMR, EGFR and VEGFR [31]. Modification of FA has great contribution to improved tumor therapy. Liu synthesized FA-modified fluorinated GO carrier for DOX delivery [32]. Subcellular organelles have great attraction for tumor therapy. GA targeted into mitochondri with combination of GO and loaded DOX as carrier is applied by Zhang et al. [33].

3.2 Gene Delivery/Therapy

Gene therapy resolves the problem of abnormalities in gene treatments methods. It is important discovery over the traditional methods of treatments and helpful in precision medicine techniques. Gene therapy is employed for treatment of genetic disorders like cancer Parkinson’s disease and cystic fibrosis. Gene therapy strategies are under research for cancer treatment. Gene therapy is very fast, less toxic, inexpensive, higher disease recovery rates and effective. Due to ameliorate research, cheaper gene vectors will be available in the market which will make treatment accessible for most of the cancer patients. As compared to chemotherapy gene therapy has been found relatively safe as side effects are tolerable [30,32].

Recent progress in developing safe and effective vectors approaches for untradeable diseases like cancer. Nucleic acids are introduced in Gene therapy through gene knockout or expression into targeted cells. GBNs are employed as carriers and interact with nucleic acids, DNA and RNA [34]. Due to physisorption properties of GO nucleobases get absorbed by π–π interaction, by these nucleotides enzymatic cleavage is protected. Basically gene delivery vector provides degradation protection to DNA and transfection efficiency is high. As a vector approach graphene oxide nanosheets are easily taken by cells [35]. There are two techniques for gene delivery systems, one is viral vector and other is non-viral vector systems. Viral vector system for transferring gene is highly efficient, but there are some limitations like insecurity, higher costing, host immune response [36,37]. Though non-virol vectors are simple but their immune response risk is low. Gene carriers are better over the viral and non-viral vectors in terms of high immune response, low toxicity, chromosomal integration, formation of mutations in DNA molecules and production cost. GO-based nanomaterials are super vehicles for drug loading due to their large surface area, good capability for adsorption, good biological-safety and biocompatibility. It is found that through π–π stacking, GO can absorb single-stranded DNA (ssDNA), but have difficulties for double-stranded DNA (dsDNA). This is resolved by Di Santo by designing GO/cationic lipid nanoparticles and plasmid DNA (pDNA) [38] to introduce artificially RNA or DNA into HeLa cells and HEK-293 cells.

For breast cancer treatment graphene-based gene therapy used many factors like siRNA [39], pDNA [40], microRNA-101 (miR-101) [41] and the HSV-TK [42]. GO derivatives help in penetration of siRNA or pDNA into cells by protecting enzyme cleavage to DNA [43]. Huang designed PEI-functionalized GO to transfect siRNA and technique is found effective and may be helpful for inhibiting breast tumor metastasis [44]. Graphene can absorb NIR light. Tian increased efficacy and taking capacity against cancer cells by localized NIR heating of GO–
PEG–Ce6.NIR heating increases membrane fluidity and hence helpful to enhance taking capacity of GO–PEG–Ce6 by [45]. According to Kim, functionalized rGO irradiation by NIR improves drug/gene delivery and intracellular lifetime [46,47].

3.3 Diagnosis and Imaging

Diagnostic and observation of the unique biological components can be ratified easily by in vivo and in vitro bioimaging techniques. Sensitivity of these bioimaging is low for small lesions, so there is need to find new technologies. GBNs technologies are found sensitive enough for these small lesions. Graphene quantum dots (GQDs) is a type of GBNs. Properties of photo-physical and fluorescence spectroscopy made GQD most widely employed for bioimaging. Bio-imaging help to detect the growth of processes which are abnormal in nature like hypoxia/hyperoxia and cancer development and or necrosis. Bio-Imaging techniques includes confocal and fluorescence imaging for graphene based structures [48], like photoacoustic imaging [49], magnetic resonance imaging [50], positron-emission tomography (PET) [51,52], ultrasound imaging [53,54], coherent anti-stokes Raman scattering imaging (CARS) [55,56], surface-enhanced Raman scattering (SERS) [53], and electron paramagnetic resonance imaging (EPRI) [57,58].

Magnetic Resonance Imaging: MRI method is a non-ionizing and non-invasive in nature. The diagnostic technique of MRI has ultra spatial and temporal resolution [59-61]. To get detailed and accurate information of image, Gd, Mn, and Fe which are contrasting elements should be accumulated in tumor region. Tumor targeting and long lasting blood circulation time vector should be developed on urgent bases. MRI combination with PTT has advantage to get diagnosis and treatment in same time. Meng prepared a GO composite nanoscaled metal–organic frames, which is applied in tumor-guided PTT with MRI [62]. In a consequent work, Usman et al. reported the synthesis of a graphene oxide (GO)-based theranostic nanodelivery system (GOTS) for magnetic resonance imaging (MRI) using naturally occurring protocatechuic acid (PA) as an anticancer agent and gadolinium (III) nitrate hexahydrate (Gd) as the starting material for a contrast agent [63,64] (Fig. 2 and Fig. 3). In addition, Lage et al. investigated the combination of diagnostics and therapy (theranostic) for nanoengineered multifunctional systems in nanomedicine (Fig. 2). From the various multimodal nanosystems for graphene-based magnetic nanoparticles (GbMNPs) as theranostic platforms. [64,65].

Fluorescence Imaging: FLI is a suitable for diagnosing tumor and its treatment. FLI technique is non-invasive in nature in which photons are emitted by fluorescent probes. High loading capability of GO leads to introduce FLI-guided theranostic by FLI agents [65]. During the therapeutic process FLI is used in observation of the pathological tissue and find distribution and metabolism of drugs [66].

Photoacoustic Imaging: PAI is dependent on the photoacoustic (PA) effect and is found to be a very effective diagnostic tool. In this imaging short pulses of non-ionizing laser are absorbed to convert them into heat and thermal expansion gives specific natural acoustic signals. PA agents are excellent in photothermal conversion. Most of the agents in application for PAI are noble metal, inorganic NPs, semiconducting NPs, and NIR dye [67-70].

Raman Imaging: Surface-enhanced Raman scattering is mostly in used for disease diagnosis and biomolecular detection due to non-invasive, ultra-sensitive and high resolution [71,72]. Noble metals like gold with specific local SPR features have excellent Surface-enhanced Raman scattering activity [73-77]. To target Raman imaging of HeLa cells, a multifunctional platform is designed by Zhang which is based on GO (GO/AuNP/FA [78,79]).

Computed Tomography: CT is a widely used non-invasive and high spatial resolution based disease diagnosis method [80]. Graphene oxide-based nanomaterials bioimaging for tumor diagnosis have more advantages than other materials. GBNs have a number of achievements in vitro cellular and in vivo bioimaging. A guided cancer therapy Imaging is designed on the bases of PVP–rGO/Bi2S3 by Dou [81]. CT has excellent performance for getting information about tumors position and details. GBNs have excellent multimodal bioimaging performance by PA/MR/CT/FL for detecting, treatment and monitoring of the tumor. Based on the above discussion, it can be hopefully assumed that the platform for green and sustainable research and development is currently focused on novel materials like, grapheme-based materials, which have soon become the prospects for
Fig. 2. [A] (a) Active agents-loaded of GAGPAu nanocomposite in a theranostic nanodelivery system. Diagnostic agents, gadolinium (green) and AuNPs (yellow) and the anticancer agent, PA are attached on a graphene sheet via hydrogen bond, π−π interaction and electrostatic interaction (GOTS); (b) Release profiles of protocatechuic acid from GO-Gd/PA nanocomposite (GAGPA) in pH 7.4 and 4.8 media; (c) Pseudo-second order kinetic plot of protocatechuic acid release data at pH 4.8 medium from GAGPA nanocomposite (Drug-GO/Gd); (d) Pseudo-second order kinetic plot of protocatechuic acid release data at pH 7.4 medium from GAGPA nanocomposite (Drug-GO/Gd); [B] FTIR spectra of GO nanosheets (A); pure protocatechuic acid (B); pure Gd(NO3)3 (C) protocatechuic acid loaded on GO/Gd nanolayers (GAGPA) (D); and gold nanoparticles coated on GAGPA nanocomposite (GAGPAu) (E); [C] Schematic representation of graphene-based magnetic nanoparticles (GbMNPs), their usual functionalization, targeting and triggering strategies, as well as their most used combined diagnostic and therapeutic applications (theranostic)
Fig. 3. (a) TEM micrographs of protocatechuic acid loaded on GO/Gd nanocarrier (GAGPA) at high and low magnifications; (b) TEM micrographs of protocatechuic acid loaded on GO/Gd nanocarrier after surface coating with gold nanoparticles (GAGPAu). ([A, B & D] Reprinted from ref. [64] under Creative Commons Attribution (CC BY) license MDPI 2018 and [C] Reprinted from ref. [65] under Creative Commons Attribution (CC BY) license MDPI 2021)
reference to the regulated and activated physical structure with diversified biomedical applications.

4. CONCLUSION AND FUTURE OUTLOOK

GBNs finds targeting several biological effects, high drug loading rate and increased sensitivity of chemotherapy as compare to other drug delivery systems. New proposals of mixing of techniques are given for cancer treatment like PTTPDT technique, chemo-PTT technique and chemo-PDT technique. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) considered graphene as a toxic product and risky for health. Toxic aspect of graphene and GBNs has great challenges and therefore it found high interest among researchers and more works are recommended. Graphene quantum dots are accepted for the use of multimodal therapies, bio-imaging regarding cytotoxic effects and little doses of special featured materials. The experimental data on the toxicity are limited more in vitro rather than to in vivo. Studies conducted are not enough for the confirmation of in vitro and in vivo toxicity but in-depth investigations is required for GO-based nanomaterials and their derivatives. In conclusion, GBNs are more crucial having many surprising advantages and also challenges such as toxicities. Researches in multiple fields in GBNs will leads to ultimate benefits for human community to cure diseases as well as disorders to a significant extent. GBNs have created interest among researchers by their unique physicochemical properties and multiple uses in different fields. 3D porous graphene materials have attracted great attention for environmental applications in the removal of pollutants of organic, inorganic and radio-pollutants. Different properties of GBNs like high surface area to volume ratio, polyatomic aromatic structure and easy multi-functional ability offers capability and flexibility for cargo-loading, transporting and tissues targeting. Furthermore, additional research is required to optimize and to evaluate their considerations on the safer synthetic strategies, toxicity and environmental appropriateness.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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