Nanotechnology-Based Graphene Oxide for Innovative Bioengineering Application

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ABSTRACT

This review paper unravels a number of novel applications of nanotechnology-based graphene oxide in bioengineering. Graphene oxide is the chemically modified version of graphene. It possesses many properties which include having a large surface area and high mechanical strength with excellent potential for functionalization. Hence, GO has become an attractive material in numerous applications in bioengineering. This paper discusses in-depth its applications in five of the most important areas of bioengineering, which include tissue engineering, drug delivery systems, biosensors, orthopedic implants, and bioimaging based on GO's structure and properties. GO promotes an improvement of scaffold properties and differentiation of stem cells in tissue engineering. GO shows high loading capacity as well as controlled mechanisms of release in drug delivery. Due to its properties, GO can enable highly sensitive and specific biosensing. Another such area wherein GO also shows promise in improving orthopedic implant integration and performance. Finally, in bioimaging, GO's optical properties make it suitable for various imaging techniques. The application of GO in all these areas holds much promise but carries challenges and considerations such as toxicity and long-term biocompatibility. The paper concludes by discussing how GO is transformative in bioengineering and the importance of ongoing research, hence unlocking the full healthcare benefits of its applications in medical technology.

Keywords: Graphene oxide, Bioengineering application, Nanotechnology.

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INTRODUCTION

Nanotechnology is a field of science and engineering and the development of objects, devices, and systems from an atomic and molecular base at the nanoscale level. Nonmaterial is defined as a particle with at least one dimension between 1 to 100 nanometres and is popularly known as Nanomaterial.^{1,2} Nanomaterials can be classified into carbon, metallic and metal oxide nanoparticles and polymer-based nanoparticles.^{3,4} One type of carbon nanomaterial is Graphene oxide (GO) which is a chemically Optimised form of graphene, which is a single layer of carbon atoms arranged in a two-dimensional honeycomb lattice.⁵ Unlike pristine graphene, GO contains various oxygen-containing functional groups, such as hydroxyl, epoxy, and carboxyl groups, which significantly alter its properties. These functional groups make GO highly dispersible in water and other solvents, enhancing its processability and

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functionalization potential. The presence of these groups also enables GO to interact with a wide range of molecules, making it a versatile material for various applications.^{5,6} GO retains exceptional mechanical strength, high surface area, and excellent thermal and electrical conductivity of graphene while also exhibiting unique optical properties and pH sensitivity.7-9 Graphene oxide (GO) has gained significant attention in bioengineering and biomedical fields due to its unique properties. The large surface area and functional groups of GO enable it to support cell adhesion and proliferation, making it ideal for tissue engineering applications where scaffolds are used to promote tissue regeneration.⁵ Its ability to mimic extracellular matrix components further enhances its potential in this area. In drug delivery, GO can be functionalized to carry therapeutic agents, allowing for targeted and controlled release, which enhances efficacy

Fig. 1: Graphene oxide (GO) in bioengineering

and reduces side effects of treatments. Its high drug-loading capacity and stimuli-responsive behavior make it particularly attractive for cancer therapeutics. GO-based biosensors are another important application, offering highly sensitive and selective detection platforms for a wide range of biomolecules, pathogens, and environmental pollutants. The exceptional electron transfer properties of GO contribute to improved sensor performance. Additionally, the incorporation of GO into biomedical devices, such as implants and prosthetics, improves their biocompatibility and mechanical performance, leading to better patient outcomes.¹⁰ GO's antimicrobial properties also make it valuable for wound healing applications and infection prevention in medical devices.⁵ Overall, the versatility and functional capabilities of GO make it a promising material for advancing bioengineering applications is explained in Fig 1.

Table 1: GO application in tissue engineering

Application	Description
Stemcell	GO enhances stem cell growth and
differentiation	differentiation ¹⁹
Scaffold material	GO incorporated into scaffolds for tissue growth ¹³
Neural tissue	GO promotes neural cell adhesion and
engineering	growth ⁸
Cardiac tissue	GO improves electrical conductivity in
engineering	cardiac patches ¹³
Bone tissue	GO enhances osteogenic differentiation and
engineering	bone formation ¹⁹
Cartilage	GO-based scaffolds support chondrocyte
regeneration	growth ¹³
Wound healing	GO accelerates wound closure and healing ³
Vascularization	GO promotes formation of blood vessels in engineered tissues ¹³
3D bioprinting	GO used as a bioink component for 3D tissue printing ⁴

Fig. 2: Structure of graphene oxide

Structure and Properties of GO

Graphene oxide (GO) is a single layer of graphite that is chemically derived and contains different oxygen functional groups, including hydroxyl (-OH), epoxy (-O-) and carboxyl (-COOH) groups, attached to the basal plane as well as to the edges of the graphene sheet.^{4,20}

The molecular formula for GO is often given as CxOyHz, where x, y and z represent different amounts of carbon, oxygen and hydrogen that may be present depending on the synthesis process and oxidation state.⁶ The history of GO dates back to 1859, when Brodie first prepared graphite oxide. However, it was not until 1957 that Hummers and Offeman developed a new method for producing graphite oxide which was safer and more efficient than previous methods used. This work paved the way for many subsequent studies on GO as a precursor for graphene or as an independent material with interesting properties. The isolation of graphene in 2004 by Geim and Novoselov sparked renewed interest in graphite oxide.¹¹ The diagrammatic representation of GO structure is shown in Fig 2. Physically, GO has a very high surface area to volume ratio ranging from approximately 300 m²/g to 2630 m²/g; therefore, GO films are stiff with reported Young's modulus around 32 GPa. It is hydrophilic, so it can be easily dispersed in water. Unlike conductive graphene, GO is insulating because its sp² hybridized carbon atoms bound with oxygen atoms cannot π -stack with each other via out-of-plane p-orbitals like pristine graphene can. GO is optically transparent in thin films while its surface is highly reactive, allowing for versatile functionalization.¹² Toxicity of GO within the body depends on particle size and shape, surface chemistry, concentration, route of exposure (inhalation/ingestion/dermal contact) and duration of exposure. In general, smaller particles or certain surface functional groups increase toxicity within the human body as do increasing concentrations and durations of exposure.^{5,9}.

Graphene Oxide in Bioengineering

Graphene oxide for tissue engineering

Graphene oxide (GO) is becoming popular for tissue engineering applications due to its uniqueness and versatility. Its large surface area, high mechanical strength, and ease of use do make it a great candidate for biocompatible scaffolds that promote cell growth and differentiation. In fact, Lee et

Fig. 3: GO application in tissue engineering

al. (2011) proved the fact that GO substrates enhance the osteogenic differentiation of human mesenchymal stem cells in an osteogenic induction media-free environment.¹⁹ The underlying mechanism could be that GO could absorb the osteogenic inducers and create an environment fit for osteogenesis. GO has been incorporated into most biomaterials to improve their properties. Shin et al. developed hydrogels with GO compounds, which increased the adhesion and proliferation of human adipose-derived stem cells.¹³ GO also improved mechanical properties of the hydrogels while at the same time providing a substrate for cell growth. Enhancing the mechanical properties in tissue engineering is further important so that scaffolds can be designed to imitate the mechanical properties of tissues in the native state. In neural tissue engineering, GO has been found to promote neurite outgrowth and neural differentiation. For instance, it was reported that GO-coated substrates promoted the differentiation of neural stem cells to neurons, which is a new strategy for treating neurodegenerative diseases and spinal cord injuries.⁴ Neuronal growth and differentiation are supported by GO either through its surface chemistry or topography, both of which may be made to replicate the properties of natural extracellular matrix. The antibacterial property is also sought for its applications in wound care. Tissue-engineered scaffolds with GO have shown an improvement in wound healing and a reduction in bacterial infections.⁵ GO's role in tissue engineering is depicted in Figure 3 with corresponding application details in Table 1. For instance, researchers have developed chitosan hydrogels containing GO for use as woundhealing agents with antibacterial effects.⁹ The antibacterial activity of GO is likely attributed to its sharp edges, which can disrupt the physical integrity of bacterial cell membranes, and its ability to generate reactive oxygen species. Due to this, GO has become an appealing material for a plethora of tissue engineering applications such as in bone, cartilage, nerve, and skin tissue regeneration, among others. $8,10,14$

Additionally, the surface of GO can be Optimised to present various biomolecules, including growth factors and peptides, enhancing its bioactivity and tissue-specific properties. Such property allows the creation of more functional scaffolds, which tend to guide tissue regeneration more effectively and induce expected cellular responses. In addition, GO has found use in tissue engineering for electrically active tissues, such as heart and nerve tissues, since it has the capability of conducting electrical signals. This allows the possibility of fabricating scaffolds that are supportive of cell growth and, at the same time, permissive for electrical signalling between them, thereby mimicking the natural behaviour of the tissues. $8,10$

GO for drug delivery systems

Graphene oxide (GO) provides an excellent opportunity to design drug delivery systems due to its unique properties and versatility. This holds good for most therapeutic applications, with high surface area, great loading capacity, and excellent functionalization capability.^{5,7,11} In 2008, Liu et al. demonstrated the potential of GO to be used as a drug delivery system by developing PEGylated nanographene oxide for water-insoluble anticancer drugs.¹⁵ The PEG-ylation of GO would better stabilize it in the physiological conditions and increase its circulation time. The developed system could effectively load and deliver the anticancer drug doxorubicin in to tumor cells more effectively compared to the free drug. Very importantly, being pH sensitive, GO realizes controlled release in selective physiological environments, a key characteristic for delivering drugs. For example, GO-loaded cancer drugs selectively release in the acidic environment of tumor cells, thus increasing therapeutic efficacy and causing minimal side effects. $8,15,\overline{17}$ Furthermore, GO is versatile in developing multifunctional drug delivery systems. For example, Zhang et al. developed a system based on GO that could be used to deliver an anticancer drug at the same time as a gene-silencing agent to bring about a synergic effect for suppressing tumors.¹⁸ This suggests a wide range of applications for GO, including combination therapy in cancer treatment. In addition, the GO surface can be easily functionalized to be very compatible with the biological system it is intended for and to further increase its targeting ability. Sun et al. wrote that PEGylated nano-GO could be utilized in cell imaging and drug delivery with an emphasis on the therapeutic application. Fig. 4 demonstrates

Graphene Oxide Applications in Drug Delivery

Fig. 4: GO application in Drug Delivery Systems

the use of GO in drug delivery systems and application description is mentioned in Table 2. The fluorophoric property of GO allows one to track it in real-time during drug delivery and provides useful information on the pharmacokinetics and biodistribution of delivered drugs.¹⁹ More importantly, a lot of studies have shown the promise in breaking biological barriers using GO-based drug delivery systems since thin and flexible GO sheets have high cellular uptake and intracellular drug transport.6,7 Furthermore, GO can be functionalized by the conjugation of targeting ligands that improve specificity with respective cells or tissues, thus improving their therapeutic activity while minimizing off-target effects.^{4,7} With regard to these properties of GO, most applications are focused on high drug loading capacity and the possibility of controlled release followed by an actuation mechanism in which it seems to be the material of choice in most drug-delivery applications, cancer therapy, gene delivery, and targeted drug delivery to some particular tissues or organs.

GO for Biosensor

Graphene oxide was a successful material for biosensors, acting on account of its amazing electronic properties, large

Graphene oxide in Biosensor application

Fig. 5: GO application in Biosensor

surface area, and easy functionality. This allows the design of highly sensitive and specific biosensors for the detection of various biomolecules like glucose, DNA, proteins, and small molecules. $8,10,21$ The most important advantage of GO with regard to biosensing is its capacity for electron transfer mediation. Liu et al. (2010) reported that the immobilization of glucose oxidase on GO-Optimised electrodes enabled the preparation of an ultra-sensitive glucose biosensor.¹⁵ The use of GO as material to facilitate electron transfer from the enzyme to the electrode surface permitted much higher sensitivity compared to traditional glucose sensors. Because of its nature as quenching, GO has been used to develop fluorescence-based biosensors. In 2010, Jung et al developed a GO-based aptasensor for thrombin detection, where thrombin binding with its aptamer made the change in fluorescence intensity.20 Sensing was given sensitive and selective protein detection. For the detection of DNA, GO showed great ability to distinguish Single-stranded and double-stranded DNA. Lu et al. developed a GO-based platform for DNA detection, differentiating even single base mismatches with high sensitivity; 22 this is very promising for genetic tests and disease diagnosis. In addition to the biological applications, GO-based biosensors were also found to be viable in the monitoring of environmental contaminants. For example, scientists developed electrochemical sensors based on GO for detecting heavy metals from water sources.⁵ The very high sensitivity and selectivity of sensors have clearly underlined the potential of GO for the design of portable devices for off-site tests. The high surface area of GO allows loading with a high quantity of biomolecules, which further leads to improved sensitivity of biosensors. Moreover, the conductance of GO can be easily Optimised by simple reduction or functionalization, paving the way to the design of high-performance electrochemical biosensors. GO is an important biosensing material as a result of its hybrid nanomaterial utilization in advanced properties. For instance, GO-particle composites have been utilized to develop biosensors with enhanced sensitivity and stability.^{8,10} Its flexibility affords it the capability to be designed into multimodal biosensors for the detection of multiple analytes. This is specifically useful in diagnostic applications where the detection of multiple biomarkers is common.^{7,8} Additionally, GO-based biosensors find extraordinary potential in pointof-care diagnostics because of the inherent property of miniaturization and thus can be easily integrated into wearable devices. Moreover, it will help GO-based systems with different recognition elements, such as antibodies, aptamers, or enzymes, to further convert into highly specific biosensors for a wide range of applications. Fig. 5 highlights the application of the GO in biosensors and the application description explained in Table 3.

GO for orthopaedic implants

Graphene oxide is considered a very promising material for orthopedic implant use due to high mechanical strength, large surface area, and biocompatibility. These properties highly attribute to GO as an attractive candidate in improving

Graphene Oxide in Cutting-Edge Bioengineering

performance and biointegration in orthopedic implants. The main use of GO in orthopedic implants is the coating for better bone integration. Lee et al. showed that the adhesion, proliferation and osteogenic differentiation of hMSCs were improved in the case of GO-coated titanium substrates. 24 GO coatings supported the formation of focal adhesions and increased osteogenic markers ex bone-implant integration. Another biocompatible polymers and prepare orthopedic applications. Shin et al. hydrogels engineered for impr properties and cell adhesion.²¹ used in cartilage tissue engineering regeneration. GO has antibacterial highly useful in preventing implant challenge in orthopedic surgery. Ak the bactericidal activity of GO against both Brand-Gram-negative bacteria.²⁴ This w implant coatings resistant to infe

Table 3: GO application in Biosensor

high sensitivity.¹⁵

hybridization.²²

specific proteins.⁸

electrochemical detection.⁶

properties used for biomolecule

detecting bacteria and viruses.²⁰

Glucose sensing GO-based glucose biosensors with

Pathogen detection GO-based immunosensors for

DNA detection GO platform for sensing DNA

Protein detection GO-based sensors for detecting

Fluorescence-based sensing GO's fluorescence quenching

Electrochemical sensing GO used in electrodes for enhanced

Application Description

Graphene Oxide in Mo

Corrosion Protection

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Drug-eluting

Implants

Wear Resistance

Osseointegration

Promotion

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to implant surfaces. For instance, GO may be functionalized with growth factors or peptides that promote osteoblast adhesion and differentiation.8,10,23 This makes it possible to develop "smart" implant surfaces that are actively stimulate bone formation and implant integration. GO-reinforced materials exhibit mechanical properties suitable for loadbearing orthopedic applications, and previous work reported mechanical reinforcement upon adding GO to polymer or ceramic matrices. This is important in the fabrication of implants that have to withstand the physiologic loading that is evident in orthopedic applications. On the other hand, for orthopedic implants, the electrical conductivity of GO can operate by enhancing bone growth stimulated with electricity. Fig. 6 illustrates the applications of Graphene Oxide (GO) in orthopaedic implants and application description is given in Table 4. Researchers have studied the GO based conductive scaffolds where the electric stimulation elicits enhanced osteogenic differentiation.7,14 GO's large surface area enables the binding of many therapeutic agents like antibiotics or growth factors with which drug-releasing implants could be developed. So, a local drug can be provided to prevent the infections or increase the growth of bone at that implantation site. $8,9$

together with a variety of biomolecules to provide bioactivity

GO for Bioimaging

Graphene oxide (GO) is a very promising material for bioimaging roperties and biocompatibility maging techniques. This was Il imaging with drugs, therefore f the material as a platform for use in biomedicine had been shown.4 Graphene oxide has a 2D for cellular uptake, and yields a alization with different imaging of these properties render GO uirements of a certain imaging ies. An example is the work of functionalized graphene oxide anhydride had been developed perties, including fluorescence red by GO, some of those used ors and imaging probes. In this he development of graphene as cognition, which could further be expanded to bioimaging applications.⁴ In addition, its sible and near-infrared regions aging since this penetration of light can go to a great depth compared with conventional fluorophores. In addition, its capability for functioning and hybridization with other nanomaterials extends further the potential for bioimaging of GO. Chung et al. (2013) gave a review of various biomedical applications of graphene and graphene oxide, one of which was the noted potential for bioimaging and drug delivery.⁸ The capability of combining the imaging functions together with the therapeutic ones is outlined in the work of Liu et al. (2008) on PEGylated nanographene

Mechanical

Biocompatibility

Enhancement

Reinforcement

Graphene Oxide in Cutting-Edge Bioengineering

Table 4: GO application in Orthopedic Implants		Table 5: GO application in <i>bioimaging</i>			
Application	Description	Application	Description		
Bone regeneration	GO enhances osteogenic differentiation of stem cells. 10	Cellular imaging	GO is used for high-contrast cellular imaging due to its fluorescence properties. [17]		
Antibacterial coatings	GO-based coatings reduce bacterial adhesion on implants. 3	Tumor imaging	PEGylated nano-GO is used for in vivo tumor imaging. $[16]$ GO functionalized with fluorescent and magnetic materials for dual-mode imaging. [8]		
Mechanical reinforcement	GO improves mechanical properties of implant materials. ¹³	Multimodal imaging			
Biocompatibility	GO surface modifications improve				
enhancement	implant integration. ⁸	Near-infrared (NIR)	GO's NIR absorbance is used for deep tissue imaging. $[6]$		
Drug-eluting implants	GO is used as a carrier for controlled	imaging			
	drug release from implants. 12	Raman imaging	GO's unique Raman signature is used for cellular tracking. [8]		
Osseointegration	GO stimulates bone cell growth and				
promotion Wear resistance	adhesion. 19	Photoacoustic imaging	GO's strong optical absorption is used for photoacoustic imaging. [6]		
	GO coatings improve wear resistance of implant surfaces. ²¹	X-ray computed tomography (CT)	GO is functionalized with iodine for enhanced CT contrast. [8]		
Corrosion protection	GO-based coatings enhance corrosion	Magnetic resonance	GO complexed with gadolinium for		
	resistance of implants. ²³	imaging (MRI)	improved MRI contrast. [17]		

oxide for drug delivery, 17 but using GO in bio-imaging is not complex. However, the toxicity of GO-based materials must be taken seriously in both in vitro and in vivo studies. On the other hand, continued research improves GO-based materials for much safer and more efficient bioimaging applications that promise to be a breakthrough in medical diagnosis and the monitoring of therapies. Fig. 7 and Table 5 showcase the applications of Graphene Oxide in orthopaedic implants.

Challenges and Limitations

Although GO can have many promising applications, there are a number of key challenges that need to be met. Yet, its alleged long-term toxicity and bioaccumulation in organisms are outlets of far more profound concern. Previous studies revealed size, surface chemistry and concentration dependence of toxicity generated by GO, which results in the difficulty for standardizing safety protocols. Toxicological studies of GO still do not fully understand the way in which it interacts with cellular components and affects gene expression. Batch-tobatch variability in the production of GO is also a significant

Graphene Oxide Imaging Applications

Fig. 7: GO application in *bioimaging*

challenge; this affects the properties and performance of the materials for applications. One important challenge in scaling up GO-based technologies is achieving a homogenous quality and properties during the mass production of these devices. Moreover, a detailed study will be followed for the *in-vivo* biodegradation pathways of GO to clarify its clearance after therapeutic use. In addition, the cost-effectiveness of GO-based products is a daunting challenge for commercial translation relying on forms of regulatory approval. Furthermore, the selectivity and stability in highly complex biological environments should be improved to enable biosensor applications; meanwhile, different tissue types may require various concentrations of GO for optimal performance in tissue engineering. Challenges and mitigation strategies are summarized in schematic representation 1 for the better understanding.

Future Perspectives

The potential future for GO in bioengineering is that it can usher vibrant advances at the level of technology and some nodal interdisciplinary areas. Recent tendencies imply the design of clever, multifunctional graphene-based total solutions integrating therapy and also refer to the simultaneous diagnostic as well as monitoring abilities in one system. This union of GO-based biosensors and artificial intelligence/ machine learning would allow a continuum from real-time health monitoring to the promise of personalized medicine. This may, in turn, contribute to the development of more complex drug delivery systems with highly efficient targeting mechanisms and controlled release behaviours by means of possessing advanced surface modification technologies. Tissue engineering: 3D bioprinting seeding of cells; the potential use for the production of highly complex and functional tissue constructs with improved mechanical as well as biological properties in combination with GO has been demonstrated.

Schematic Representation 1: Challenges and Mitigation Strategies

Material		Sensitivity Specificity	Detection Limit	Cytotoxicity (IC50)	Genotoxicity	In Vivo Toxicity	Cell Uptake	Protein Binding	Blood Circulation
Graphene Oxide (GO) 90-95%		85-90%	$10 - 100$ ng/mL	$20-50 \mu g/mL$	Low	Moderate	High	Moderate	Short-term
Carbon Nanotubes (CNT _s)	80-90%	75-85%	$1-10$ ng/mL	$10-30 \text{ ug/mL}$	Moderate	High	High	High	Short-term
Quantum Dots (QDs)	95-99%	90-95%	$0.1 - 1$ ng/mL 5-20 μ g/mL		High	High	Low	Low	Long-term
Gold Nanoparticles (AuNPs)	85-95%	80-90%	$0.1 - 10$ ng/mL	$50-100 \mu g/mL$	Low	Low	Low	Moderate	Long-term
Magnetic Nanoparticles (MNPs)	80-90%	75-85%	$1-10$ ng/mL	$30-50 \mu g/mL$	Moderate	Moderate	Moderate	Moderate	Short-term

Scheme 2: Comparative analysis with other materials

GO-based theranostic platforms can be a breakthrough in cancer treatment where imaging and therapy combine into one system. Future studies should concentrate on the standardization of methods for GO characterization, safety considerations and simplified production strategies. The incorporation of GO into other nanomaterials to form hybrid materials will likely lead to better properties and novel applications. In addition, the antimicrobial applications and wound-healing potential of GO may help address increasing healthcare problems in terms of fighting antibiotic-resistant bacteria. New potential in regenerative medicine, tissue suppleness and formation of interfaces with the nervous system may form as we dig further into how GO interacts within biological systems… many times even changing the way neurological pathology can be targeted for treatment or tissues regenerated. The comparative chart of GO for diagnostic performance, toxicity and interactions with biological systems with other materials has been illustrated in Scheme 2

CONCLUSION

Graphene oxide has emerged as one of the most versatile and promising nanomaterials in bioengineering. With its particular structure and chemical and physical properties, it can be considered an excellent candidate in a wide range of applications, starting with tissue engineering up to drug delivery systems. GO improves the differentiation of stem cells cell adhesion properties, and enhances scaffold properties. GO in drug delivery systems presents high drug load, controlled release manners, and the capability of bio-barrier overcoming. Its electronic properties and high surface-to-mass ratio enable the development of highly sensitive and specific sensors for biosensing in a wide range of biomolecules and environmental contaminants. GO has presented excellent performance in orthopedic implants, enhancing bone integration and mechanical properties and providing antibacterial coatings. Bioimaging preparation,

GO optical properties, and functionalization capability make GO suitable for cellular imaging, tumor detection, and also for different modes of imaging techniques. However, despite these promising applications, there are yet a good number of challenges to be resolved in terms of long-term toxicity and biocompatibility issues of GO-based materials. Further research is required to only achieve complete understanding but also mitigate the possible risks. As this field continues to grow, the expectation will be that GO will go a long way in embedding new bioengineering technologies and making major improvements in healthcare, diagnostics, and therapeutic intervention.

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