Health and environment

Environmental and health effects of engineered materials are of concern to the general public, regulatory authorities, industry, and the materials science community responsible for their creation. Health and safety can never be separated from technology development, and so Graphene Flagship researchers are assessing the safety of graphene and related materials. The small size and unique properties of 2D materials pose potential risks to human health and the environment.

Resolving safety and toxicity issues associated with graphene and related materials will not only be beneficial when it comes to their integration into structural composites and electronics – they will also be critical in biomedical applications, and especially devices which interface directly with biological cells and tissues.

As a first step, flagship researchers have created a classification system for 2D materials. Classification is based on the number of layers and lateral length of 2D material sheets, and the oxygen content of the materials relative to that of carbon.

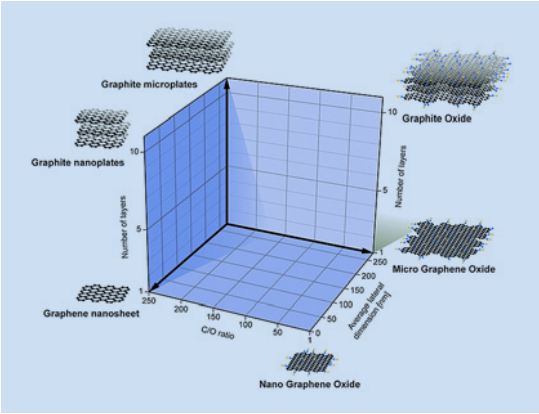
During the first year of the flagship, researchers carried out experimental studies of graphene dispersion in cell cultures. This is not an easy task, as graphene is hydrophobic, and cannot be dispersed in water without the help of additives that reduce the surface tension of the liquid. Maurizio Prato, an organic chemist at the University of Trieste, comments on the results…

> Initial studies of cellular uptake and the toxicity of graphene and graphene oxide using primary human macrophages and rodent neuronal cells have shown low impact on cell viability and dose-dependent cellular activation. With aquatic microorganisms and terrestrial algae, the observed effects are rather limited.

As the development of graphene and related 2D materials progresses, health and environmental research will increase in line with it. Risk assessments of 2D materials will be disseminated widely, so that we can all be confident as to their safety.

> Risk assessments of 2D materials will be disseminated widely, so that we can all be confident as to their safety.

Work package leader
Prof. Maurizio Prato.



Classification of graphene according to the number of layers, oxygen content and dimensions. © 2014 Wiley-VCH Verlag.

Work Package 2: Health and Environment
Work package leader: Prof. Maurizio Prato, University of Trieste, Italy
Deputy leader: Prof. Alberto Bianco, CNRS National Centre for Scientific Research, France

Article: Wick et al., Classification framework for graphene-based materials, Angew. Chem. Int. Ed. 30, 7714 (2014); doi: 10.1002/anie.201403335


Health and
Environment

As all groups within the Flagship move towards the goal of transitioning graphene from the laboratory and into commercialised products, nanosafety has never been more important. The mission of the Work Package Health and Environment is to assess and resolve health and toxicity issues associated with graphene and other 2D materials and to enable integration of graphene with biomedical applications.

Prof. Maurizio Prato

WORK PACKAGE LEADER
Prof. Maurizio Prato,
University of Trieste, Italy

WORK PACKAGE DEPUTY
Prof. Alberto Bianco, CNRS National Centre for Scientific Research, France

Over the past year researchers from within the group have made big steps forward on both of these goals. However, before the biological interactions can be studied, an important hurdle needs to be overcome as the Work Package Leader, Prof. Prato from the University of Trieste in Italy, who is also a member of the Graphene Flagship Executive Board, explains:

"As a first achievement of this last year of intense research, we have established an original protocol for the production of graphene that can be easily dispersed in water and allows an easier development of the biological studies."

This relatively simple sounding improvement has paved the way for many different biological studies into the health and toxicity issues surrounding graphene. For example, using these water dispersions, researchers from within the Flagship have published work[(1)] which provides a previously unavailable pharmacological understanding of how graphene oxide (GO) sheets are transported within the body of a mouse; primarily accumulating in the spleen and excreted in the urine.

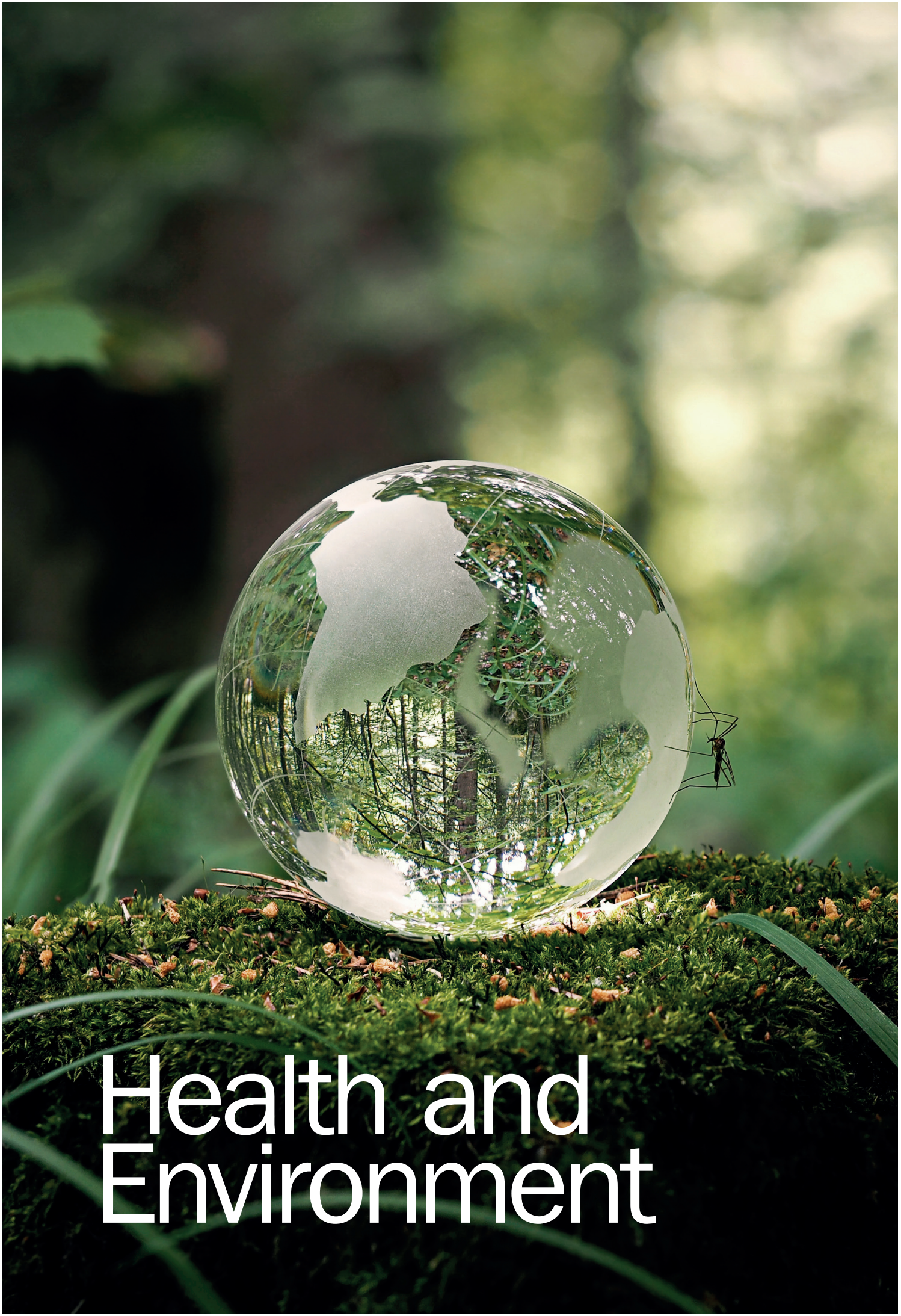
Also, work package researchers have shown for the first time that is is possible to interface untreated graphene with neuron cells whilst maintaining the integrity of these vital cells[(2)]. This result is a significant first step towards using graphene to produce better deep brain implants which can both harness and control the brain. The work, published in the journal ACS Nano, was a truly interdisciplinary collaboration between the University of Trieste in Italy, the University Castilla-La Mancha in Spain and the Cambridge Graphene Centre, with nanotechnologists, chemists, biophysicists and neurobiologists all playing an important role.

Prof. Prato, commented that *"We are currently involved in frontline research in graphene technology towards biomedical applications, exploring the interactions between graphene nano- and micro-sheets with the sophisticated signalling machinery of nerve cells. Our work is only a first step in that direction."*

Prof. Ferrari, Director of the Cambridge Graphene Centre, and Chair of the Graphene Flagship Executive Board, stated that *"The Flagship will support biomedical research and development based on graphene technology with a new work package and a significant cash investment from 2016. These initial results show how we are just revealing the tip of an iceberg when it comes to the potential of graphene and related materials in bio-applications."*

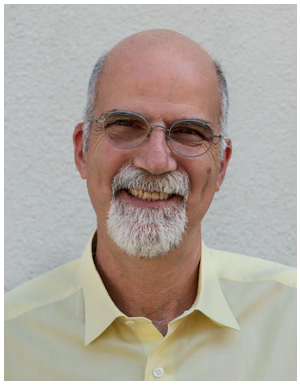
[(1)] Jasim D. A., et al., Chem. Sci., 6, 3952 (2015)

[(2)] Fabbro A., et al., ACS Nano, 10 (1), 615 (2016)



Health and Environment

Identifying and controlling any possible safety and toxicity issues regarding the use of GRMs in humans, animals and the environment is of paramount importance, and cannot be separated from the development of new, GRM-based technologies. GRMs must be well characterised for the different applications, as the physical and chemical properties of the materials strongly affect their interactions with biological materials.



Maurizio Prato

WORK PACKAGE LEADER
Maurizio Prato, University of Trieste, Italy

WORK PACKAGE DEPUTY
Alberto Bianco, National Centre for Scientific Research (CNRS), France

One important potential application of GRMs in nanomedicine is drug delivery, with GRMs acting as vehicles to carry and deliver therapeutic molecules to specific targets within the body. For this, it is very important that GRMs do not induce unwanted effects within the body, and that they can be safely excreted through the body's normal functions. The University of Manchester and the National Centre for Scientific Research (CNRS) investigated the effect of graphene oxide (GO) sheets on the function of mouse kidneys when injected intravenously [1]. Importantly, they found that not only are the GO sheets readily excreted in urine, the excreted sheets are intact, confirming stability within the body.

Hexagonal boron nitride (hBN) is another layered material is promising for use alongside graphene in a wide range of areas. It is chemically inert and strongly resistant to oxidation, meaning that biopersistance could be a potential problem with its use. CNRS and Trinity College Dublin explored the degradation of hBN when exposed to peroxidases – enzymes produced by microorganisms and in the human immune system – and under a UV-assisted Fenton reaction [2]. Biodegradation of hBN is quite different to that of graphene and GO. It was found that hBN can be degraded by myeloperoxidase, an enzyme expressed in activated neutrophils, white blood cells present in the lungs. Significantly, the UV-assisted Fenton reaction is highly effective, suggesting a route to treating waste hBN on an industrial scale. Maurizio Prato, Leader of the Health and Environment Work Package, said *"The Health and Environment Work Package is widening its research horizons – extending the studies to other GRMs that may be interesting for their chemical and physical properties. It is an important result that hBN, a very robust material, with increasing appeal for applications, is relatively easily degraded by specific enzymes. This may avoid the accumulation and persistence of hBN in the environment in the perspective use of this fascinating material."*

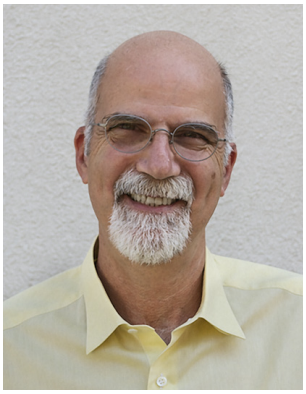
CNRS, the French Alternative Energies and Atomic Energy Commission (CEA) and the University of Castilla-LaMancha performed an investigation into the effect of various different types of carbon nanoparticles, including graphene, on the growth of larval *Xenopus laevis*, an aquatic organism. Their results show that it is the size of the carbon nanoparticle that affects the larval growth, rather than the morphology. This study is a step towards a realistic metric of assessing the dose of nanoparticles in the environment.

[1] D. A. Jasim et al. ACS Nano 10, 10753 (2016)

[2]] R. Kurapati et al. Angewandte Chemie 128, 5596 (2016)

[3] A. Mottier et al. Nano Letters 16, 3514 (2016)

The Graphene Flagship is committed to responsible development of technologies based on graphene and related materials (GRMs). Within the Health and Environment Work Package, potential risks to humans, animals and the environment are assessed to guide the development of safe materials and technologies.


Maurizio Prato

WORK PACKAGE LEADER
Maurizio Prato, University of Trieste, Italy

WORK PACKAGE DEPUTY
Alberto Bianco, National Centre for Scientific Research (CNRS), France

For human health, there are several possible routes of exposure to through use of GRM-based technologies or in their manufacturing processes: skin contact, inhalation and ingestion. Flagship researchers have conducted studies into the effects of various GRMs on different skin cells to understand potential health risks and inform safety-by-design approaches.

Researchers from the University of Trieste, the University Castilla-La Mancha and CIC BiomaGUNE have studied the effects of different types of graphene or graphene oxide (GO) flakes on skin cells [1]. The researchers found that skin cells were damaged only after contact with very high concentrations and extended exposure times, indicating an acceptable biocompatibility after both short and long-term exposure. These results indicate that the cytotoxicity of these graphene-based materials is lower than other carbon-based nanomaterials such as nanotubes.

"We have found that graphene is not devoid of safety concerns. However, the amounts that are necessary to stimulate a reaction, at the skin level, are so high and the exposure so persistent, that these conditions are very unlikely to occur," said Maurizio Prato, leader of the Health and Environment Work Package.

One important aspect of understanding the effect of GRMs in the human body is the immune response, the body's first line of defence against foreign materials. Research from Flagship scientists working at Karolinska Institute and the University of Manchester has shown that graphene oxide (GO) can be rapidly degraded by human neutrophil cells, part of the body's immune response [2]. Further, the researchers also investigated the effect of the by-products of this degradation on lung cells, finding that the degraded GO is non-toxic for human lung cells and does not cause damage to DNA.

GO is more reactive than pristine graphene and can be more easily functionalised, making it highly attractive for a range of applications including biomedicine and drug delivery, antimicrobial coatings and water purification. Flagship researchers working at University of Strasbourg, CNRS and the University of Manchester have demonstrated that biodegradation of GO by enzymes is strongly dependent on the type of functionalisation and can be enhanced using specific functional groups [3]. This opens the path to designing safer GRMs that break down more readily in the environment.

[1] M. Pelin *et al., Sci. Rep.* **7**, 40572 (2017)

[2] S. P. Mukherjee *et al., Nanoscale* **10**, 1180 (2018)

[3] R. Kurapati *et al., 2D Mater.* **5** 015020 (2018)

ROADMAP

Over the past year, Work Package Industrialisation has implemented a new method for conducting focused value chain analyses of promising GRMs applications. The approach consists of three main steps: the first step comprises the elaboration of a GRMs-based value chain starting from the assessment of promising application areas within the overview roadmap. The second step focuses on identifying the key stakeholders along the value chain. The third step is the joined elaboration of the different stages of the value chain including specific KPIs and milestones.

In 2018 three topics have been elaborated upon following this scheme:

- Graphene-based materials for next generation neural interfaces
- Perovskite solar cells using GRMs materials
- Integrated graphene-based photonics for data communication

In some cases, the outcomes of these analyses have already been taken up directly by industry-led consortia in order to implement this innovation approach. An example is the perovskite solar cell topic where a team was set up including researchers and industries, covering the whole value chain starting from material preparation until system integration.

Work Package Leader:
Kostas Kostarelos

Work Package Deputy:
Jose A. Garrido

Biomedical Technologies

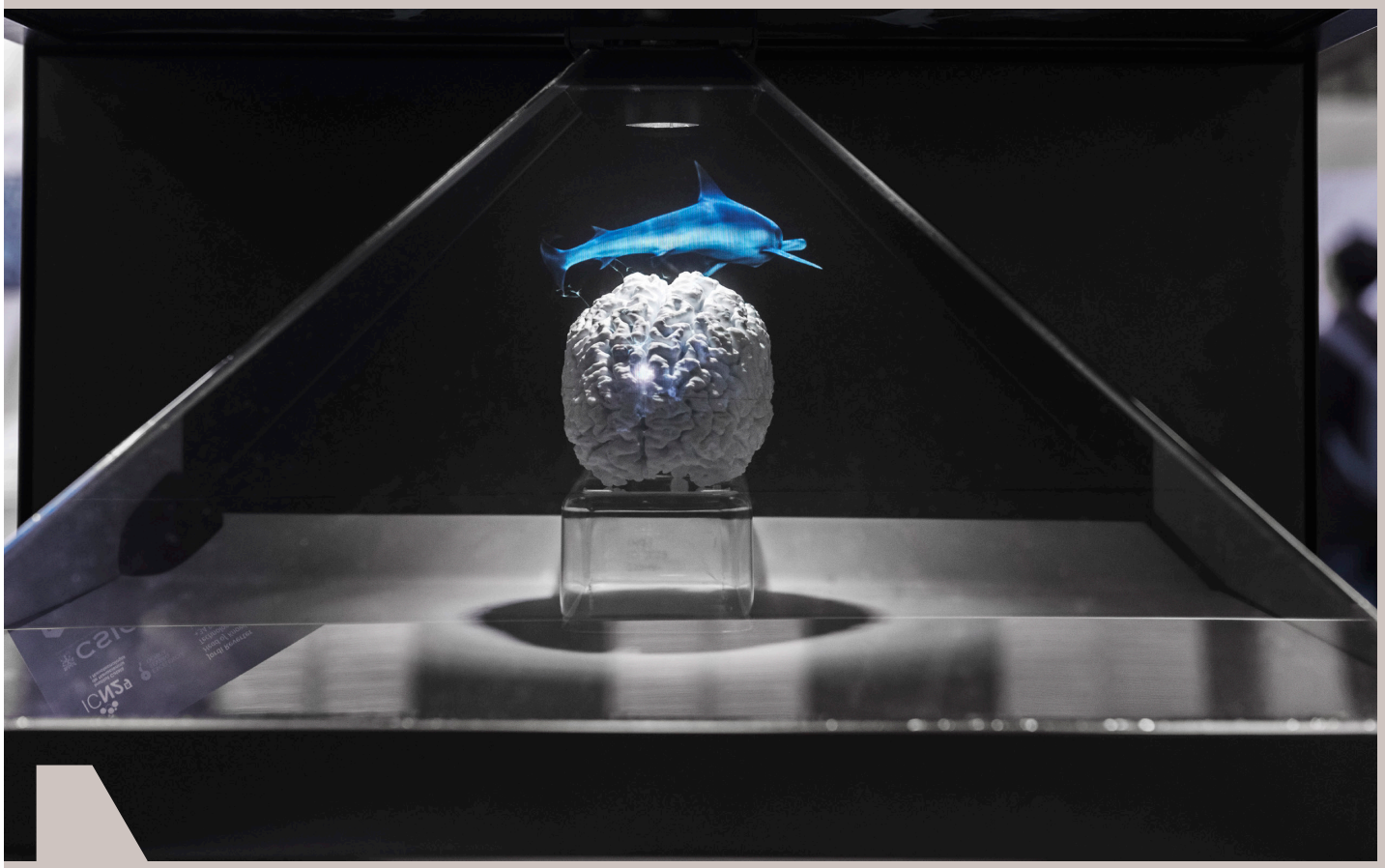
The Graphene Flagship's Biomedical Technologies Work Package aims to develop medical devices using graphene and related materials as well as advanced tools to monitor and influence the nervous system. This work package explores the use of graphene and related materials (GRMs) in the design of neural implants for recording and stimulating electrical activity, combined with localised drug delivery.

"Our work package is highly multidisciplinary, composed of engineers, chemists, neuroscientists and clinicians. In order to perform multidisciplinary research with such a large consortium, large-scale funding, such as that provided by the Graphene Flagship structure, is necessary," said Jose A. Garrido, work package deputy leader from Catalan Institute of Nanoscience and Nanotechnology (ICN2), Spain. "During the past three years, we demonstrated that graphene-based devices can have important applications in neuroscience. This work package contributed to pushing the state-of-the-art of the technology worldwide."

YEAR IN REVIEW

The success stories and technological breakthroughs over the past year are numerous. Here we highlight

Graphene Flagship researchers have developed a sensor that records brain activity at extremely low frequencies and could lead to new treatments for epilepsy. Credit: ICN2



Flexible arrays of graphene solution-gated field effect transistors can be used to monitor brain activity. Credit: Alexandra Csuport

> We have demonstrated that graphene-based devices can have important applications in neuroscience, pushing the state-of-the-art of the technology worldwide."
>
> **Jose Garrido**

three papers on neural implants and on the understanding of how graphene can be used at the cutting edge of this field.

- A paper summarising the fabrication of flexible arrays of graphene solution-gated field effect transistors and their use for monitoring brain activity. This provides a benchmark with respect to standard passive electrodes used to monitor the activity within the brain.
- A paper demonstrating the impact of single layer graphene on the communication between neurons. This describes how single-layer graphene under cultures of neurons can influence the membrane characteristics, resulting in an augmented neuronal activity.
- An article summarising the impact of graphene solutiongated field effect transistors and how they can be used to map infraslow cortical activity *in vivo*.

LOOKING FORWARD

The work package plans, by the end of March 2020, to complete several chronic studies in order to understand the impact of long-term implantation of graphene based neural probes. The main objectives are to assess both the impact of the implant on the tissue and the change of the implant functionality with time.

Thinking in both the short and mid-term, Garrido anticipates the future innovations to appear from the work package: "In the short term (1–2 years), I hope that the development of novel tools (neural probes for monitoring and stimulating nervous activity) reaches the neuroscience research market. In the mid-term (2–4 years), we should be able to demonstrate our neural probes in first-of-its-kind human trials. This will allow us to break the ceiling for using graphene in clinical settings, opening up graphene use to investors in the fields of health and medial devices."

REFERENCES:

"Flexible Graphene Solution-Gated Field-Effect Transistors: Efficient Transducers for Micro-Electrocorticography", Advanced Functional Materials 28, 1703976 (2018)

"Single-layer graphene modulates neuronal communication and augments membrane ion currents" Nature Nanotechnology (2018), 13 (8), 755-765 (2018)

"High-resolution mapping of infraslow cortical brain activity enabled by graphene microtransistors" Nature Materials, 2019, 18, 280–288

https://doi.org/10.1038/s41563-018-0249-4

Work Package Leader:
Maurizio Prato

Work Package Deputy:
Alberto Bianco

Health and Environment

As the Graphene Flagship continues its drive to commercialise graphene and related materials (GRMs) technologies it is important that all safety aspects are thoroughly researched and understood. The Health and Environment Work Package targets safety by design as a core part of innovation.

Fulfilling a fundamental role within the Graphene Flagship, to assess the potential risks associated with the development of GRMs, the findings from the Health and Environment Work Package play a crucial role in technology development across all the technical work packages.

Alberto Bianco, work package deputy leader from CNRS National Centre for Scientific Research, France, stated, "One of the objectives of our work package is to explore appropriate chemical strategies to enhance the biodegradability of GRMs. Towards this goal, we demonstrated that artificial enzymes based on appropriate DNA sequences can be an alternative to natural peroxidases (like the plant enzyme horseradish peroxidase) for the oxidation and degradation of graphene oxide (GO). Our results open new possibilities for the degradation of GRMs."

YEAR IN REVIEW

Graphene has long been thought of as an important material for neuroengineering; however, research into how graphene interacts with neural tissue is still in its

Artificial enzymes based on appropriate DNA sequences can be an alternative to natural peroxidases for the degradation of graphene oxide.

Experiments have shown that the acidic properties of GO affect pollen performance in seed plants.

> “Graphene might represent an unconventional tool to gain insights into genuine biological processes.”
>
> **Maurizio Prato**

infancy. “Over the past year, we have been able to show that single layer graphene, when engineered on an insulating glass substrate, is able to tune neuronal excitability. Graphene properties might thus affect neuronal information processing through its physical interaction with the biological environment. This indicates that graphene might represent an unconventional tool to gain insights into genuine biological processes,” said Maurizio Prato, work package leader from University of Trieste, Italy.

“A particularly important aspect in graphene technology is the impact of GRMs on the environment,” continued Prato. “GO is among the most active GRMs, since it causes widely varying effects on the vegetative body of seed plants. In addition, GO is prepared under strongly acidic conditions with this acidity being maintained in most commercial products. In a study on the reproductive process, the effects of GO on pollen germination and pollen tube elongation were investigated. The results reveal that GO affects the intracellular pH homeostasis with experiments showing that the main factor influencing pollen performance is the acidic properties of GO. This might affect the reproductive process of numerous seed plants thus being relevant from an environmental point of view.”

LOOKING FORWARD

Over the next year, this work package will investigate GRMs’ impact on both health and environment, monitoring effects on the skin, lungs, intestines, brain, plant pollination and fertilisation, and aquatic ecosystems.

WHY THE GRAPHENE FLAGSHIP?

“The large-scale funding of the Graphene Flagship allows us to conduct our research in a highly competitive way, putting us firmly at the forefront of discovery and innovation within the field of toxicology investigation for both health and the environment,” said Bianco. “Safety issues related to technologically advanced materials such as graphene represent an important matter and the results of research in this area should not be assumed. Our research points to a clear indication of biodegradability of GRMs, which is important information. However, more work is needed to fully understand the effects of GRMs on health and the environment.”

"Ein besonders wichtiger Aspekt in der Graphen-Technologie sind die Auswirkungen von GRM auf die Umwelt", so Prato weiter. "GO gehört zu den aktivsten GRMs, da es sehr unterschiedliche Auswirkungen auf den vegetativen Körper von Samenpflanzen hat. Darüber hinaus wird GO unter stark sauren Bedingungen hergestellt, und dieser Säuregehalt wird in den meisten kommerziellen Produkten beibehalten. In einer Studie über den Fortpflanzungsprozess wurden die Auswirkungen von GO auf die Pollenkeimung und die Verlängerung der Pollenschläuche untersucht. Die Ergebnisse zeigen, dass GO die intrazelluläre pH-Homöostase beeinflusst, wobei Experimente zeigen, dass der Hauptfaktor, der die Pollenleistung beeinflusst, die sauren Eigenschaften von GO sind. Dies könnte sich auf den Fortpflanzungsprozess zahlreicher Samenpflanzen auswirken und ist daher aus ökologischer Sicht relevant."

Biomedical Technologies

Work Package Leader
Kostas Kostarelos, University of Manchester, United Kingdom

Work Package Deputy
Jose Garrido, ICN2, Spain

Designing graphene-enabled technology for diagnosis, medical monitoring and treatment

In the Biomedical Technologies Work Package, we take advantage of the unique properties of graphene, such as its ability to interact with electro-active cells and tissues in the body, for medical monitoring, diagnosis and neuropathic therapy.

The graphene-based neural interface devices developed in our Work Package offer the capability to record brain activity, allowing neuroscientists and clinicians to access previously untapped bandwidths of electrophysiological signals in serious brain disorders, such as epilepsy. We aim to translate this technology into graphene-based products in the biomedical field, enabled by our industrial partners.

We are hopeful that our research will allow other scientists around the world to use graphene to better understand neural signaling and physiology at the pre-clinical level, and in parallel, offer clinicians and patients improved treatment options for blindness, epilepsy and other neurological diseases.

NEW HORIZONS IN NEUROLOGICAL DIAGNOSIS

Neurological diseases like epilepsy and Parkinson's disease are currently not well-understood, and they affect millions of people worldwide. For many of these patients, the treatment options available are limited – but the graphene technologies we develop have the potential to change the way we diagnose, treat and monitor these diseases.

Our Work Package will organise and execute a clinical study in the next phase of the project using graphene-based electrodes. They allow for the wireless recording of neurons and brain activity for the diagnosis of diseases of the brain. This will be the first in-human trial of a graphene-based medical device, and we believe the findings of this study will open up new avenues for graphene to be translated into a clinical environment.

WORKING WITH KEY PLAYERS IN INDUSTRY

We have a number of industrial partners who are excited to join us in taking advantage of graphene's properties for brain recording and stimulation.

We worked with Graphene Flagship partner MultiChannel Systems, based in Germany, on our graphene-based neural interfacing electrodes for experimental neuroscience. Furthermore, Graphene Flagship partner Guger, based in Austria, created hardware to process electrophysiological data recorded using field-effect transistor technology. Both of these partners aim to make these products available to consumers in 2020.

2019: A YEAR IN REVIEW

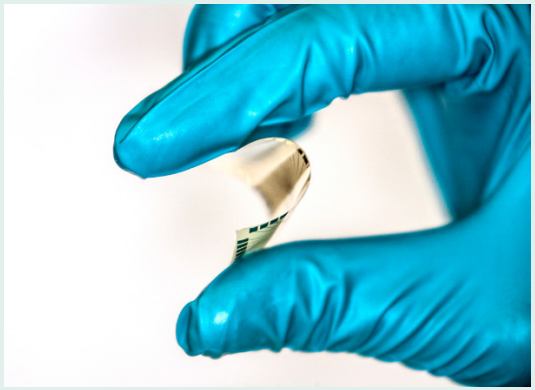
We were very pleased to collaborate with Nature Nanotechnology to run a workshop during Graphene Week 2019: The Science and Science Fiction of Nano-neuro Interfaces. We combined this with a number of public engagement and outreach opportunities to discuss graphene and layered materials with the wider public.

Furthermore, scientists from our Work Package pioneered graphene-enabled retinal implants to provide artificial vision to patients with retinal degeneration. The success of this project has attracted investments and awards from external agencies and has also prompted the creation of a spin-off company, InBrain Neuroelectronics, who will commercialise the technology and guide its transition into the biomedical sector.

OUR VISION

We anticipate that graphene-based devices for pre-clinical use will be on the market in the next three years, and we aim to have a working device approved for human use within five years.

We are also very conscious of the need for continual basic research on graphene to continue. We believe that the biological properties of graphene have great potential, and that they must be further understood before we can fully harness them in biomedical applications. While graphene-based technology for diagnosis has come a long way, our main goal for the next phase of the project is to get graphene-based treatments for neurological diseases to the pre-clinical stage within the next few years.



Above
Flexible neural interface device developed by our spin-off company. Image credit: InBrain Neuro-electronics

Left page
Smart bandage with built-in biosensor for at-home healing monitoring using a smartphone app. Image credit: Grapheal

Bottom
Neural interface device to record neuron activity. Image credit: ICN2

The graphene-based neural interface devices developed in our Work Package offer the unprecedented capability to record brain activity, allowing neuroscientists and clinicians to diagnose serious brain disorders."

Kostas Kostarelos

Health and Environment

Work Package Leader
Maurizio Prato, University of Trieste, Italy

Work Package Deputy
Alberto Bianco, CNRS, France

Profiling graphene's biosafety and setting standards for its safe use in research and industry

In the Graphene Flagship's Health and Environment Work Package, we assess the safety profile of graphene and layered materials and define protocols for their safer use.

We start by looking at the physicochemical properties and bio-nano interactions of graphene and layered materials when they are introduced to biosystems, considering all routes of exposure. We believe that three fundamental steps are necessary for the safety profiles of graphene and layered materials to be fully characterised:

- Maximise the synergy and coordination between different research strategies.
- Monitor toxicity at different levels: cellular, organ, tissue and environmental.
- Define protocols for the materials' safer use, where necessary.

SAFETY FIRST

Graphene and layered materials are rapidly approaching working applications in many areas: electronics, optoelectronics, photonics, energy, materials and composites, and biomedicine, just to cite a few. For these new materials to be viable for industrial and clinical applications, it is fundamental to fully understand their impact on health and the environment.

We still have gaps to fill in risk-related knowledge, and this is what drives us. It is therefore crucial to develop a thorough and reliable safety profile of graphene and layered materials and offer solutions if any risks are identified.

In the EU, all manufactured materials and nanomaterials must follow REACH regulations, established by the European Chemicals Agency, in order to be authorised for industrial production and commercialisation. In 2020, our Work Package will work with the European Chemicals Agency to develop and implement the official guidelines for the safety assessment of graphene and layered materials.

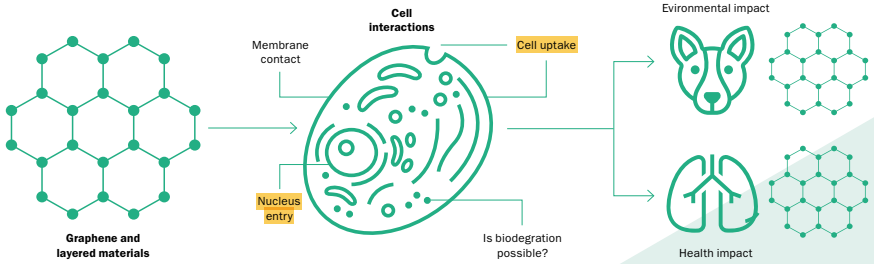
2019: A YEAR IN REVIEW

We studied the degradability of graphene and layered materials, and designed a graphene oxide conjugate, with increased biodegradability, that can stimulate neutrophils to secrete myeloperoxidase: the human peroxidase that degrades unwanted molecules, materials and microorganisms in the lungs.[1]

We investigated ways to revert hyperexcitability, which is one of the causes of epilepsy and other similar conditions, using small-size graphene oxide. In the future, this material could play a neuroprotective role in the treatment of brain-related diseases.[2]

We also looked at the effects of graphene oxide on a microcosm consisting of algae and bacteria as primary producers, chronomid larvae as primary consumers and decomposers, and larvae of the Iberian ribbed newt as secondary consumers. We fully characterised the toxicological effects of graphene oxide on the system and reported genotoxicity in the top predators and significant effects on communities in the sediment.[3]

Graphene and layered materials could interact with cells in different ways, and this may have consequences for the world around us



Studies like these, which are the focal point of our Work Package, are extremely important for scientists to fully understand the biological and environmental safety of graphene and layered materials.

WHY IS THE GRAPHENE FLAGSHIP IMPORTANT TO US?

The Graphene Flagship provides a fantastic opportunity to explore new fields of technology with the potential to cause a deep impact on the future and quality of life of humankind. This unique framework makes it possible to unite and merge the strength and expertise of researchers with very different scientific backgrounds, and it brings together cutting-edge fundamental and applied research, helping to bridge the gap between academia and industry.

The Health and Environment Work Package is a network of scientists who all share a common aim: ensuring the safety of graphene and layered materials. Our work in harmony with the Graphene Flagship enabled us to produce two extremely important publications on graphene classification and safety,[4,5] which will be critical to the field for years to come.

WORKING TOWARDS A SUSTAINABLE FUTURE

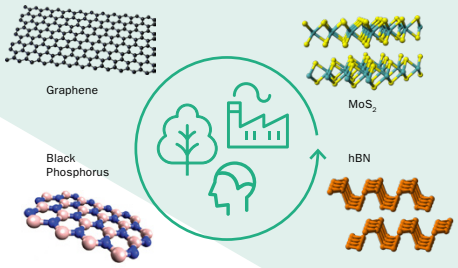
As scientists, we have a responsibility to work consciously towards an environmentally friendly and sustainable future. For both research and industry involving graphene and layered materials, we must achieve this by developing an exhaustive understanding of all possible interactions with human health and our ecosystem.

Only by fully understanding all of the properties of these novel materials, including any potential impacts on health and the environment, will we be able to overcome any risk at any point in the research and production chain. This is the principal goal of our Work Package.

REFERENCES

1. C. Martín et al., Adv. Funct. Mater., 39, 1901761 (2019)
2. R. Rauti et al., Nano Lett., 19, 2858 (2019)
3. L. Evariste et al., Carbon, 156, 261 (2020)
4. P. Wick et al., Angew. Chem. Int. Ed., 53, 7714 (2014)
5. B. Fadeel et al., ACS Nano, 12, 10582 (2018)

The effects of graphene and layered materials on health and the environment must all be fully characterised



Only by fully understanding all of the properties of these novel materials, including any potential impacts on health and the environment, will we be able to overcome any risk at any point in the research and production chain."

Maurizio Prato

Bottom
Could the Graphene Flagship help to mitigate pollution in urban environments?

Health and Environment

Work Package Leader
Maurizio Prato, University of Trieste, Italy

Work Package Deputy
Alberto Bianco, CNRS, France

Assessing the safety and environmental impact of graphene and layered or two-dimensional materials

In the Health and Environment Work Package, our main objective is to assess the safety of graphene and other layered or two-dimensional materials in relation to their use and manipulation. To do this, we investigate any potential risks to health and the environment across all Graphene Flagship Work Packages and Spearhead Projects.

We believe that the comprehensive evaluation of the impact of layered or two-dimensional materials is vital for the protection of human health and the environment, as well as to secure the future applications of graphene and layered or two-dimensional materials, both within the Graphene Flagship and beyond.

OUR STORY

The first and second phases of the Graphene Flagship project, Core 1 and Core 2, laid the foundations for the full safety evaluation of graphene and layered or two-dimensional materials. In 2018, we released a comprehensive review article reporting current knowledge of graphene's health and safety. It contained a thorough analysis of the interactions of graphene and other layered or two-dimensional materials with different types of cells and organs, and formed the basis for further studies into health and safety.

Overall, our investigations study both acute and chronic exposure to different cellular models, and the results we obtain are fundamental for the future of the Graphene Flagship.

OUR RESEARCH

Our Work Package provides information fundamental to the production of safe materials. In particular, we focus on the study of the interaction of graphene and layered or two-dimensional materials with natural barriers. Furthermore, we study new production methods that are greener and more sustainable.

We are also investigating composites and fragments of materials, as well as the effects of material decomposition and factors relating to a material's lifetime. These studies are vital to understand for which applications graphene and layered or two-dimensional materials are in accordance with the OECD regulations on safety.

> Our investigations study both acute and chronic exposure to different cellular models, and the results we obtain are fundamental for the future of the Graphene Flagship."
>
> **Maurizio Prato**
> Work Package Leader

HIGHLIGHTS FROM 2020

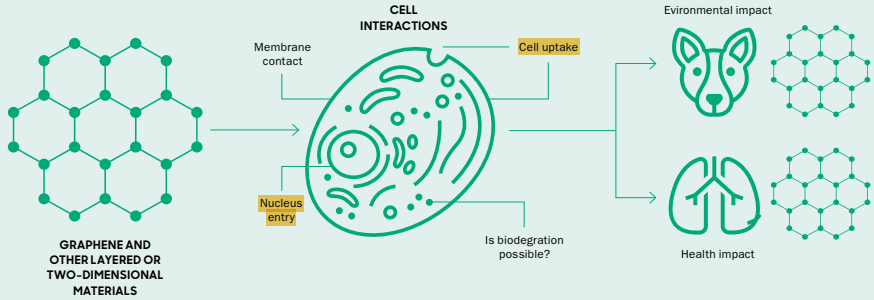
We characterised graphene's interactions with the skin, its size-dependent interactions with the lungs, and published many studies into the interactions between graphene and natural barriers.

One of our most important accomplishments over this unusual year was to see partners of our Work Package join the Graphene Flagship COVID-19 Working Group. It is encouraging to see how our work can help in the worldwide collaborative efforts to mitigate the effects of pandemics such as COVID-19. Expanding our studies into new potential applications of graphene-based technologies to protect people from the virus, and products to help improve the human way of life and the world around us, make us proud to be part of the Graphene Flagship family.

WORKING TOWARDS A SUSTAINABLE FUTURE

Our studies are fundamental for the green future of graphene and layered materials. New products from the Graphene Flagship need to be safe before they can enter the market: this is a vital step that is entirely contingent on our Work Package.

For instance, we recently found that graphene is biodegradable by human enzymes and in zebrafish. These results represent the first steps towards biodegradable-by-design graphene materials.



POWERED BY THE GRAPHENE FLAGSHIP

Collaboration is key, and the importance of our studies spans the entire consortium. We work with many other Work Packages and partners to assess the safety profiles of materials and products generated by the Graphene Flagship. For instance, data on the neurotoxicity of graphene materials enabled our Biomedical Technologies Work Package to study the use of graphene oxide to treat diseases like Parkinson's and anxiety.

We also tested materials for our industrial partners, like Graphenea, Grupo Antolin and BeDimensional, to make sure they are safe. Furthermore, we used reduced graphene oxide from partner company Avanzare to study a composite material developed by our Composites Work Package, demonstrating the sheer degree of interconnectivity fostered by the Graphene Flagship.

SAILING FORWARD

Our next steps are to focus on implementing OECD guidelines for graphene and layered or two-dimensional materials. We are investigating ways to obtain safe-by-design functional materials. We plan to develop a library of robust, verified assays that can be used by researchers, not just experts in the field, to determine the impact of their research or products on human health and the world around us.

Biomedical Technologies

Work Package Leader
Kostas Kostarelos, The University of Manchester, UK and ICN2, Spain

Work Package Deputy
Serge Picaud, Sorbonne University, France

Now we are performing the first in-human clinical trials of graphene-based neural interface devices. We are at the cutting edge of this technological field."

Kostas Kostarelos
Work Package Leader

Developing technologies to treat neurological diseases like epilepsy and Parkinson's

In the Biomedical Technologies Work Package, we design devices to manage diseases of the central nervous system, all based on various types of graphene and layered materials. We develop flexible graphene-based devices to record brain activity and explore our technologies for the treatment of blindness, epilepsy and Parkinson's using pre-clinical disease models.

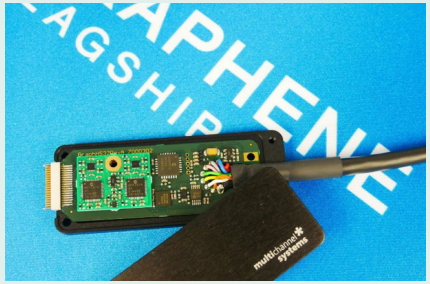
We worked with our industrial partners to launch two commercial products by the end of the Core 2 phase of the Graphene Flagship. This is a great achievement, as the Graphene Flagship roadmap predicted applications in medical technology by 2023 to 2024. In addition, we are now performing the first in-human clinical trials of graphene-based neural interface devices. These activities show that our Work Package is at the cutting edge of this exciting technological field.

OUR STORY

Our Work Package was established three years after the Graphene Flagship's inception. Initially, the Graphene Flagship focused more on physics and materials, so graphene's biomedical applications came along a bit later. However, the great potential of graphene and layered materials for next-generation medical technologies became evident very early on. So, in 2016, the Graphene Flagship launched the Biomedical Technologies Work Package.

In these four years, our Work Package focused on investigating several graphene and layered material-based applications in the central and peripheral nervous system. Our goal was to identify the most promising applications of these materials in the field.

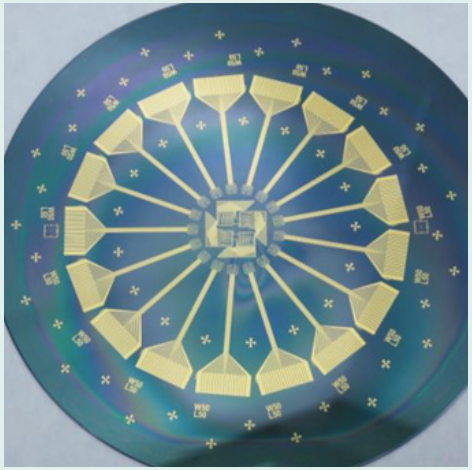
In Core 3, our focus is to further raise the technology readiness level (TRL) of our devices for selected, targeted areas, ensuring they are adopted by clinics and industry. To reach this goal sooner, we work closely with a Business Developer focused exclusively on this field. The Graphene Flagship's industrial and clinical partners are becoming increasingly important for Biomedical Technologies.

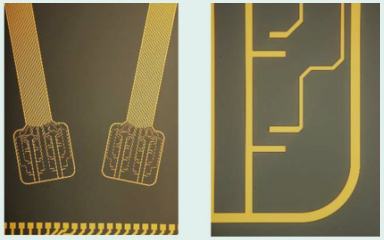


Commercially available neural headstages which amplify, record and analyse data from the brain in vivo, developed by Multi Channel Systems within the Biomedical Technologies Work Package.

WORKING TOWARDS A SUSTAINABLE FUTURE

The scientific output of our work Package directly contributes to the UN's Sustainable Development Goal 3: ensuring healthy lives. Furthermore, through our conscious and ethical hiring, purchasing and waste disposal practices, we also ensure that our research meets SDGs 5, 8 and 12: for gender equality, sustainable economic growth and sustainable production.





Commercial headstages – devices which amplify, record and analyse data from biological systems in vivo – developed by Multi Channel Systems within the Biomedical Work Package.
Credit: Multi Channel Systems

ON THE ROAD TO COMMERCIALISATION

Partner company Guger Technologies, Austria, developed a biosignal amplification and acquisition system that enables recording from graphene transistors. The device has a TRL of 7, meaning their prototype has been demonstrated in an operational environment.

Multi Channel Systems, also a Graphene Flagship partner, used graphene-based neural probes and headstages to develop headstages: all-in-one solutions for amplifying, recording and analysing data in living organisms or cells. The devices have a TRL of 8, showing that the devices are complete and qualified. They plan to market the devices in 2021.

OUR RESEARCH

The applications of our research in the field of neuroscience are already moving forward. For instance, two industrial partners of our Work Package, Multi Channel Systems GmbH, Germany, and Guger Technologies OG, Austria, launched commercial products developed within our Work Package.

Now, we aim to design graphene-enabled sensors that record seizures in patients with epilepsy. Together with our partners ICN2 and ICREA, we launched our spin-off company INBRAIN Neuroelectronics, which will use this graphene-enabled technology to detect seizures moments before they happen, with greater precision than current technologies.
In addition, we are investigating how graphene can improve the electrical stimulation of neuronal circuits to rehabilitate patients with blindness or Parkinson's. This technology could open the door to brain-machine interfaces that reintroduce lost brain function.

HIGHLIGHTS FROM 2020

We launched the first graphene-based neural interface device, in collaboration with our partner Multi Channel Systems, aimed at the neuroscience market. This development will allow neuroscientists around the world to use graphene for their own research, and lead to new discoveries about the human brain beyond the scope of our project.

An additional and remarkable accomplishment during such a challenging year was the creation of INBRAIN Neuroelectronics, a new start-up company. INBRAIN uses licensed technology owned by ICN2, ICREA and the University of Manchester, all three of which are partners of the Graphene Flagship. Furthermore, INBRAIN also recently joined the Graphene Flagship as a full partner.

POWERED BY THE GRAPHENE FLAGSHIP

Our technologies have all significantly benefited from the synergy with other Work Packages enabled by the Graphene Flagship. We all belong to the same large consortium, meaning we can easily communicate with each other, sharing know-how, results and more. In this way, our close interactions with other teams working on materials, electronics and industrialisation have accelerated the maturity of our Work Package's technology.

SAILING FORWARD

In Core 3, our objectives have advanced considerably – to the point that several companies are now involved with commercialising our graphene-enabled products, or commencing investigations in clinical settings. In a few years, we are pleased to have achieved many of our short-term goals.

But our ambitious long-term objectives, like using electrical stimulation to rehabilitate patients, will require long-term studies to assess safety and efficacy. These technologies are poised to be included in medical devices expected to last several decades, so it is important for them to be safe and durable. Therefore, in this funding phase, we expect to provide proof-of-concept devices ready to enter clinical trials. These studies will also provide highly valuable information on graphene's performance in a real clinical setting.

Health and Environment

Work Package Leader
Maurizio Prato, University of Trieste, Italy

Work Package Deputy
Alberto Bianco, CNRS Strasbourg, France

> We are proud to be able to say that our Work Package has contributed enormously to reassuring the public on the safety of GRMs”
>
> **Maurizio Prato**
> Work Package Leader

Ensuring the safe and environmentally friendly commercialisation of graphene

The main objective of the Health and Environment Work Package is to evaluate the safety profile of graphene and related materials (GRMs) with regard to health and the environment in relation to their production, handling, manufacturing and use.

THE LAST 10 YEARS

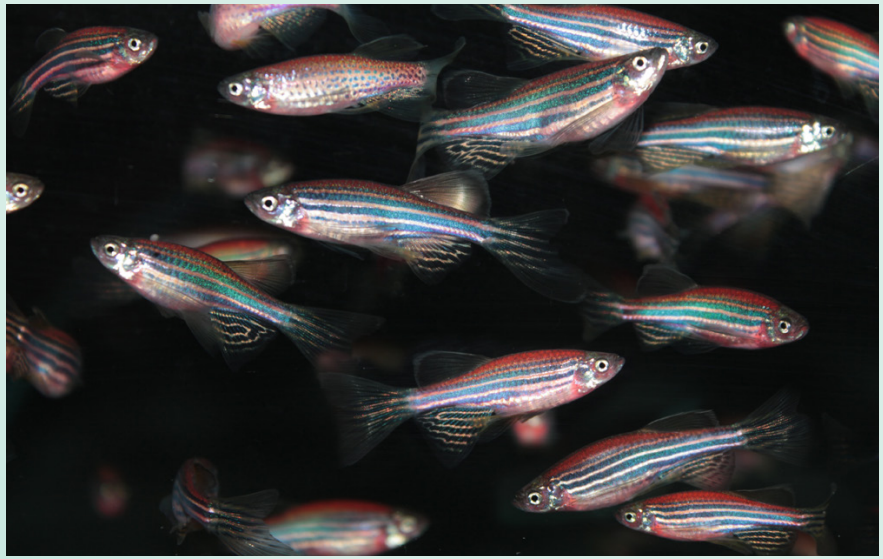
The Health and Environment Work Package has been responsible for performing a series of *in vitro* and *in vivo* studies to prove the safety of GRMs use, identify possible risks and propose solutions for avoiding these risks. During the ramp-up phase of the project, we began to evaluate the impact of graphene oxide and few-layer graphene using several *in vitro* and *in vivo* models, including lung, kidney, gastrointestinal tract, skin, immune system, brain, plants, algae, and aquatic organisms. In Core 1, we expanded the studies on the assessment of the life cycle of GRMs and their (bio)degradability. During Core 2, we enlarged the toxicity studies to other layered materials and evaluated the impact of composites containing graphene that underwent a process of degradation, mimicking their aging, to understand possible risks related to their disposal. Finally, in Core 3 we addressed the issue of occupational health and hazards to workers in daily contact with these materials. Researchers evaluated the applicability of the Organization for Economic Cooperation and Development (OECD) guidelines to GRMs in view of the European Union’s Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and future commercialisation of graphene materials. We believe that this comprehensive evaluation of the impact of graphene and layered materials has been fundamental to the protection of human health and the environment as well as to the current and future commercial applications of these materials.

Our review article on health and safety of graphene-based materials (see page 28) published in 2018 and co-authored by all the partners in the Health and Environment Work Package scores in the top 1% of the academic field of Materials Science, according to the Web of Science. Other Graphene Flagship Work Packages and various companies have been interested in the results of these studies. For example, the University of Castilla-La Mancha, Spain, created with the spin-off company BioGraph Solutions in 2019 with the aim of producing graphene and 2D materials free of any type of contaminants and suitable for physiological media.

THIS YEAR’S DEVELOPMENT

This year’s most important achievements were published in Nature Nanotechnology. The study shows that the gut microbiome needs to be taken into consideration when evaluating the impact of graphene and related materials, specifically graphene oxide (GO). Using zebrafish as a model, the researchers showed that GO modulates the composition of the gut microbiome. Furthermore, using germ-free zebrafish, the authors found that GO in combination with certain microbial metabolites triggered the induction of an innate immune response. This study has shown that GO can influence the crosstalk between the microbiome and immune system, thus opening a new horizon with respect to layered material-host interactions.

Additionally, a joint paper coordinated by Graphene Flagship Partner EMPA reported on the toxicological impact of reduced GO-reinforced composites.



Researchers tested the effect of graphene oxide on zebrafish gut and immune system.

References
Peng, G. et al. Nat. Nanotechnol. 2022, DOI: 10.1038/s41565-022-01260-8
Loret, T. et al. Adv. Sci. 2022, DOI:1 0.1002/advs.202104559
Chortarea, S. et al. Mater. 2022, DOI: 10.1016/j.jhazmat.2022.129053

Biomedical Technologies

Work Package Leader
Kostas Kostarelos, The University of Manchester, UK and ICN2, Spain

Work Package Deputy
Serge Picaud, Sorbonne University, France

Coordinating the first-ever clinical investigation for graphene-based technologies is challenging, complex and thrilling."

Kostas Kostarelos
Work Package Leader

Graphene biosensors for biomedical applications

The Biomedical Technologies Work Package innovates and pushes the technological boundaries for the engineering and biological performance of neural interface devices for the diagnostics and treatment of neuropathologies.

THE LAST 10 YEARS

The Biomedical Technologies Work Package became part of the Graphene Flagship at the start of Core 1 with 14 partners. We focused on the utilisation of different types of graphene and other layered materials in the design of devices for the management of neuropathologies. During Core 1 and Core 2, we concentrated on the development and initial preclinical proof-of-concept studies of graphene-based neural interface technologies for the central and peripheral nervous system. The goal was to identify the most promising applications to take forward in the clinical translation pipeline.

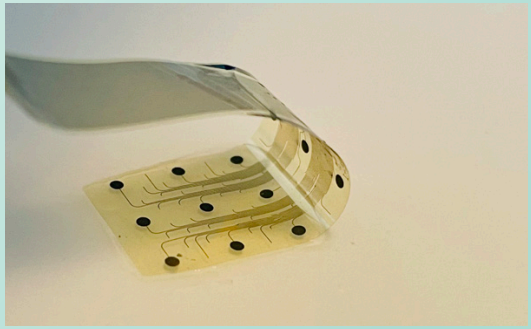
The Work Package has grown to incorporate 18 partners and, more importantly, has evolved to advance the most promising technologies to higher TRL levels. During Core 3, the role of our industrial and clinical partners has become increasingly important to ensure the translation of these technologies by industry and adoption by clinics. For example, the spin-off company INBRAIN Neuroelectronics was created in 2020 and raised more than €17.5M in venture capital investment to champion the clinical translation of our graphene-based technologies for the benefit of patients suffering from serious neuropathologies.

Critical research highlights include high-profile publications detailing graphene-based field-effect transistor (GFET) technologies in Nature Communications and Nature Nanotechnology. We will culminate Core 3 with a world-first: the first in-human clinical study of graphene-based electrocorticography (ECoG) microelectrode arrays in patients undergoing brain cancer resection surgery performed at NHS Royal Salford Hospital, UK.

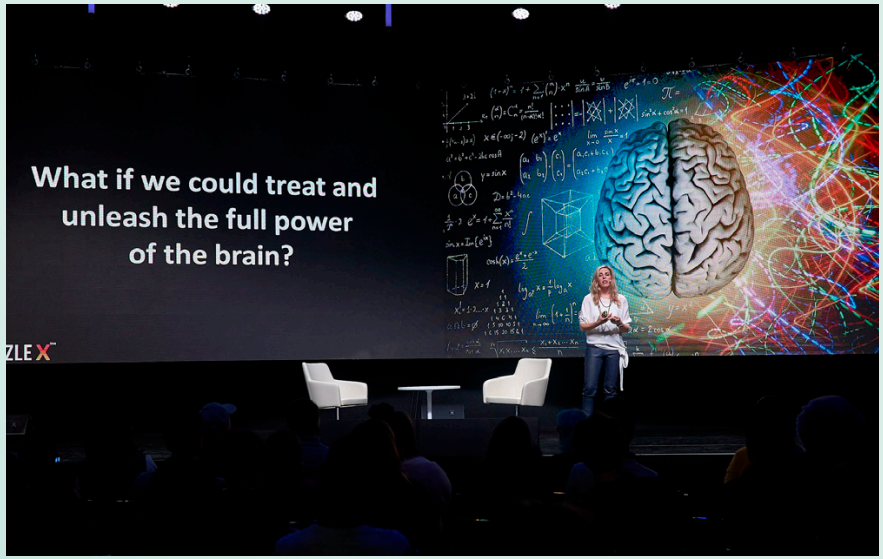
THIS YEAR'S PROGRESS

Multi Channel Systems GmbH, an industrial partner in the Biomedical Technologies Work Package, developed new tools to record brain electrical activity via GE2100 head stages. These are all-in-one solutions for amplifying, recording and analysing preclinical *in vivo* data from GFET probes with 16 or 32 channels, 24-bit resolution and 50 kHz sampling rate. The GE2100 HS16 and HS32 are currently technology readiness level (TRL) 8 and are planned to be marketed in 2023.

INBRAIN Neuroelectronics and its subsidiary INNERVIA Bioelectronics signed a strategic collaboration programme with Merck, Germany, to develop next-generation bioelectronics. G.tec medical engineering GmbH worked on a biosignal amplification and acquisition system with graphene-based electrodes, which is used to decode signals from GFETs.



INBRAIN graphene-based, high-resolution cortical brain interface. Credit: INBRAIN Neuroelectronics



INBRAIN Neuroelectronics' CEO Carolina Aguilar presents the possibilities for future brain research. Credit: INBRAIN Neuroelectronics

The INBRAIN Neuroelectronics team. This Graphene Flagship spin-off company exemplifies the project's commercialisation progress. Credit: INBRAIN Neuroelectronics

References
Viana, D. et al. bioRxiv 2022. DOI: 10.1101/2022.11.16.515761
Zummo, F. et al. Front. Neurosci. 2022, DOI: 10.3389/fnins.2021.811348
Di Mauro, G. et al. ACS Nano, 2023, DOI: 10.1021/acsnano.2c06609

GRAPHENE FLAGSHIP ANNUAL REPORT 2022