PREPARATION OF NANOPARTICLES BY GREEN SYNTHESIS AND A STUDY ON THEIR ANTIBACTERIAL AND ANTICANCER PROPERTIES

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"Research is what I'm doing when I don't know what I'm doing."

Wernher von Braun
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ABSTRACT

With a continuous improvement in living standards, humankind’s desire for a healthy living environment has become increasingly attractive. However, with the rise of a globalized world and the increase in live-expectancy, contact and exchanges among individuals, and the probability of slight genetic changes, respectively, have become more and more frequent, which also virtually facilitates the spread of infectious diseases and the development of cancer. The rise of medicine has been exposed to every single human being including the possibility of using antibiotics and access to a healthcare system, but it has also failed in other ways. The continued misuse and overuse of antibiotics have led to the spread of antibiotic resistant-microbes (AMR), triggering our jump to the edge of a time in which common diseases that were once easily treated, may kill again. Moreover, cancer, as one of the most lethal diseases in the world, is experiencing an emergence of multidrug resistance behavior towards chemotherapeutic drugs that were designed to inhibit their proliferation. Current treatments based on chemotherapy and radiotherapy are often insufficient, while now they face barriers.

Because of the need of new and alternative treatments to fight AMR and cancer, more researchers and physicians have begun to pay attention to nanotechnology, a nanosized world where materials start showing outstanding properties never achieved by their macroscopic configurations. Nanoparticles can destroy the structure of bacterial membranes by binding themselves to the cells, while also they can release metallic ions that will damage the genetic content of cancerous cells. Hence, a broad variety of possibilities and biomedical applications for nanomaterials is now present in front of all of us. Nevertheless, sometimes it is essential to pay attention to how things are made instead of what things are used.

Therefore, the traditional synthesis of nanomaterials, based on the use of synthetic chemistry and physics, presents questionable methods related to environmental concerns, such as the production of toxic by-products, or end-user issues, like the lack of biocompatibility towards the target biological tissue. Consequently, new and alternative approaches have been studied, such as those based on the use of natural materials for the generation of nanomaterials, giving rise to what is called “Green Nanotechnology.” Therefore, an environmentally-friendly and cost-effective way
of synthesizing nanoparticles takes advantages of natural sources, such as bacteria, biomolecules or waste materials.

From all of these methods, plant-based and plant-derived biomolecule-based processes are among the most reported and successful, due to their feasibility and reproducibility. Therefore, this dissertation is divided into two parts. In the first section, a starch-derived tellurium (Te) nanowire is used as a template for the \textit{in situ} generation of noble metallic nanoparticles (palladium (Pd) and platinum (Pt)) and for the generation of a synergetic nanosized tellurium-based nanostructure with powerful antibacterial and anticancer properties, with a promising future as a coating for implantable devices. PtNPs-TeNWs and PdNPs- demonstrated antibacterial properties in a range of concentrations between 10 and 25 \(\mu\text{g/mL}\), triggering no cytotoxicity toward healthy epithelial cells above the same period of time. Moreover, both nanostructures were discovered to have anticancer activity toward melanoma cells in a range of concentrations between 10 and 15 \(\mu\text{g/mL}\) with no alteration of healthy skin cells' usual proliferation. On the other hand, the second section is based on the quick and environmentally friendly synthesis of TeNPs using Aloe Vera as a unique reducing and stabilizing agent, showing the generation of a strong synergetic effect between the phytochemicals of the plant and the novel biomedical properties of the metalloid. TeNPs' antimicrobial activity was studied, demonstrating antibacterial activity toward both Gram-positive bacteria and Gram-negative for a range of nanoparticle concentrations between 5 and 50 \(\mu\text{g/mL}\).
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LIST OF TERMS

Ag. Silver.
AgNPs. Silver nanoparticles.
AMPs. Antimicrobial peptides
AMR. Antimicrobial resistance to antibiotics.
ATCC. American Type Culture Collection.
ATR. Attenuated total reflectance.
AV. Aloe Vera.
AV-TeNPs. Aloe Vera tellurium nanoparticles.
BE. Absolute binding energies
C. Carbon.
°C. Celsius.
CFU mL-1. Colony forming units per milliliter.
Cl. Chlorine
CO2. Carbon dioxide.
Cu. Copper.
CVD. Chemical vapor deposition.
DCFDA. 2’,7’ –dichlorofluorescin diacetate.
DMEM. Dulbecco’s Modified Eagle Medium.
DNA. Deoxyribonucleic acid.
EDX. Energy-Dispersive X-Ray Spectroscopy.
EMS. Electron Microscopy Sciences.
eV. Electron-volt.
FBS. Fetal bovine serum.
FE-SEM. Field Emission Microscope.
FQ. Fluoroquinolones.
FTIR. Fourier-transform infrared spectroscopy.
GRG. Generalized Reduced Gradient.
h. Hour.
HDF. Human Dermal Fibroblasts.
HIV. Human immunodeficiency virus.
HPV. Human papillomavirus.
K. Potassium.
K₃PtCl₄. Potassium tetrachloroplatinate.
K₂TeO₃. Potassium tellurite.
KV. Kilovolt.
LB. Luria-Bertani.
MDR E.coli. Multidrug-resistant Escherichia coli.
MDR. Multidrug-resistant.
Mg. Magnesium.
MIC. Minimum inhibitory concentration.
Min. Minute.
Ml. Milliliter.
Mm. Millimeter.
MRSA. Methicillin-resistant Staphylococcus aureus.
MTS. (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium).
N. Nitrogen.
Na₂TeO₃. Sodium tellurite.
NaOH. Sodium hydroxide.
NGS. Next-generation sequencing
NONPs. Nitric oxide-releasing nanoparticles
NPs. Nanoparticles.
O. Oxygen.
OD. Optical density.
OsO₄. Osmium tetroxide.
PBP. Penicillin-binding proteins.
Pd. Palladium.
PdCl₂. Palladium chloride.
PdNPs. Palladium nanoparticles.
PdNPs-TeNWs. Palladium Nanoparticles-Tellurium Nanowires.
Ph. Power of hydrogen.

PtNPs. Platinum nanoparticles.

PtNPs-TeNWs. Platinum Nanoparticles-Tellurium Nanowires.

PVP. Polynynylpolirridone.

RNA. Ribonucleic Acid.

ROS. Reactive oxygen species.

Rpm. Revolutions per minute.

SA. Staphylococcus aureus.

SEM. Scanning Electron Microscopy.

Si. Silicon.

SNV. Single nucleotide variants.

Te(OH)₆. Tellurium hydroxide.

Te. Tellurium.

TEM. Transmission Electron Microscopy.

TeNDs. Tellurium nanodots

TeNPs. Tellurium nanoparticles.

TeNWs. Tellurium nanowire.

TeO₂. Tellurium dioxide.

The US. The United States.

Ti. Titanium.

TSB. Tryptic Soy Broth.

UV. Ultraviolet.

XPS. X-ray photoelectron spectroscopy.

XRD. X-Ray photoelectron spectroscopy.

Zn. Zinc.

λ. the lag time.

μl. Microliter.

μm. Micrometer.
1. GENERAL INTRODUCTION

1.1. Antimicrobial resistance (AMR)

1.1.1. What is AMR?

Antimicrobial resistance (AMR) is the capacity of a microorganism to cease the activity of a drug designed to inhibit its proliferation, resulting in a lack of effectivity and an increase in the chances of infections to persist and spread to other living organisms.\(^1,2\)

1.1.2. Brief historical context

In the 19th century, microbiologists Louis Pasteur and Jules Francois Joubert observed some antagonistic effects between bacteria and considered the qualities of managing these interactions in medicine.\(^3\) In 1928, Alexander Fleming discovered a natural antimicrobial agent, penicillin, that was generated by the fungus *Penicillin Rubens*, which was utilized in 1942 to treat streptococcal contagion, in what we now term the birth of the antibiotic era.\(^4\)

The detection, improvement, and employment of antibacterial agents in the 20th century decreased the death rate from bacterial infections, as well as stopped the increased spreading that was observed just years before.\(^5\) In the US for instance, the leading causation of death changed from infectious diseases to non-communicable diseases, such as cardiovascular disorders, cancer, and strokes, and the average life expectancy at birth rose to 78.8 years.\(^6\) From 1945 to 1970, proficient drugs were grown and became the "golden bullets" against bacterial infections.\(^7\) However, bacteria found the way to fight back, and the first cases of antibiotic resistance were observed a few years after their first massive use in society, a fact that has been shadowing more and more what is called the death of the antibiotic era.

While the employment of new antibacterial agents for clinical use has been shrinking since 1980, in part because of the high cost of strengthening and testing new drugs, the resistance of bacteria has increased significantly.\(^8\)–\(^10\). Probably, the most shocking case is ligated to the bacterium *Staphylococcus aureus* (SA), and its most severe antibiotic-resistant phenotype, Methicillin-
resistant *Staphylococcus aureus*, or MRSA\textsuperscript{11,12}. In the late 1940s, *Staphylococcus aureus* began a dangerous evolution when it became resistant to penicillin. With a main weapon towards killing the organism taken out of commission, clinicians began using methicillin to treat infections. However, in 1961, experts got regretful news with the discovery of SA strains that had become resistant to beta-lactams, including methicillin, giving MRSA its name\textsuperscript{13,14}. The first infection related to MRSA in the United States was diagnosed in 1968, and the organism has persisted ever since. Beginning in 2002, there have been some of the incidents documented in which the bacterium was also detected to be resistant to one of the last available drugs being used to treat it, vancomycin\textsuperscript{15}.

### 1.1.3. Mechanisms of AMR in bacteria

It is important to define that there are two types of AMR, depending on the source of this activity: natural- and human-induced AMR. While the first one has been around since the beginning of time, the second one is a consequence of the relatively new interaction between bacteria and humans.

Bacteria have developed natural mechanisms of resistance to avoid being killed by antimicrobial molecules generated by other bacteria or fungi, a process that has likely happened over millions of years.\textsuperscript{16} However, these mechanisms are similar to the ones developed after exposure to human-derived antibiotics. It is well known that a bacterial cell can use various biochemical pathways that will trigger several mechanisms to shield and avoid the influence of an antibiotic\textsuperscript{17}. As an example, we will use fluoroquinolones (FQ), antibiotics that are commonly used to treat a variety of bacterial illnesses such as respiratory and urinary tract infections. FQ resistance can take place due to three separate biochemical ways, all of which may exist together in the same bacteria at a given time (generating an addictive impact and, usually, raising the levels of defense): 1) mutations in genes encoding the target site of FQs; 2) over-expression of efflux pumps that force out the drug from the cell; and 3) safeguard of the FQ target site by a designated protein. These mechanisms can be found all over bacterial strains those are resistant to one or other antibiotics, found in either nature or as a consequence of research in laboratories\textsuperscript{18}. 

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Bacteria seem to have developed a favor for some mechanisms of resistance over others. For example, the predominant mechanism against β-lactams in Gram-negative bacteria is the production of β-lactamases that can diminish the antibiotics efficiency, while resistance to these compounds in Gram-positive organisms is often attained by adjustments of their target site, the penicillin-binding proteins (PBPs). These behaviors are due to primary differences in the cell envelope between Gram negative and positive bacteria.

1.1.4. The present and future of AMR

In the United States, antibiotic-resistant organisms have led to over 2 million infections and are connected with roughly 23,000 deaths every year, whereas, in Europe, AMR is associated with around 25,000 deaths every year. AMR’s economic losses are substantial, estimated at $20 billion in medical spending in the United States each year.

The development of antimicrobial-resistant bacterial species increasingly derives from a vast quantity of factors including the widespread and sometimes inappropriate use of antimicrobials (as seen in Figure 1), the full consumption of these agents as growth enhancers in animal feed, and, with the rise in international and regional travel, the relative ease with which antimicrobial-resistant bacteria cross geographic barriers.

Back in the 1980s, the world saw tens of new antibiotics being approved every year, but now society is lucky to see a few products trickle through. Although pharmaceutical companies are researching new antibiotics, policy-makers do not want healthcare professionals to use them. In other words, products should sit on the shelf until patients need them because of the aforementioned antimicrobial resistance problem through the imprudent use of antibiotics over the years.
Therefore, alternative approaches have been followed, such as the use of antimicrobial peptides (AMPs). Evidence advises us that naturally occurring or synthetic AMPs could be a model for the creation and design of new functional classes of antibiotics. AMPs are selective agents that are based on their action against the prokaryotic membrane. Bacterial membrane modifications are induced by these agents ranging from small lipid bending to complete membrane dissolution, and this last event is similar to a detergent-induced micelle formation resulting in complete membrane disintegration. Besides AMPs, protein synthesis inhibitors (for instance, macrolides or tetracyclines) are bacteriostatic, while aminoglycosides are bactericidal antibiotics affecting translational fidelity and have been used to deal with mainly Gram-negative infections for more than 40 years, with kanamycin, gentamicin, amikacin, and tobramycin being the most popular in the clinic. When combined with traditional antibiotics, aminoglycosides have been used to deal with enterococcal endocarditis and staphylococcal infections.

Infection constraint, antibiotic stewardship, and new antibiotic evolution are cornerstones of how society can fight against resistance. It is, however, reported by the World Economic Forum that, despite ongoing attempts, antibiotic resistance, antibiotic study breakdown, and pipeline evolution continues to worsen. Beyond new agents, the healthcare system should look for developing innovative diagnostic tools which would need to be uncomplicated, quick, precise, and inexpensive, to detect and profile microorganisms or antibiotic resistance genes. Diagnostic tests are vital to infectious disease management and combat the rise in antibiotic resistance, and the introduction of accurate and rapid diagnostics tests to clinicians will influence their prescribing of
more rational antibiotics \(^{26,34,35}\). Besides, to enhance the efficacy of current antibiotics with antimicrobial action, multiple modes of action would help decrease antibiotic resistance, while possibilities could be broadened by screening for new antibiotics from natural sources for dealing with infections.

1.2. Cancer

1.2.1. What is cancer?

Cancer is a collection of diseases that consist of significant cell functional increases with a capability to invade or disperse to other portions of the body \(^{36-38}\). It is usually not possible to know precisely why one person gets cancer, and another does not. Nevertheless, research has shown that certain risk factors may increase a person's chances of developing cancer. Cancer risk factors comprise vulnerability to chemicals or other materials. They also include things people cannot manage, like age and family records. Moreover, diet, physical activity, weight, alcohol consumption, sun exposure, and exposure to infections like hepatitis, HPV, and HIV are likewise risk factors \(^{39-41}\).

1.2.2. Current cancer treatments

There are many types of cancer therapies. The types of treatment that a patient would receive depends on the kind of cancer the patient develops and in which stage it is. While some people with cancer will have only one treatment, most patients have a combination of treatments, such as surgery followed by chemotherapy.

Chemotherapy is the use of any drugs to kill cancer cells, operating by stopping the cancer cells from dividing and growing. It has a more significant impact on tumoral cells than regular cells; nevertheless, it is commonly known that the drugs used for chemotherapy can cause damage to healthy tissue. Therefore, there are often numerous side effects related to the use of chemotherapeutics like nerve damage, fertility problems or heart damage \(^{42}\).
On the other hand, radiation is a form of cancer treatment using high doses of radiation to destroy cancer cells and reduce tumors. The term "radiation remedy" most frequently relates to external beam radiation treatment. Throughout this kind of radiation, the high-energy beams aim at a point on the target tissue. Several technological improvements, together with a more excellent understanding of neoplasm biology at the molecular, cellular, immunological and physiological levels, have enhanced the therapeutic efficacy of radiotherapy. For instance, the overall survival rates for cancer radiation therapy have ameliorated from around 30% to approximately 80% nowadays in some malignancies, like neck and head cancers.

1.2.3. *Chemotherapy-resistant cancer*

Chemotherapy resistance occurs when tumors that have been reacting to a therapy abruptly begin to grow. Consequently, the tumor cells are holding the consequences of chemotherapy and when chemotherapy fails, the drugs need to be changed. There are undoubtedly many similarities between chemotherapeutic cancer cell resistance and antibiotic bacterial resistance.

The reasons behind this particular behavior of cancer cells are not fully understood. However, extensive research is being accomplished in this matter. What we do know so far is that some of the cells that are not killed by the chemotherapy, mutate and become resistant to the drug. Once they reproduce, there may be more resistant cells than cells that are susceptible to chemotherapy. Besides, it is widely known that a cancer cell may produce hundreds of copies of a particular gene, and after exposure to chemotherapy drugs, a particular gene triggers an overproduction of a protein that renders the anticancer drug ineffective. Furthermore, cancer cells may pump the drug out of the cell as fast as it is going in using a molecule called p-glycoprotein, and they may stop taking in the drugs because the protein that transports the drug beyond the cell wall stops operating. It has also been reported that cancer cells may learn how to repair the DNA breaks caused by some anti-cancer drugs.

1.2.4. *The present and future of cancer*

It is estimated that the world-wide, cancer cases have increased to 18.1 million new cases and 9.6 million deaths in 2018, while 1,735,350 new cancer cases were diagnosed and 609,640 cancer
deaths occurred in the United States alone. One out of 5 men and one out of 6 women worldwide get cancer throughout their existence, and one in 8 men and one in 11 women pass away from the illness. Worldwide, it is estimated that 43.8 million people are currently within five years of a cancer diagnosis and alive.

The future for cancer goes beyond enhancements in current treatments. For instance, next-generation sequencing (NGS) can look into the molecular machinery inside cancer cells and can enhance the current understanding of the disease. Apart from gene expression profiling and transcription as well as finding alternative splicing, NGS has allowed for the identification of single nucleotide variants (SNV), insertions, deletions, amplifications and inter-chromosomal rearrangements in the transcriptome and entire genome. The development of a more safe, efficient and effective personalized cancer therapy has been led by the advent and improvements in the NGS bioinformatic algorithm predicting mutated gene immunogenicity.

On the other hand, in the progression of cancer, somatic mutations will be acquired by tumors, and those cells acquiring specific mutations have survival advantages that will control localized tumor areas through displacing those that lack these genomic alterations. Driver mutations dominate in cancer in all metastatic sites, and the heterogeneousness will affects subclonal mutations positively. Tumor heterogeneousness (both intra-tumor and inter- heterogeneousness), together with clonal mutations are the main challenges. Repeated biopsies at biomarker-driven personalized therapies and progression are consequently needed to determine resistant mechanisms, and their potential targeted inhibition. Next-generation clinical trials that are taking this into consideration are being developed.

Besides, new techniques such as immunotherapy (a type of cancer treatment that helps one’s immune system fight the disease) or genetic therapy (using genes that should be introduced inside the cancer cells using vectors) are revolutionizing the cancer field. However, most of them are still in the research stage and are extremely expensive.
1.3. Nanotechnology as a biomedical tool

1.3.1. Nanotechnology and nanomedicine

Nanotechnology can be defined as the science and engineering that is involved in the design, synthesis, characterization, and use of materials and devices whose small-scales (in no less than one dimension) is of nanometer size or one billionth of a meter \(^61\). At these scales, one considers individual molecules and interactions in comparison with bulk macroscopic properties of the device or material. At the nanoscale, atomic interactions become significant, as they possess control over fundamental molecular structures permitting control over macroscopic physical and chemical properties \(^62\).

With applications of nanotechnology into medicine, nanomedicine was born, seeking to deliver a valuable set of research tools and clinically useful devices. Therefore, biomedical applications like AMR and cancer treatment have been receiving plenty of nanomedicine related research for use as active agents towards fighting these diseases \(^63\).

1.3.2. Nanotechnology in the treatment against AMR
Many inorganic and organic nanomaterials have been demonstrated to possess potent inherent antimicrobial properties that are rarely expressed in their bulk form. More importantly, some of these nanomaterials can combat antibiotic resistance by compromising existing resistance mechanisms. Furthermore, nanoparticles for antimicrobial drug delivery also offer distinct advantages in overcoming resistance and causing fewer side effects than conventional antibiotics. Besides, the incorporation of antimicrobial nanomaterials in medical devices can prevent microbial adhesion and infection. Last, but not least, using nanomaterials as vaccine adjuvants and delivery vehicles can evoke more efficient immune responses against microbial infection ⁶⁴.

Several kinds of nanoparticles (NPs) including nitric oxide-releasing nanoparticles (NONPs), chitosan-containing, and metallic nanoparticles, have been used to fight against microbes simultaneously by multiple mechanisms ⁶⁵, making it hard for microbes to generate resistance to these nanoparticles. Nowadays, with nanotechnology being more widespread and applied in medicine, it is not surprising to see nanoparticle technologies being applied to combat antibiotic resistance ⁶⁶.

There are several approaches to use NPs for AMR treatments. Therefore, NPs can be applied by amalgamation with existing clinically related antibiotics to adjust and increase their physiochemical properties to defeat anti-microbial resistance mechanisms; or as antimicrobial agents themselves, as the colloidal formulations of silver (Ag), zinc (Zn), copper (Cu), titanium (Ti) and gold (Au), among others ⁶⁷. The three most important objectives for antibiotic action are the synthesis and inhibition or disruption of translation and transcription during protein synthesis and the synthesis of nucleic acids as well as cell wall structure. Nanoparticle technologies have also been used to influence the bacterial respiration system, inducing the generation of reactive oxygen species (ROS) in bacteria via compromising the bacterial antioxidant system ⁶⁴,⁶⁸.

Consequently, nanotechnology can offer a new therapeutic approach to overcome AMR ⁶⁹–⁷¹. Nanoparticles can also overcome microbe drug resistance mechanisms, including reducing uptake and increasing efflux of drugs from the microbial cell, biofilm formation, and intracellularly in bacteria. Lastly, nanoparticles can be applied to target antimicrobial agents to an infection site and
higher drug doses can be provided at the infected site, thereby overcoming resistance with fewer side effects to the patient 72.

1.3.3. Nanotechnology in the treatment against cancer

Starting a few years ago, nanotechnology has attracted significant attention in cancer therapeutics because of its vast potential to contribute an innovative model to overcome the problems of existing chemotherapeutic tools 73. For example, a variety of nano-vehicle platforms with nanoscale sizes favor intracellular endocytic uptake, high drug packing, and precise targeting to tumor networks. These artificially engineered nanomaterials will significantly improve the therapeutic efficiency of the chemotherapy loaded drugs while decreasing non-specific toxicity, thereby making it likely to produce reliable and useful cancer treatments. Furthermore, tremendous efforts have been newly devoted to producing multi-functional theragnostic nanosystems for both cancer diagnosis and therapy, which can deliver drugs precisely to tumors and concurrently monitor their therapeutic answer by visualizing tumor legions in the body 74,75. Therefore the convergence of nanotechnology and biological sciences will revolutionize the entire discipline of cancer medicines.

Nanoparticles have displayed a significant commitment to enhancing cancer therapy efficiency while reducing treatment side effects and toxicity. Forecasting the therapy result for nanoparticle systems by measuring nanoparticle biodistribution has been challenging because of the ordinarily unmatched, heterogeneous distribution of nanoparticles that was about free drug distribution 76. Nanotechnology holds in potential minimizing systemic toxicity through functionalized particles' development for targeted treatment 73. They also supply an alternative strategy to circumvent multidrug resistance, as they possess a capability to bypass the drug efflux mechanism. Except for the advantages, they provide in therapy; nanoparticles are also emerging to be valuable diagnostic entities 77,78.

Concerning cancer treatment, most current anticancer regimes do not differentiate between healthy and cancerous cells effectively. This indiscriminate action leads to systemic toxicity and debilitating adverse effects in normal body tissues frequently including bone marrow suppression, neurotoxicity, and cardiomyopathy. A more targeted approach was promising significant
improvements in the remedy of cancer can be offered with nanomedicine and nanotechnology 79–81.

1.3.4. Drawbacks in the use of nanotechnology for biomedical applications

One of the most significant troubles about nanotechnology is related to safety. The nanoparticles' increased surface area leads to an augmented chemical reactivity. This reactivity brings into question as for how NPs will react under varying conditions within biological tissue, and whether they will be able to cross cell membranes to get into cells 82. Nanoparticles’ increased chemical reactivity brings about the production of reactive oxygen species (ROS), which may cause oxidative stress, inflammation, and damage to proteins, membranes, and DNA, leading to cell death 82–84.

A significant disadvantage of nanomedicine is that nanoparticles possess no common feature other than their size. Hence, each particle needs to be evaluated separately. Likewise, changes in size and shape can cause various chemical and physical interactions. Another limitation is these particles’ dependency on the surrounding environment—particles may disintegrate or aggregate resulting in changes in size leading to toxicity 84. Other determinants that may influence toxicity are the chemical composition, surface structure, surface charge, solubility and the presence of functional groups on the nanoparticles 85,86.

Furthermore, nanoparticles can result in effects not considered with traditional therapy. For instance, they can obtain access into the various kinds of cell organelles such as mitochondria or nucleus and trigger harm. They can in a similar way initiate blood coagulation pathways and cause platelet aggregation. Toxicity may be caused by the carrier systems themselves, though designed to reduce the drug's systemic adverse effects 87. Following research, nanoparticles can accumulate in various animals' organs.

Furthermore, it cannot be predicted if the organisms will be capable of excreting them or if they will remain accumulated in the body. Whereas the biodegradable nanoparticles are usually excreted, the non-biodegradable ones may accumulate in organs, possibly harmful 88. The particles
that do not decompose or break down gradually may develop at the drug administration's site covering chronic inflammation\textsuperscript{89}.

1.4. Green nanotechnology

How nanomaterials are synthesized has a significant impact in their applications, and especially for biomedical application. For a long time, the unique answer for a quick, straightforward and efficient synthesis of structures in the nanoscale community has been traditional synthesis, taking knowledge from both physics and chemistry with techniques such as chemical vapor deposition (CVD), laser ablation, redox reactions or electrochemical mechanisms\textsuperscript{90,91}.

These protocols are not free of drawbacks, and often, there is an essential generation of toxic by-products can be released to the environment. Therefore, alternative approaches for generation of nanomaterials are needed, and that is how green nanotechnology appeared.

Green nanotechnology is based on the application of the green chemistry principles to the generation of nanomaterials in a safe, environmentally friendly, cost-effective. Through the use of living organisms, such as bacteria or fungi, biomolecules coming from different natural sources, like polysaccharides or proteins, and waste material, green nanotechnology offers the possibility to overcome main disadvantages of traditional synthesis, with a low impact in the environment, the economy, and society in general\textsuperscript{92,93}.

Numerous applications exploit biosynthesis over chemical or physical nanoparticles syntheses, including lower capital and operating costs. Also, they showed superior biocompatibility and stability of the nanomaterials, mainly due to the application of biosurfactants or capping agents on their surfaces. Size, morphology, and properties of nanoparticles can be controlled by changing the temperature, pH, reaction time, metal ion concentration and amount of organic matter present in the reaction\textsuperscript{93,94}.

1.5. Tellurium-based green nanotechnology
Tellurium (Te) is a metalloid element whose name is originated from “tellus” meaning earth, whose properties are in between metals and non-metals. It is a greyish white substance in appearance, with metallic brightness, that can conduct electricity like a metal. Chemically it behaves as a non-metal, with intermediate ionization energy. Its oxides are amphoteric - able to react both as a base and as an acid. The unique features of tellurium are essential to develop a multitude of applications in fields of metallurgy, semiconductors, biological, and other developing technologies. Currently, this rare metal is mostly involved in metallurgy, to produce useful alloysand in solar cells to raise photovoltaic efficiency. Some biological applications are investigated, but the study are not extensive.

However, everything changes when going to the nanoscale. The synthesis and features' controls of Te nanomaterials have been extensively reported over the years, establishing protocols for quick synthesis of nanomaterials with a broad range of applications. Tellurium ions are easily reduced elemental tellurium structures showing different sizes, shapes, and dimensionality depending on the synthetic routes. The reducing agent, as well as the tellurium precursor, strongly influence the nucleation and next nanoparticle generation.

Many microorganisms have shown the ability to reduce metallic ions to elemental nanoparticles as part of their natural detoxification processes. For instance, Zonaro et al. reported the use of the tellurite-reducing bacterial strain Ochrobactrum sp. MPV1 isolated from polluted sites. Fungi are another kind of microorganisms that show resistance to tellurium, whose ability is used by researchers to develop alternative synthetic methods. For instance, Abo Elsoud et al. used six fungal isolates to reduce potassium tellurite ($K_2TeO_3$) into elemental tellurium nanoparticles with potential biomedical applications. Hydrothermal methods are also considered green pathways for the generation of nanoparticles, based on a clean and water-based reaction. In this line of research, Medina Cruz et al. developed an environmentally-friendly approach for coating nanocolumnar titanium with Te nanorods with enhanced antibacterial properties against Gram-positive and harmful bacteria.
Despite the diversity and reactivity of tellurium compounds, the rare metalloid is having trouble finding its position as an essential biological agent. Living organisms and biomolecules try to avoid the presence of the metalloid in their mechanisms and actions with feasibility. Therefore, a few examples of the interaction of the element and a range of organisms can be found in nature.

A critical remark should be stated from the beginning: tellurium and its compounds are known to be rather toxic. This is one of the reasons why tellurium has not been considered in drug development is related to its early association with toxic and otherwise undesired effects on humans. Indeed, it has been known since the 19th century that humans and animals who ingest tellurium compounds, such as TeO$_2$ or tellurite, breathe out a "disagreeable garlic-like odor." More severe clinical manifestations may appear including a metallic taste, nausea, and vomiting $^{101}$. Nevertheless, there is no generalized toxicity of tellurium. The toxic effects associated with specific tellurium compounds depend on the chemical form in which the metalloid is present. For instance, inorganic and organic tellurium compounds do not behave the same inside the body. Besides, the oxidation state has a strong dependence on its biochemistry.

Therefore, the primary goal of this thesis research is to develop a study and show the abilities of nanosized tellurium materials as essential agents for biomedical applications. Two approaches are developed for the green synthesis of tellurium nanomaterials with two particular morphologies – nanowires and nanoparticles- that were able to show antibacterial, anticancer and redox activities by themselves. We further explored the potential of tellurium nanowires as templates for the in-situ generation of noble palladium and platinum nanoparticles, generating a synergetic structure that was able to enhance the biomedical applications of the template alone further. Therefore, we demonstrated that both hydrothermal and plant-based reactions are suitable examples for the generation of tellurium nanomaterials with potential as biomedical agent adding our contribution to the successful clarification of the biological role of tellurium.
2. FIRST AIM: PREPARATION OF PLATINUM AND PALLADIUM NANO Particles
BY TELLURIUM NANOWIRES AND DETECTION OF A SERIES OF FUNCTIONS
SUCH AS ANTIBACTERIAL AND ANTICANCER

2.1. Objective

Tellurium (Te) is a metalloid element whose properties are in between those of metals and non-
metals. It is a greyish white substance in appearance, with metallic brightness, that can conduct
electricity like a metal. Despite the diversity and reactivity of tellurium compounds, the rare
metalloid has trouble finding its position as an essential agent in biology, which is the reason
tellurium nanomaterials have been poorly studied as biomedical agents. 102,103

Noble nanoparticles made of gold (Au), silver (Ag), palladium (Pd), and platinum (Pt) have been
widely used in the literature for biomedical applications because of their antibacterial, anticancer,
and antioxidant properties.104,105 Although Au- and AgNPs have been studied for a long time, Pd-
and PtNPs have not received the same level of attention. The application of these metallic
nanomaterials in biomedicine is still debated because of their unclear toxicological
characterization.106,107 Pd- and PtNP cytotoxicity are still being studied. Their possible harmful
mechanisms are not entirely understood, and exposure to them has been reported to induce
different toxic effects on various cellular models. However, results from available studies
demonstrated the potential for these chemicals to affect the ecosystem function, exert cytotoxic
and pro-inflammatory effects in vitro, as well as induce early alterations in different target organs
in vivo models.106,108 However, there is a strong need for future studies aimed at clarifying the role
of the NP physicochemical properties in determining their toxicological behavior as well as
investigations focused on environmental and biological monitoring to verify and validate
experimental biomarkers of exposure and early effect in real exposure contexts, elucidating the
role of these noble nanomaterials within biological interactions.

In this study, green-synthesized TeNWs are generated using purified starch as a unique reducing
agent. The purified starch is used as a template for the growth of Pd- and PtNPs on top in a reaction
that took place in seconds with no need for temperature or stirring with an additional reducing
agent. The novel heterogeneous structure is extensively characterized in terms of morphology, composition, and surface chemistry using TEM, SEM, EDX, XPS, XRD, and FTIR techniques. Pd- and Pt-TeNWs were tested for their potential biomedical applications as antibacterial, anticancer, and antioxidant agents with low cytotoxicity for healthy human cells. The synergetic structure showed an enhancement in their biomedical applications compared to only TeNWs, demonstrating that the combination of the metal nanoparticles and the metalloid nanowires can be used as a therapeutic agent.

2.2. Materials and Methods

2.2.1. Instruments and characterization

A thorough morphological characterization of the synergetic structures was accomplished using transmission electron microscopy (TEM) (JEM-1010 TEM (JEOL USA Inc., MA). The nanoparticles were dried on 300-mesh copper-coated carbon grids (Electron Microscopy Sciences, Hatfield, PA) to prepare the samples for imaging. Additionally, an FEI Verios 460 field-emission microscope (FE-SEM) (FEI Europe B.V., Eindhoven, Netherlands) with selective secondary/backscattered electron detection was used for morphological characterization. The subsequent observation was done using 7 μL of a solution of Pt- and PdNP-TeNWs in distilled water that was deposited on clean Si substrates and allowed to dry for more than 24 h. The images were taken with 2 kV acceleration voltage and a 25 pA electron beam current. Energy-dispersive X-Ray spectroscopy (EDX) was performed using an EDX detector (EDAX Octane Plus, Ametek B.V., Tilburg, Netherlands) coupled with the SEM previously mentioned to verify the presence of elemental tellurium in the structures. SEM conditions for EDX measurements were 10 kV acceleration voltage and 400 pA beam current.

The structural analysis of the nanostructures was carried out by infrared spectroscopy using a Fourier transform infrared spectrometer (Perkin Elmer 400 FT-IR/FT-NIR) in attenuated total reflectance (ATR) mode. The samples for FT-IR analysis were prepared by drop casting the nanostructure colloids on a sample holder heated at 50 ºC. The IR spectra were scanned in the range of 500–4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$. The spectra were normalized and the baseline corrected using Spectrum software from Perkin-Elmer.
Crystal structure characterization of the synthesized TeNWs-based nanomaterials was carried out by powder XRD. The X-ray diffraction patterns were obtained with a Rigaku MiniFlex 600 operating with a voltage of 40 kV, a current of 15 mA, and Cu-Kα radiation (λ = 1.542 Å). All XRD patterns were recorded at room temperature with a step width of 0.05 (2θ) and a scan speed of 0.2°/min. The samples for the XRD analyses were prepared by drop-casting 8 mL of the colloids onto the sample holder.

In the X-ray photoelectron spectroscopy (XPS), drops of both compounds dispersed in water were deposited on clean copper substrates for sample preparation. After water evaporation, the samples were loaded in a vacuum load lock chamber and then transferred to the XPS ultrahigh vacuum chamber with a base pressure of $1 \times 10^{-10}$ mbar. The XPS chamber is equipped with a hemispherical electron energy analyzer (SPECS Phoibos 100 spectrometer) and an AlKα (1486.29 eV) X-ray source. The angle between the hemispherical analyzer and the plane of the surface was kept at 60°. Broad scan spectra were recorded using an energy step of 0.5 eV and a pass-energy of 40 eV, whereas specific core levels spectra (Te 3d, Pd 3d, Pt 4f, O 1s, and C 1s) were recorded using an energy step of 0.1 eV and a pass-energy of 20 eV. Data processing was performed with CasaXPS software (Casa software Ltd, Cheshire, UK). The absolute binding energies (BE) of the photoelectron spectra were determined by reference to the Pt 4f$_{7/2}$ at 71.2 eV in one of the samples and Pd 3d$_{5/2}$ at 335.1 eV in the other. The contributions of the AlKα satellite lines were subtracted, and the spectra were normalized to the maximum intensity.

A SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA) was used to measure the optical density (OD) of the bacterial cultures. Growth curves and other bacterial analysis were performed in a plate reader SpectraMax Paradigm multi-mode detection platform.

For cell fixation studies, a Cressington 208HR high-resolution sputter coater and a Samdri PVT-3D critical point dryer was used to prepare the samples, which were imaged using a Hitachi S-4800 SEM instrument was used with a 3 kV accelerating voltage and 10 µA of current.
2.2.2. Synthesis of the synergetic structures Pt- and PdNPs-TeNWs

The tellurium nanowire (TeNWs) template was synthesized following the protocol described by our group and used as unique reducing agent for nanoparticle production. Briefly, a solution 1:1:15 by volume of newly synthesized TeNWs, 5 mg/mL metallic salt precursor, and distilled water was prepared. The metallic precursors employed were palladium chloride (PdCl$_2$) (Sigma Aldrich, St. Louis, MO) and potassium tetrachloroplatinate (K$_2$PtCl$_4$) (Sigma Aldrich, St. Louis, MO) for the preparation of palladium and platinum nanoparticles, respectively. The mixture was then allowed to react for 1 min with no heat or agitation. The volume was transferred and centrifuged and washed twice with distilled water at 10,000 rpm for 20 min. A final pellet was collected from the bottom of the centrifuge tube and suspended in distilled water. The solution was lyophilized overnight, resulting in a black powder that was resuspended in the desired amount of water for further characterization and experiments.

2.2.3. Stability analysis

TEM and zeta-potential measurements were completed in fresh and 120-day-old PdNPs- and PtNPs-TeNWs to measure their stability.

2.2.4. Preparation of the bacterial cultures

Two bacterial strains that are resistant to antibiotics were employed for the study: multidrug-resistant Escherichia coli (MDR E. coli) (ATCC BAA-2471; ATCC, Manassas, VA) and methicillin-resistant Staphylococcus aureus (MRSA) (ATCC 4330; ATCC, Manassas, VA). These strains were selected for the antimicrobial tests to determine the effect of both Pt- and PdTeNWs on the population’s growth. The cultures were maintained on agar plates at 4 °C. Bacteria were inoculated into 5 mL of sterile tryptic soy broth (TSB, Sigma) in a 50 mL Falcon conical centrifuge tube and incubated at 37 °C/200 rpm for 24 h. The optical density was measured at 600 nm (OD 600) using a spectrophotometer. The overnight suspension was diluted to a final bacterial concentration of $1 \times 10^6$ colony forming units per milliliter (CFU mL$^{-1}$) prior to measuring the optical density.
2.2.5. Testing the antimicrobial effect of the nanostructures

A colony of each bacterial strain was re-suspended in TSB media and then placed in a shaking incubator to grow overnight remaining at constant 200 rpm and 37 °C. The overnight suspension was diluted to a bacterial concentration of $1 \times 10^6$ colony forming units per milliliter (CFU mL$^{-1}$), and optical density measurements at 600 nm (OD 600) were performed using a spectrophotometer. Furthermore, the seeding density was determined in each experiment using a colony forming unit assay. Different concentrations of both synergetic structures—Pd- and PtNP-TeNWs—were mixed with 100 µL of the different bacteria in TSB medium and added to each well of a 96-well plate for the specific antimicrobial assay (Thermo Fisher Scientific, Waltham, MA). As a control group, the bacteria were mixed with 100 µL of TSB culture media in the absence of any nanosystem, reaching a final volume of 200 µL per well. After the plate was prepared, the absorbance values of all samples were measured at 600 nm every 2 min on the absorbance plate reader for 24 h. The absorbance values related to the synergetic nanostructures were measured by preparing negative controls made by mixing of TSB medium and nanostructures only. For the conversion of OD to CFU/mL, standard curves were used for each one of the bacteria.

The bacterial growth curves were obtained and fitted into the Gompertz model$^{110}$ by subtracting the initial values to the entire curve and shifting them to the starting point. For the application of Gompertz distribution, re-parametrization was needed in order to describe the biological parameters ($A$, $\mu$, and $\lambda$) (Eq. 2) into mathematical ones ($a$, $b$, $c$...) (Eq. 1). The estimation of initial values in addition to the 95% confidence intervals were difficult to calculate, as it is not directly estimated into the equation.

$$y = Ae^{-e^{(b-ct)}}$$

Equation 1. Gompertz equation in terms of geometrical parameters.

The Gompertz equation in terms of mathematical parameters was modified through a series of derivations to obtain the modified equation that was used for the fitting of the curves. The resulting equation describes a sigmoidal growth curve.

$$y = Ae^{-e^{{\mu \lambda (\lambda-\mu) + 2}}}$$

Equation 2. Gompertz equation in terms of biological parameters.
The parameter $y$ is related to the number of bacteria (corresponding to the optical density reading), $A$ is the maximal possible value of $y$, $\mu$ is the maximal growth rate, and $\lambda$ is the lag time. The parameters $A$, $\mu$, and $\lambda$ were estimated according to a least-squares estimation algorithm using a GRG nonlinear solver.

The colony counting assays were done by seeding the bacteria in a 96-well plated and adding different concentrations of the synergetic structure. The plates were incubated at 37 °C for 8 h and were then removed from the incubator and diluted with PBS in a series of vials until a concentration diluted $1 \times 10^5$ and $1 \times 10^6$ times. Three drops of 10 µL were taken of each dilution and deposited in a TSB-agar plate. After a final period of incubation of between 8 and 10 h at 37 °C, the numbers of colonies formed were counted at the end of the incubation.

2.2.6. Testing the effect of the nanomaterial towards human cells

Cytotoxicity assays were performed with primary human dermal fibroblasts (TCC PCS-201-012TM, Manassas, VA) and melanoma (ATCC CRL-1619, Manassas, VA) cells. Cells were cultured in Dulbecco’s modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA), supplemented with 10% fetal bovine serum (FBS; ATCC 30-2020, American Type Culture Collection, Manassas, VA) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA). MTS assays (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI) were carried out to assess cytotoxicity. Cells were seeded onto tissue-culture-treated 96-well plates (Thermo Fisher Scientific, Waltham, MA) at a final concentration of 5,000 cells per well in 100 µL of cell medium. After an incubation period of 24 h at 37°C in a humidified incubator with 5% carbon dioxide (CO$_2$), the culture medium was replaced with 100 µL of fresh cell medium containing concentrations from 5 to 100 µg/mL of both synergetic nanostructures.

Cells were cultured for different periods of time in order to evaluate the performance and interaction of the nanoparticles with the cells. Biocompatibility assays were done during 1, 3, and 5 days at the same conditions and then washed with PBS. Afterward, the medium was replaced with 100 µL of the MTS solution (prepared using a mixing ratio of 1:5 of MTS:medium). Subsequently, the 96-well plate was incubated for 4 h in the incubator to allow for a color change in the MTS solution. Finally, the absorbance was measured at 490 nm on an absorbance plate...
reader (SpectraMAX M3, Molecular Devices) for cell viability after exposure to the NP-NW concentration. Cell viability was calculated by dividing the average absorbance obtained for each sample by the one achieved by the control sample and then multiplied by 100. Controls containing cells and media, and just media, were also included in the 96-well plate to identify the average growth of cells without nanoparticles and to determine the absorbance of the media itself.

2.2.7. Cell fixation and SEM imaging (for bacteria and human cells)

For the fixation of bacterial cells, both bacterial strains (*MDR E. coli* and *MRSA*) were inoculated into 5 mL of sterile TSB media in a 50 mL Falcon conical centrifuge tube and incubated at 37°C/200 rpm for 24 h. The optical density was then measured at 600 nm (OD600) using a spectrophotometer. The overnight suspension was diluted to a final bacterial concentration of $10^6$ colony forming units per milliliter (CFU/mL) prior to measuring the optical density. A selected 75 µg/mL concentration of PdNPs- and PtNPs-TeNWs was mixed with TSB media and bacterial solution in a 6-well plate with a glass coverslip attached to the bottom. The coverslips were pre-treated with poly-lysine to enhance cell adhesion right before the experiment. The plate was placed inside an incubator for 8 h at 37 °C.

For the fixation of primary human dermal fibroblasts and melanoma, the cells were seeded in a six-well plate with a glass coverslip (Fisher Brand) attached to the bottom. After an incubation period of 24 h at 37°C in a humidified incubator with 5% carbon dioxide (CO$_2$), media was removed and replaced with a fresh one containing a concentration of 50 µg/mL of the different synergies. Cells were cultured for another 24 hours at the same conditions.

After the experiments, the coverslips were fixed with a primary fixative solution containing 2.5% glutaraldehyde and 0.1 M sodium cacodylate buffer solution for 1 h. Subsequently, the fixative solution was exchanged for 0.1 M sodium cacodylate buffer, and the coverslips were washed 3 times for 10 min. Post-fixation was done using 1% osmium tetroxide (OsO$_4$) solution in the buffer for 1 h. Afterward, the coverslips were washed three times with buffer and dehydration was progressively achieved with 35, 50, 70, 80, 95, and 100% ethanol (three times for the 100% ethanol). Finally, the coverslips were dried by liquid CO$_2$–ethanol exchange in a Samdri PVT-3D critical point dryer. The coverslips were mounted on SEM stubs with carbon adhesive tabs.
(Electron Microscopy Sciences, EMS) after treatment with liquid graphite and then sputter-coated with a thin layer of platinum using a Cressington 208HR high-resolution sputter coater. Digital images of the treated and untreated bacteria were acquired using SEM.

2.2.8. **ROS analysis**

To assess intracellular reactive oxygen species (ROS) in melanoma cells, we used the ROS detection assay kit with 2′,7′-dichlorodihydrofluorescein diacetate (ab113851, DCFDA Cellular ROS Detection Assay Kit, Abcam). DCFDA is a fluorescent probe that can detect reactive oxygen species such as hydrogen peroxide, peroxyl radical, and peroxynitrite. Briefly, melanoma cells were seeded into 96-well plates at a cell density of 2.5 × 10⁴ cells per well in 100 μL working media (DMEM media with 10% FBS and % P/S (penicillin-streptomycin)). After 24 h of incubation at 37 °C in a 5% CO₂-humidified atmosphere, ROS was detected at 485 nm excitation and 535 nm emission wavelengths. Four hours prior to completion of treatment, TBHP (tert-butyl hydrogen peroxide) solution was added to 3 wells to the final concentration of 500 μM as a positive control. Forty-five minutes before completion of the incubation, 100 μL of 50 μM DCFDA in working media was added to each well to make the final concentration of 25 μM DCFDA. End-point fluorescence from triplicate wells for each experimental condition was measured in a fluorescence microplate reader at 485 nm excitation and 535 nm emission wavelengths.

2.2.9. **Statistical analysis**

All experiments were repeated in triplicate (N = 3) to ensure the reliability of results. Statistical significance was assessed using Student's t-tests, with a p < 0.05 being statistically significant. Results are displayed as mean ± standard deviation.

2.3. **Results and Discussion**
2.3.1. Synthesis of the synergetic nanostructures Pt- and PdTeNWs

The ability of green-synthesized TeNWs as a template for the reduction of Pt and Pd ions to their elemental form was successfully accomplished upon addition of the metallic salt into a mixture of previously synthesized TeNWs and water. The reaction was conducted at room temperature, with no stirring and no need for additional reducing or capping agent. In a standard reaction that will lead to the production of nanoparticles, both reducing and capping agents are needed to reduce the ions to elemental valence state and bond to the nanostructure and avoid aggregation of the nanoparticles, respectively. These agents, whose precedence can be either chemical or natural, should be added to the reaction, a process that was avoided here.

The role of starch in the reduction and capping of the nanoparticles is clear because characterization through TEM before and after the reaction reveals the disappearance of the starch coating surrounding the nanowires. The hydroxyl groups of starch possibly facilitated palladium and platinum ions in solution to be reduced on the surface of the nanowires by electrostatic binding in the helical structure of amylose chains. As a consequence, a part of starch granules is converted to glucose that has an aldehyde group. The aldehyde functional terminal allows glucose to act as the reducing sugar, which can reduce the ions and trigger the generation of small nuclei all over the nanowires that will give rise to the nanoparticles. Thus, we hypothesized that both Pd- and PtNPs could be formed by simultaneous in situ reductions without the addition of any other reducing agent.

Besides, the tellurium itself might be involved in the reduction. However, no reports of this behavior are found in the literature; this is the reason why a clear hypothesis cannot be stated.

2.3.2. TEM characterization of the samples

The synergetic structures were characterized using transmission electron microscopy right after the samples were purified. As can be seen in Figure 3, the TeNWs served as a template for the growth of Pd- and PtNPs. The structures grew all over the length of the nanowires, developing a narrow size distribution and definite shape; PdNPs showed a square shape and an average size of 80 ± 26 nm, spherical PtNPs showed an average size of 68 ± 29 nm. As can be seen, the
nanoparticles grow on the surface of the nanowires. When they reach a large enough size, some of them are released, whereas others remain attached to the structure.

Further characterization was conducted to observe the strength of the bonding between the nanoparticles and nanowires. Upon sonication, release of the structures was observed (Figure 3). The nanoparticles remained monodispersed in solution with a low degree of aggregation, remaining stable.

Figure 3. Transmission electron microscopy (TEM) characterization of (A) PdNPs- and (B) PtNPs- TeNWs. Different morphologies and features were found when the nanomaterials were characterized by TEM.

2.3.3. SEM characterization of the samples

SEM characterization over Pd-TeNWs (Figure 4A) showed a similar cubic-shaped structure, whereas for the Pt-TeNWs (Figure 4B), spherical-shaped structures were observed along the surface of the elongated nanowires.
Figure 4 Scanning electron microscopy (SEM) characterization of (A) PdNPs- and (B) PtNPs-TeNWs.

2.3.4. EDX characterization of the samples

EDX analysis was performed on different points on the sample to appreciate the composition of the synergy. Figure 5 shows the presence of Pt nanoparticles all over the surface of the nanowire. Although spot 1 focuses on the metallic nanoparticle structure, spot 2 is centered on the nanowire structure; therefore, the composition between them mismatch. In general, it can be said that high quantities of platinum and tellurium come from the nano-synergetic structure. Moreover, carbon and oxygen peaks can be associated with residual starch—present on the initial TeNWs—covering the surface of the structure.
Figure 5. EDX characterization of PtNPs-TeNWs on different spots. Spot 1 (left) focused on the Pt structure, and Spot 2 (right) focused on the TeNW structure.

Similarly, Figure 6 shows Pd nanoparticles growing on the top of the nanowire. Again, tellurium and palladium peaks confirm the synergetic structure. Nonetheless, even though different spots of the sample were analyzed, a higher amount of Pd was found all over the sample. It has been hypothesized that because of the lower power of reduction of Pd in comparison with Pt, more
nanoparticles were formed. Hence, less carbon and oxygen, previously related to the starch-coating, was found on the solution, as it was used in the reduction process.

**PdNPs-TeNWs**

**Figure 6. EDX characterization of PdNPs-TeNWs on different spots.** Spot 1 (left) focused on the TeNW structure, and spot 2 (right) focused on the Pd structure.

In addition, and further discussed in XPS and XRD analysis, the high amount of oxygen present on the sample can be related to the partial incorporation of it into the metallic structure making PdO and PtO nanoparticles instead of Pd and Pt nanoparticles.
Other elements present on the sample such as silicon come from the support used for the imaging. Other peaks such as sodium or chloride one might come from residual salts in water.

2.3.5. FTIR characterization of the samples

The FT-IR spectra of samples PdNPs-TeNWs and PtNPs-TeNWs and potato starch are depicted in Figure 7. In each TeNWs-based sample, the weak and broad vibrational band at around 3300 cm\(^{-1}\) and the medium vibrational signals found in the region between 800 and 1150 cm\(^{-1}\) were assigned to the O–H stretching and glycosidic linkage, respectively.\(^{116,117}\) The origin of these signals could be related to the starch-functionalized Te-NWs. In the case of PdNPs-TeNWs, the strong peak at 534 cm\(^{-1}\) was related to the Pd–O bond,\(^{118}\) whereas in PtNPs-TeNWs, the strong peak at 545 cm\(^{-1}\) was assigned to the vibration of the Pt–O bond.\(^{119}\) As commented beforehand, these findings suggest the synthesis of oxide nanoparticles of both metallic elements, platinum oxide, and palladium oxide nanoparticles.

![FT-IR spectra](image)

*Figure 7. FT-IR spectra of a) PtNPs-TeNWs, b) PdNPs-TeNWs and c) potato starch.*

2.3.6. XRD characterization analysis
In Figure 8, the experimental XRD patterns of the PtNP-TeNW and PdNP-TeNW samples were compared with the calculated XRD patterns of cubic PdO with NaCl-type structure\textsuperscript{120} and NaCl (space group $Fm\bar{3}m$).\textsuperscript{121} Both experimental diffraction patterns may be principally indexed to their corresponding metal oxides, i.e., cubic PtO and PdO with NaCl-type structures for PtNP-TeNWs and PdNP-TeNWs, respectively. The lattice parameter ($a$) calculated for the cubic TeNW-based Pt nanostructures was $a = 5.665 \pm 0.007$ Å, which is in agreement with the reported values for a cubic PtO NaCl-type ($a = 5.65 \pm 0.05$ Å). In the case of the sample PdNP-TeNWs, the calculated lattice parameter was $a = 5.613 \pm 0.015$ Å, which deviates significantly (around 9%) from the reported values for a cubic PdO NaCl-type ($a = 5.15 \pm 0.05$ Å).\textsuperscript{120} Interestingly, in both experimental diffraction patterns (Figure 6), the relatively low-intensity diffraction peak at $2\Theta = 28.21^\circ$ may be indexed to the (011) plane of elemental hexagonal Te in sample.\textsuperscript{122} Moreover, the presence of further low-intensity peaks in both experimental XRD patterns may suggest the minor presence of crystalline phases related to bimetallic Pt/Te\textsuperscript{123} and Pd/Te structures,\textsuperscript{124} for PtNPs-TeNWs and PdNPs-TeNWs, respectively.
2.3.7. XPS characterization analysis

XPS analysis has been used to characterize the chemical composition and electronic states of synergetic structures for both PtNPs- and PdNPs-TeNWs. Figure 9 displays the full scans that have been normalized arbitrarily to the C 1s core level peak for the sake of comparison. Moreover, Table 1 presents the composition of the samples extracted from the analysis of the wide energy range scans. In the two samples O, C, N, and Te were detectable. The amount of oxygen in the PdNPs-TeNWs was 1.5 times higher than in the PtNPs-TeNWs. In addition, the amount of Pd detected was approximately 5 times higher than the Pt in the other sample. These results were found in concordance with the information extracted from EDX and XRD analysis, suggesting that although the same concentration was used in both processes, the lower power of reduction for Pd allowed for the generation of a higher number of nanoparticles. Moreover, the more significant presence of Pd surrounding the Te rods could be the reason for the smaller XPS signal of Te in this sample (nearly half of the PtTe sample). Note that the PdTe sample presents 3% of chlorine, probably arising from the aqueous solvent. In addition, Cu signals from the substrate were detected, but not considered in the quantification.
Figure 9. XPS scans recorded on both PtNPs-TeNWs and PdNPs-TeNWs compounds.

Table 1. The composition of the samples extracted from the full energy range scans.

<table>
<thead>
<tr>
<th>Composition % at</th>
<th>O</th>
<th>C</th>
<th>N</th>
<th>Te</th>
<th>Pd</th>
<th>Pt</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNP-TeNWs</td>
<td>48.5</td>
<td>31.8</td>
<td>1.6</td>
<td>2.7</td>
<td>12.4</td>
<td>-</td>
<td>3.0</td>
</tr>
<tr>
<td>PtNP-TeNWs</td>
<td>31.0</td>
<td>59.7</td>
<td>2.2</td>
<td>4.7</td>
<td>-</td>
<td>2.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Detailed analysis of the core level peaks enables the observation of individual differences between the samples. Figure 10 (left) presents the Te 3d core level peak of the samples. Three components were found to be present. Referencing the values to the Te 3d5/2, the first component at 573 eV corresponds to Te⁰. The second component at 575.7 eV corresponds to the oxidized component. The same oxide structure was previously registered by the group for the TeNWs structure. A third and final component appeared at a binding energy (BE) of 578.0 eV. This energy,
which is too high for standard Te compounds, was associated with Te(OH)$_6$ because of its tabulated BE at 577.1 eV. It has been hypothesized that this structure could come from the further reduction of the tellurium from the nanowires due to the presence of the coating starch acting as a reducing agent. From these analysis, it could be observed that the PdNPs-TeNWs presented a more significant proportion of non-oxidized Te, whereas the PtNPs-TeNWs sample presents more Te(OH)$_6$. As said previously, the higher power of reduction of Pt in comparison to Pd allows the starch to further reduce tellurium into the hydroxide structure instead of reducing the Pt.

Additionally, the component on the PdNP-TeNW sample at 570.2 eV was related to the Cu$_{LMM}$ auger peak from the substrate. The summary of the proportion of each component can be found in Table 2. The analysis of the Pd 3d core level presented two components: one at 335 eV that corresponds to the metallic component in the Pd 3d$_{5/2}$, whereas the other component at 336.8 eV arises from the oxide. From this analysis, it is clear that most of the Pd is oxidized (68%), showing the formation of palladium oxide nanoparticles. On the contrary, the analysis of the Pt 4f core level of the PtTe sample showed that all the Pt was present only in metallic form, as reflects the component at 71 eV of the Pt 4f$_{7/2}$. A small component of oxide was found on the sample but without enough contribution to be considered. One explanation could be that Pt was found in lower proportion than Pd in the top of the nanowires, and hence if the structure is covered with an organic coating (starch), it is possible that the oxide compounds are not adequately described with this characterization technique.

In addition, the two small components at 76 and 78 eV corresponded to Cu 3p.
Figure 10. XPS core level spectra of Te 3d (left), Pd 3d (center) and Pt 4f (right) samples.

Figure 11. XPS core level spectra of C 1s (left) and O 1s (right) samples.
Table 2. Components extracted from the analysis of the Te 3d, Pd 3d, O 1s, and C 1s core level peaks of the PdTe and PtTe samples.

<table>
<thead>
<tr>
<th></th>
<th>Te⁰</th>
<th>TeOx</th>
<th>TeOH</th>
<th>Pd⁰</th>
<th>PdOx</th>
<th>O1</th>
<th>O2</th>
<th>C-C/ C-H</th>
<th>C-O</th>
<th>C=O</th>
<th>O-C=O</th>
<th>sp2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNP-TeNWs</td>
<td>15.5</td>
<td>43.4</td>
<td>41.1</td>
<td>32.1</td>
<td>67.9</td>
<td>39.3</td>
<td>60.7</td>
<td>32.3</td>
<td>22.9</td>
<td>12.2</td>
<td>5.4</td>
<td>27.1</td>
</tr>
<tr>
<td>PtNP-TeNWs</td>
<td>6.8</td>
<td>64.9</td>
<td>28.3</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>51.9</td>
<td>25.6</td>
<td>12.0</td>
<td>8.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

The analysis of the C 1s core level spectra of the samples revealed specific differences between the samples. Five components were used for the fitting. All of them were forced to have the same full width at half maximum (FWHM). These components correspond to C–C/C–H bonds at 285±0.2 eV; C–O/C–N bonds at 286.3 ± 0.2 eV; C=O bonds at 288.1 ± 0.2 eV and O–C=O bonds at 289 ± 0.2 eV. These four components are typical from organic compounds. The fifth component that appeared at a lower BE (284 ± 0.2 eV) is attributed to carbon in the sp² configuration. The relative intensity of each component varies depending on the sample. Table 2 displays the proportion of each component. The main difference between both samples relies on the abundance of the sp² component, which is much higher in the PdNP-TeNW sample. The proportion of the oxidized compounds is similar in both samples. Mainly, the organic structure was related to the presence of starch coating both synergetic structures, also found previously in the nanowire structure.

The O 1s core level of the samples presents significant differences related to the oxide and hydroxide structures discussed beforehand. In the case of the PdNPs-TeNWs sample, two different contributions can be observed, one at 533.6 eV and another at 530.6 eV. The first component could be attributed to the organic compounds, whereas the second at lower BE is related to the presence of palladium and tellurium oxides in the sample (at 530 and 530.8 eV, respectively), previously observed in the analysis of the Te 3d and Pd 3d core levels. The third component included in the fitting at 530.6 eV corresponds to the Pd 3d that overlaps in this BE range. On the other hand, for PtNPs-TeNWs, the oxygen peak can be fitted with a single component at 531.4 eV, intermediate energy that can include the oxidized organic compounds (usually around 532.3 eV) and the Te oxide (usually at 530.8 eV).
As the characterization shows, the nanoparticles are made of palladium and platinum oxide. In a standard procedure for generation of these nanostructures, strong reducing agents, such as NaBH₄, sodium hydroxide (NaOH), sodium benzoate, or trioctylphosphine, and additional stabilizing agents, such as polynynylpolirridone (PVP) are needed. Besides, reaction conditions that are far away from standards of temperature and pressure are also needed, such as calcination at 500 °C under synthetic air conditions, reflux conditions combined with microwave, or an argon atmosphere set up. In contrast, we present here a natural reaction at atmospheric pressure and room temperature, with no need for stirring or addition of reducing/capping agents using water as a single solvent.

2.3.8. Stability analysis

To verify the stability of the nanostructures, we carried out TEM imaging on the samples after 120 days of synthesis (Figure 12). It is evident that the samples kept their original morphologies and features. For instance, the 120-day-old PtNPs-TeNWs sample is composed of partial agglomerated thin nanowires (50–200 nm length and 2–15 nm full), as seen in Figures 3 and 4B. Moreover, PdNPs-TeNWs showed a slight change in morphology, as the particular cubic form was finally turned into a more spherical form.
Stability analysis of the freshly synthesized and 120-day-old Te-based nanomaterials was also carried out through measurement of the Z-potential. In general, a colloid or suspension is considered stable if the Z-potential is above a critical value of ±30 mV. Given the measured Z-potential values for the colloids (fresh and 120-day-old samples, see Table 3), they can be considered stable.

Table 3. Zeta-potential values for fresh and 120-day old Pd and PtNPs-TeNWs. The pH of the colloids was 7.0±0.2.

<table>
<thead>
<tr>
<th>Nanostructure</th>
<th>As-synthesized</th>
<th>120 days old</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNPs-TeNWs</td>
<td>-27.21 ± 1.81</td>
<td>-25.55 ± 1.49</td>
</tr>
<tr>
<td>PtNPs-TeNWs</td>
<td>-28.02 ± 2.02</td>
<td>-25.09 ± 1.33</td>
</tr>
</tbody>
</table>

2.3.9. Testing the antimicrobial effect of the nanostructures

The antibacterial effect of the PdNPs- and PtNPs-TeNWs structures was first studied using 24 h-growth curve analysis to observe the potential changes in the bacterial growth when cultured with different concentrations of the nanomaterials.
Figure 13. Effect of PdNPs – TeNWs on MDR E. coli (A), PtNPs-TeNWs on MDR E. coli (B), PdNPs-TeNWs on MRSA and Ecoli (C), and PtNPs-TeNWs on MRSA (D). The growth of a 1 × 10^6 CFU ml⁻¹ suspension of S. aureus (A) and E. coli (B) for 24 hours in the presence of different concentrations of PtNPs-TeNWs and PdNPs-TeNWs. The values represent the mean ± standard deviation.

Nanoparticle concentrations between 5 and 100 µg/mL produced a delay in the growth of MDR E. coli (Figure 13A, B) for both entities, with a relative dose-dependent inhibition. On the other hand, the nanostructures seem not to have a significant effect on bacterial growth, with a slight inhibition at the end of the exponential phase.
Figure 14. Analysis of parameters A, λ and μ from the modified Gompertz equation. All parameters were analyzed in relation to the PdNPs – TeNWs and PtNPs-TeNWs concentrations. The effect of the NPs-TeNWs was analyzed, taking data from the Gompertz equation fitting of bacterial growth curves.
The parameters of the modified Gompertz equation (Eq. 3) were calculated and plotted for analysis (Figure 14) to further investigate the effects of the nanostructures on the bacterial growth. The parameter $A$, which represents the maximum bacterial growth, decreased at increasing concentrations, a behavior that was especially visible in experiments with MDR *E.coli*. On the other hand, changes in the maximum bacterial growth rate were determined by analyzing the parameter $\mu$. This analysis demonstrated that higher nanostructures concentrations resulted in an overall lower growth rate of the bacteria. The decay was especially visible in experiments with MDR *E.coli* even at the minimum concentration. Last, the parameter $\lambda$, which represents the lag time in the bacterial growth or the duration of time where bacteria are adapting themselves to the growth conditions offered by the media, was analyzed (Figure 14E,14F). This analysis showed that higher nanostructure concentrations led to a shorter lag phase in bacterial growth. This was especially visible with *E.coli*. This suggests that the presence of both PdNPs- and PtNPs-TeNWs delays bacterial maturation, therefore inhibiting bacterial growth.
Figure 15. Colony counting assay of (A) multidrug-resistant Escherichia coli treated with PdNP-TeNWs, (B) Escherichia coli treated with PtNP-TeNWs, (C) methicillin-resistant Staphylococcus aureus treated with PdNP-TeNWs, and (D) methicillin-resistant Staphylococcus aureus treated with PtNP-TeNWs. The treatment time is 8 h. N = 3. *p < 0.05 versus control, **p < 0.01 versus control.

Colony counting unit assay conducted over MDR E. coli (A, B) and MRSA (C, D) showed a dose-dependent inhibition of the bacterial growth when exposed to different concentrations of both PdNPs- and PtNPs-TeNWs. The palladium-containing nanostructures were useful toward MDR E. coli at a range of concentrations between 10 and 100 µg/mL, whereas a concentration range between 25 and 100 µg/mL was useful toward MRSA. On the other hand, PtNPs-TeNWs produced a delay in MDR E. coli proliferation in a range of concentrations between 5 and 100 µg/mL, whereas a concentration range between 10 and 100 µg/mL successfully inhibited the proliferation of MRSA. Therefore, a wide range of concentrations was shown to be effective in both antibiotic-resistant phenotypes.
MIC values were calculated as an extent of the antibacterial behavior.

Table 4. MIC values for different nanoparticles against MDR E. coli and MRSA.

<table>
<thead>
<tr>
<th>MIC values (µg/mL)</th>
<th>MDR-Escherichia coli</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNPs-TeNWs</td>
<td>24.79</td>
<td>26.45</td>
</tr>
<tr>
<td>PtNPs-TeNWs</td>
<td>15.24</td>
<td>15.86</td>
</tr>
</tbody>
</table>

These values differ from others found in the literature, showing either a decrease in or similar MIC values for both our nanosystems. For example, Tahir et al. showed that PdNPs produced by the *Sapium sebiferum*, tested against *SA*, rendered a MIC of 45.4 µg/mL,\(^{132}\) whereas Dhanavel et al. reported the MIC of chitosan-supported PdNPs toward *E.coli* with a value of 25 µg/mL.\(^{133}\) On the other hand, Khan et al. reported the synthesis of PtNPs using pectin and sodium borohydride, which were tested against *E.coli*, with a MIC around 12 ug/mL,\(^{134}\) whereas PtNPs prepared from marine actinobacteria (*Streptomyces* sp.), showed a MIC of 20 ug/mL when tested toward the activity of *SA*.\(^{135}\)

2.3.10. Testing the effect of the nanomaterials towards human cells

MTS assays were done with HDF and human melanoma cells to assess the potential cytotoxicity of the nanostructures.
Figure 16. HDF assays on human dermal fibroblasts cells in the presence of (A) PdNPs-TeNWs and (B) PtNPs-TeNWs, at concentrations ranging from 5-100 μg/mL. N=3. Data are represented as mean ± SD; *p<0.05, **p<0.01.
A dose-dependent cell proliferation decay was found when the two nanosystems were cultured with HDF cells over a period of time of 5 days. For PdNPs-TeNWs, a low cytotoxic effect was found in a range of concentrations between 5 and 25 µg/mL at 24 h, whereas the range was reduced at concentrations up to 10 µg/mL on the fifth day. When PtNP-TeNWs were present in the cell media, the optimum range of concentrations was found to be 5–15 µg/mL in experiments up to 5 days. Therefore, the PdNPs-TeNWs and PtNPs-TeNWs can be considered biocompatible in a range of concentrations up to 10 and 15 µg/mL, respectively.
Figure 17. HDF assays on Melanoma cells in the presence of PdNPs-TeNWs (A) and PtNPs-TeNWs (B), at concentrations ranging from 5-100 µg/mL. N=3. Data is represented as mean ± SD; *p<0.05, **p<0.01
Moreover, a dose-relative cell proliferation decay was found when both nanostructures were cultured with melanoma cells for a pried time of 3 days, and a dose-dependent cell proliferation decay was found when both nanostructures were cultured with melanoma cells for 5 days. For PdNPs-TeNWs, the anticancer effect towards melanoma cells was found in a range of concentrations up to 25 µg/mL at 24 h with low cytotoxic effect, whereas the range was reduced at a concentration only 15 ug/mL at the third day. When PtNPs-TeNWs were present in the cell media, the optimum range of concentrations was found to be 5–15 ug/mL in experiments up to 5 days. Thus, the PdNPs-TeNWs can be considered to have an anticancer effect at a concentration of 15 ug/mL for a 3-day treatment, and PtNPs-TeNWs can be considered as such at between 10 and 15 ug/mL for a 5-day treatment.

IC₅₀ values were calculated to further study the response of the cells to the nanostructures.

**Table 5. IC₅₀ values for different nanoparticles cultured with HDF cells.**

<table>
<thead>
<tr>
<th>IC₅₀ values (µg/mL)</th>
<th>1 day</th>
<th>3 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNPs-TeNWs</td>
<td>21.25</td>
<td>17.64</td>
<td>38.75</td>
</tr>
<tr>
<td>PtNPs-TeNWs</td>
<td>30.46</td>
<td>24.37</td>
<td>35.85</td>
</tr>
</tbody>
</table>

**Table 6. IC₅₀ values for different nanoparticles cultured with melanoma cells.**

<table>
<thead>
<tr>
<th>IC₅₀ values (µg/mL)</th>
<th>1 day</th>
<th>3 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdNPs-TeNWs</td>
<td>8.00</td>
<td>26.55</td>
<td>30.74</td>
</tr>
<tr>
<td>PtNPs-TeNWs</td>
<td>7.57</td>
<td>10.04</td>
<td>12.45</td>
</tr>
</tbody>
</table>

These values differ from others found in the literature, showing a decrease in IC₅₀ values for both of our nanosystems. For example, Sathishkumar et al. have investigated the anticancer effect of PdNPs produced by the aqueous fruit extract of *Couroupita guianensis* Aubl.¹³⁶ The nanoparticles were tested against a lung cancer cell line (A549), with an IC₅₀ value of 121 µg/mL for a 1-day treatment. Moreover, Vivek synthesized PtNPs from *Saccharomyces boulardii*, and the nanostructures were tested against epithelial carcinoma (A431) and breast cancer (MCF-7) cell
line, that showed IC₅₀ values greater than 100 and 70 µg/mL, respectively, after 24 h of treatment.¹³⁷

2.3.11. Cell fixation and SEM imaging for bacteria and human cells

Characterization indicated that treatment with the synergetic structures induced changes in both bacterial strains. Disruption of other cell membrane and cell lysis was seen after treatment with both nanostructures. As a result, clear cell damage was observed, with an abundant presence of holes and cracks all over the cell membrane along with bacterial deformation and collapse. Cell membrane damage is commonly found to be a cause of ROS. Nevertheless, other mechanisms can also be inferred, as the direct damage of the cells due to the morphology of the nanostructures. From the SEM images of the bacteria, we can see that the membrane damage occurs and that there is the attachment of nanoparticles to bacteria, but the exact mechanism of how damage occurs could not be identified.

![SEM micrographs of control MDR E.coli and MRSA (A, D) and bacteria after treatment with PdNPs- (B, E) and PtNPs- (C, F) TeNWs.](image)

Besides, as can be seen, HDF cells were able to successfully proliferate in the presence of the nanostructures, that were either deposited on top of the cell membrane or underneath the structure, with no apparent disruption or alteration of the membrane or normal growth which indicated
biocompatibility with the nanostructures. On the other hand, the presence of the nanomaterials induced a severe presence of bubbles and swelling of the membrane before final disruption within the melanoma cell population which can be related to an apoptosis mechanism of cell death. 

**Figure 19.** SEM micrographs of HDF and melanoma cells with (A, D) no nanoparticle treatment and after treatment with (B, E) PdNPs- and (C, F) PtNPs-TeNWs were obtained.

### 2.3.12. ROS study

**Figure 20.** ROS study of (A) PdNPs-TeNWs and (B) PtNPs-TeNWs analysis. **p < 0.01** versus control.
The production of ROS was evaluated in response to the exposure of two different concentrations of PdNPs- and PtNPs-TeNWs, 25 and 100 ug/mL, to explore the mechanism of NP toxicity toward human cells. As such, melanoma cells were exposed for 24 h to the concentrations of NPs and ROS was quantified in both the cell media. Both nanostructures showed a similar production of ROS, with a more significant release of species for PtNPs-TeNWs.

The contribution of ROS might be related to both tellurium and the noble metal presented on the structure, either palladium or platinum. Tellurium oxyanions have been found to trigger the generation of ROS, with the ability to react with intracellular thiols and forming intermediates that cause oxidative stress as a consequence of the formation of superoxide radicals. The contribution of palladium and platinum to the production of ROS must be significant, as there are a few reports that show this mechanism. For instance, Alarifi et al. reported that the mode of cell death for melanoma exposed to PdNPs was apoptosis, which was mediated by the ROS-triggered cleavage of caspase-3 enzyme, whereas Almeer et al. found that the generation of ROS in HEK293 cells after exposure to green PtNPs increased in a concentration- and time-dependent manner, explaining that a high quantity of free radicals can inflict direct damage to the lipids of the cells.

Because the ROS generation is similar for both structures, but different results were obtained for the cytotoxicity analysis, there must be other mechanisms responsible for the anticancer activity of these nanostructured materials. Some proposed mechanisms that will be checked in future reports are related to the nanostructures themselves, contributing to the cell damage by disrupting the integrity of the envelope, or to the surface chemistry and features of the nanostructures.

2.4. Conclusion

In this report, green synthesized starch-mediated TeNWs were successfully used as nanometric templates for the in situ generations of noble Pd- and PtNPs in a quick, environmentally friendly, and cost-effective reaction with no need for additional reducing or capping agent. The nanoparticles composed of palladium and platinum oxide were extensively characterized in terms of composition and surface chemistry. In addition, the structures were tested as biomedical agents.
PdNPs- and PtNPs-TeNWs showed antibacterial properties in a range of concentrations between 10 and 25 μg/mL, triggering no cytotoxicity toward healthy epithelial cells over the same period of time. Furthermore, both nanostructures were found to have anticancer activity toward melanoma cells in a range of concentrations between 10 and 15 μg/mL with no alteration of the usual proliferation of healthy skin cells. Therefore, we can conclude that our systems can be successfully used at low concentrations as biomedical agents with antibacterial and anticancer properties, as they are biocompatible in the same range of concentrations.
3. SECOND AIM: A NEW METHOD FOR PREPARING TELLURIUM NANOPARTICLES BY ALOE EXTRACT USING A GREEN SYNTHESIS METHOD

3.1. Introduction

Of all the green chemistry approaches, plant-based synthesis has been shown to be a quick and very reproducible group of techniques. Aloe vera has been used as a medical tool since ancient times. For instance, aloe vera gel has been used for the treatment of severe skin cuts, burn abnormalities, constipation, colic, skin diseases, and worm infestation (Rahmani et al. 2015; Foster, Hunter, and Samman 2011). The name aloe vera derives from the Arabic word “alloeh” meaning “shining bitter substance,” whereas “vera” means “true” in Latin. In addition, the term aloe refers to a solid residue obtained by evaporating the latex derived from the outer layers of the plant leaf. It has stiff, green, lance-shaped leaves. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed, a clear mucilaginous substance appears that contains fibers, water, and the ingredient to retain the water in the leaf. The gel consists of 99.3% water, with the remaining 0.7% comprising solids with a high content of glucose and mannose (Rahmani et al. 2015; Hashemi, Madani, and Abediankenari 2015; Surjushe, Vasani, and Saple 2008).

The leaves of this shrubby, perennial succulent plant of the Liliaceae family possess anti-inflammatory activity, UV protection, and antiarthritic properties; promote wound and burn healing; and have reported antibacterial properties. There are a number of biologically active constituents in aloe vera leaves, such as lignin, hemicellulose, and pectins. Nanotechnology has used them to produce nanomaterials. The mentioned compounds are directly involved in the reduction of metallic ions into elemental nanostructures. Once the ions are reduced and start to nucleate, enzymes and proteins are weakly bound to the ions and function as a complexing agent, stabilizing the nanostructures and avoiding aggregation due to surface charges. Furthermore, many other different phytoconstituents such as vitamins, minerals, sugars, anthraquinones, saponins, salicylic acid, and amino acids are found in the extracts, which may have a direct role in the nanoparticles’ formation and applications, as they are also responsible for their medicinal and therapeutic properties.
Microwave heating is a fast emerging and widely followed new processing technology for a variety of nanostructured materials with smaller size, narrower size distribution, and different shape. Microwave-assisted synthesis is accessible in areas ranging from biochemical processes to nanotechnology. Chemical reactions are often faster than traditional convection heating methods and have higher yields and fewer side products. Current microwave reactors provide excellent control over reaction mixing, withstand high temperatures and pressures, and demonstrate exceptional reproducibility from reaction-to-reaction.\textsuperscript{151–153} For nanomaterials’ production, in which the initial parameters, including temperature, heating rate, and precursor reactivity and concentration, define what the nucleation events are, and thus the products, careful attention to the choice of solvent, and reactants may yield alternate reaction pathways. In addition to heating induced by the precursors/reactants, the reaction products can provide an additional handle for tunability of reaction parameters, namely spatially selective heating.\textsuperscript{153,154}

Because of their low cost and environmentally friendly nature coupled with their reducing properties, aloe vera was selected as the reducing and stabilizing agent to prepare tellurium nanoparticles (TeNPs) through a microwave-assisted method. To the best of our knowledge, this is the first time that a plant has been used for the synthesis of TeNPs. The nanostructures were purified and extensively characterized in terms of surface chemistry, composition, and morphology and subsequently tested for their biomedical applications as antibacterial and anticancer agents with low cytotoxicity for healthy human cells.

3.2. Materials and Methods

3.2.1. Instruments and characterization

A thorough morphological characterization of the synergetic structures was accomplished using transmission electron microscopy (TEM) (JEM-1010 TEM (JEOL USA Inc., MA). To prepare the samples for imaging, the nanoparticles were dried on 300-mesh copper-coated carbon grids (Electron Microscopy Sciences, Hatfield, PA). Additionally, an FEI Verios 460 field-emission microscope (FE-SEM) (FEI Europe B.V., Eindhoven, Netherlands) using selective secondary/backscattered electrons detection was also used for morphological characterization. The subsequent observation was done using 10 µL of a 5 mM solution of AV-TeNPs in distilled water.
that was deposited on clean Si substrates and allowed to dry for more than 24 h. The images were taken with 2 kV acceleration voltage and a 25 pA electron beam current. Energy-dispersive X-Ray spectroscopy (EDX) was performed using an EDX detector EDAX Octane Plus, Ametek B.V., Tilburg, Netherlands) coupled to the SEM previously mentioned, for the verification of the presence of elemental tellurium in the structures. SEM conditions for EDX measurements were 10 kV acceleration voltage and 400 pA beam current.

The X-ray diffraction pattern was recorded using a Rigaku Miniflex 600 operating with a voltage of 40 kV, a current of 15 mA, and Cu-Kα radiation (λ = 1.542 Å). The measurement was done at room temperature with a step width of 0.005 (2θ) and a scan speed of 0.25°/min. The sample for the XRD analyses was prepared by drop-casting 5 mL of the AV-TeNP colloid onto the sample holder.

Drops of the 5 mM preparation were deposited on the clean conductive copper substrate. After water evaporation, the sample was loaded in a vacuum load-lock chamber and then transferred to the ultra-high vacuum XPS system. The XPS chamber has a base pressure of 1 × 10⁻¹⁰ mbar and is equipped with a hemispherical electron energy analyzer (SPECS Phoibos 100 spectrometer) and an AlKα (1486.29 eV) X-ray source. The angle between the hemispherical analyzer and the plane of the surface was kept at 60°. Broad scan spectrum was recorded using an energy step of 0.5 eV and a pass-energy of 40 eV, whereas specific core levels spectra (Te 3d, O 1s, and C 1s) were recorded using an energy step of 0.1 eV and a pass-energy of 20 eV. Data processing was performed with CasaXPS software (Casa software Ltd, Cheshire, UK). The absolute binding energies of the photoelectron spectra were determined by reference to the C 1s core level at 285 eV. The contributions of the AlKα satellite lines were subtracted.

Structural analysis of the AV-TeNPs was completed by infrared spectroscopy using an FT-IR spectrophotometer PerkinElmer Spectrum 400 FT-IR/FT-NIR in attenuated total reflectance (ATR) mode. For FT-IR spectroscopy analysis, 5 μg of the dried sample was used. The FT-IR spectrum was scanned in the range of 500 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. The spectrum was normalized and the baseline corrected using Spectrum software from PerkinElmer.
A SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA) was used to measure the optical density (OD) of the bacterial cultures. Growth curves and other bacterial analysis were performed in a plate reader SpectraMax Paradigm multi-mode detection platform.

For cell fixation studies, a Cressington 208HR high-resolution sputter coater and a Samdri PVT-3D critical point dryer were used to prepare the samples, which were imaged using a Hitachi S-4800 SEM instrument was used with a 3 kV accelerating voltage and 10 µA of current.

3.2.2. Tellurium nanoparticle synthesis and purification

The precursors employed for the green synthesis of tellurium nanoparticles were sodium tellurite (Na₂TeO₃) and aloe vera extracts. Aloe vera leaves were purchased from a local vendor and sterilized to remove potential contaminants. For the extract preparation, 100 g of aloe vera leaves were finely cut into small pieces and boiled in a 500 mL beaker together with 100 mL of deionized water for 30 min. During that time, the initially clear water turned brownish. After boiling, the solution was cooled and filtered using a 0.2 µm pore size filter coupled with a vacuum. The cooled brownish leaf broth was then stored in the refrigerator at 4°C prior to use in experiments as the unique liquid medium for the reaction.

A stock solution of 10 mM sodium tellurite was prepared in deionized water, and a final concentration of 2 mM in 20 mL of deionized water was mixed with the same amount of aloe vera extract. The mixture was introduced in a microwave and heat for 10 s, after which it is cooled to room temperature. The solution turned black in a few seconds, indicating the reduction of tellurium ions to elemental particles. The resulting black solution was centrifuged at 10,000 rpm for 20 min. After that time, a black precipitate formed on the bottom of the container and was collected and washed twice with deionized water to remove potential additional compounds from the reaction. After both washes, the pellet was lyophilized. The powder was then collected, weighed, and dissolved in autoclaved water for further experiments.
3.2.3. Stability analysis

To analyze the stability of the samples, TEM and zeta-potential measurements were completed in fresh and 60-day-old AV-TeNPs.

3.2.4. Bacterial cultures

Strains of both Gram-negative and Gram-positive bacteria were used in this study to determine the antibacterial activity of the tellurium nanorods. Methicillin-resistant *Staphylococcus aureus* (MRSA)(ATCC 4330; ATCC, Manassas, VA), and multidrug-resistant *Escherichia coli*(MDR E. coli) (ATCC BAA-2471; ATCC, Manassas, VA) bacteria were used. Prior to inoculation, the bacterial cultures were maintained on agar plates at 4 °C. Bacteria were introduced into 6 mL of sterile Luria-Bertani (LB) (bioPLUS, bioWORLD) medium in a 15-mL Falcon centrifuge tube and incubated at 37 °C/200 rpm for 24 h. The optical density (OD) of the bacterial cultures was measured at 600 nm (nm) using a spectrophotometer (SpectraMax M3, Molecular Devices, Sunnyvale, CA). The bacterial suspension was then diluted to a concentration of 106 colony forming units per milliliter (CFU mL⁻¹) and stored at 4 °C until use.

3.2.5. Determining the antimicrobial activity of biogenic AV-TeNPs.

Two different bacteria were used for the bacterial assays (*E. coli* and *SA*). A colony of each was re-suspended in LB media. The bacterial suspension was placed in a shaking incubator to grow overnight at 200 rpm and 37°C.

After optical density measurements at 600 nm (OD₆₀₀) were taken to determine bacterial concentration, the overnight suspension was diluted to 1 × 10⁶ colony forming units per milliliter (CFU mL⁻¹). For the antimicrobial assay, different concentrations of nanoparticles were mixed with 100 μL of bacteria in LB medium and were then added to each well of a 96-well plate (Thermo Fisher Scientific, Waltham, MA). For the untreated controls, bacteria were mixed with 100 μL of LB media without nanoparticles. The final volume per each well was 200 μL. Once the plate was prepared, the absorbance of all samples was measured at 600 nm on an absorbance plate reader every 2 min for 24 h. Negative controls containing only nanoparticles and medium were used to determine the absorbance caused by the nanoparticles.
Colony counting assays were also performed as follows: bacteria were seeded in a 96-well plate and treated with different concentrations of nanoparticles for 8 h inside an incubator at 37 ºC. The 96-well plate was then removed from the incubator, and all the samples were diluted with PBS in a series of vials to either x100, x1,000, or x10,000. Three drops of a 10 µL aliquot of each dilution were then placed in an LB agar plate and incubated for 8 h inside the incubator at 37 ºC. The resulting number of colonies formed in each plate was counted at the end of the incubation.

3.2.6. In vitro cytotoxicity assay with biogenic AV-TeNPs

Cytotoxicity assays were performed with primary human dermal fibroblast cells (Lonza, CC-2509, AMP) and melanoma cells (ATCC CRL-1619, Manassas, VA). The cells were cultured in Dulbecco’s modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA), supplemented with 10% fetal bovine serum (FBS; ATCC 30-2020™, American Type Culture Collection, Manassas, VA) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA). MTS assays (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI) were carried out to assess cytotoxicity. Cells were seeded onto tissue-culture-treated 96-well plates (Thermo Fisher Scientific, Waltham, MA) at a final concentration of 5,000 cells per well in 100 µL of cell medium. After an incubation period of 24 h at 37° C in a humidified incubator with 5% carbon dioxide (CO₂), the culture medium was replaced with 100 µL of fresh cell medium containing concentrations ranging from 25 to 175 µg/mL of biogenic AV-TeNPs.

Cells were cultured for another 24 h in the same conditions, followed by washing the cells with PBS and replacing the medium with 100 µL of MTS solution (prepared using a mixing ratio of 1:5 of MTS:medium). After the addition of the solution, the 96-well plate was incubated for 4 h to allow for a color change. The absorbance was measured at 490 nm on an absorbance plate reader (SpectraMAX M3, Molecular Devices) for cell viability after exposure to the AV-TeNP concentration. Cell viability was calculated by dividing the average absorbance obtained for each sample by the one obtained for the control sample and then multiplying by 100. Controls containing either cells and media or just media were also included in the 96-well plate to identify the healthy growth of cells without nanoparticles and determine the absorbance of the media. Cell experiments were carried out for 24 and 48 h.
3.2.7. SEM study of the interaction between nanoparticles and bacteria

Both bacterial strains (MDR Escherichia coli and MRSA) were inoculated into 5 mL of sterile LB media in a 50 mL Falcon conical centrifuge tube and incubated at 37°C/200 rpm for 24 h. The optical density was then measured at 600 nm (OD600) using a spectrophotometer. The overnight suspension was diluted to a final bacterial concentration of 106 colony forming units per milliliter (CFU/mL) prior to measuring the optical density. A selected 10 µg/mL nanoparticle concentration was mixed with LB media and bacterial solution in a 6-well plate with a glass coverslip attached to the bottom. The coverslips were pre-treated with poly-lysine to enhance cell adhesion right before the experiment. The plate was placed inside an incubator for 8 h at 37 °C. After the experiment, the coverslips were fixed with a primary fixative solution containing 2.5% glutaraldehyde and 0.1 M sodium cacodylate buffer solution for 1 h. Subsequently, the fixative solution was exchanged for 0.1 M sodium cacodylate buffer, and the coverslips were washed 3 times for 10 min. Post-fixation was done using 1% osmium tetroxide (OsO₄) solution in the buffer for 1 hour. Subsequently, the coverslips were washed three times with buffer and dehydration was progressively achieved with 35, 50, 70, 80, 95 and 100% ethanol (three times for the 100% ethanol). Finally, the coverslips were dried by liquid CO₂-ethanol exchange in a Samdri PVT-3D critical point dryer. The coverslips were mounted on SEM stubs with carbon adhesive tabs (Electron Microscopy Sciences, EMS) after treatment with liquid graphite, and then sputter-coated with a thin layer of platinum using a Cressington 208HR high-resolution sputter coater. Digital images of the treated and untreated bacteria were acquired using an SEM.

3.2.8. SEM study of the interaction between nanoparticles and human cell

Primary human dermal fibroblasts and melanoma cells were seeded in a 6-well plate with a glass coverslip (Fisher Brand) attached to the bottom. After an incubation period of 24 h at 37 °C in a humidified incubator with 5% carbon dioxide (CO₂), media was removed and replaced with fresh one containing a concentration of 50 µg/mL of tellurium nanoparticles. Cells were cultured for another 24 h at the same conditions. After, the coverslips were fixed with a primary fixative
solution containing 2.5% glutaraldehyde (Electron Microscopy Sciences, EMS) and 0.1 M sodium cacodylate buffer solution (Electron Microscopy Sciences, EMS) for 1 h. Subsequently, the fixative solution was exchanged for 0.1 M sodium cacodylate buffer, and the coverslips were washed 3 times for 10 min. Post-fixation was done using 1% osmium tetroxide (OsO₄) solution (Electron Microscopy Sciences, EMS) in the buffer for 1 h. Subsequently, the coverslips were washed three times with buffer and dehydration was progressively achieved with 35, 50, 70, 80, 95, and 100% ethanol (three times for the 100% ethanol). Finally, the coverslips were dried by liquid CO₂–ethanol exchange in a Samdri PVT-3D critical point dryer. The coverslips were mounted on SEM stubs with carbon adhesive tabs (Electron Microscopy Sciences, EMS) after treatment with liquid graphite and then sputter-coated with a thin layer of platinum using a Cressington 208HR high-resolution sputter coater. Digital images of the treated and untreated bacterial cells were acquired using an SEM microscope.

3.2.9. Statistical analysis

All experiments were repeated in triplicate (N = 3) to ensure the reliability of results. Statistical significance was assessed using Student's t-tests, with an alpha value <0.05 being statistically significant. Results are displayed as mean ± standard deviation.

3.3. Results and Discussion

3.3.1. Synthesis of AV-TeNPs

The experiments demonstrated the ability of aloe vera extract to reduce tellurite (TeO₃²⁻) ions dissolved in the liquid medium to elemental tellurium (Te⁰) in the form of nanoparticles, with a quick reaction rate and a facile and straightforward procedure.

Active components that are present in the aloe vera extract include vitamins, enzymes, minerals, sugars, lignin, saponins, and several amino acids, which represents a standard composition for many other plant extracts. Sugars derived from the mucilage layer of the plant, known as mucopolysaccharides,¹⁵⁷,¹⁵⁸ are responsible for the ionic reduction. The presence of a free aldehyde
group or an open ketone group within the structure of the mucopolysaccharide allows them to reduce metallic ions.

Once the ions have been reduced, nanoparticles form from small metallic nuclei, which tend to naturally arrange themselves via a process called “Ostwald ripening.” \(^{159}\) A stabilization of the structures is achieved for aloe vera extracts due to (a) the presence and action of the same sugars that lead to the reduction, or (b) the work of other organic molecules, such as fatty acids (cholesterol, campesterol, beta-sitosterol, and lupeol), with a high in the extracts. \(^{146,148,157}\) The selective interaction of these organic compounds with the forming nanoparticle nuclei leads to the specific crystallographic shapes present in the nanostructures.

3.3.2. TEM characterization

The nanoparticles were characterized using TEM observe the size and morphology of the structures. Small amorphous nanoparticles were found to be aggregated in clusters of different extension. The individual nanoparticles had a size between 3 and 24 nm. These clusters were easily disrupted, and small aggregates were pulled apart after a few minutes of sonication, indicating a weak interaction between the nanostructures.

![Figure 21. Transmission electron microscopy (TEM) characterization of AV-TeNPs. Different morphologies and features were found when the nanomaterials were characterized by TEM.](image)
It has been proposed that the benzoquinones found in the aloe vera extract might act as reducing agents during the formation of the nanoparticles, whereas proteins and enzymes are responsible for the isotropic growth of the nuclei that will lead to the formation of the final nanoparticles that were found in TEM characterization.\textsuperscript{157,160}

### 3.3.3. SEM characterization

Figure 22 shows SEM imaging of AV-TeNPs where spherical nanoparticles were observed. Higher agglomeration of nanoparticles could be seen in comparison to TEM imaging. Nonetheless, the structures presented as the same size. Moreover, the morphology of the particles could be more clearly observed than in TEM, confirming a final spherical shape.

![SEM imaging of AV-TeNPs](image)

*Figure 22. Scanning electron microscopy (SEM) characterization of AV-TeNPs.*

### 3.3.4. EDX characterization

In the image taken at EDX conditions (Figure 23), it is possible to see the uniform distribution of NPs on top of the substrate. EDX has been measured integrating the X-ray signal coming to the full area, as well as with the electron beam stationary on the bright NP surrounded by the red circle in the image.

The elements distribution obtained from the fitting is shown, both for the full image area and for the spot. As the presence of the Si peak is due to the substrate used for sample preparation but is not relevant to the analysis of the obtained NPs and surrounding matrix composition, the element
distribution has been renormalized by removing the Si content obtained and rescaling the rest of elements to add to 100% composition.

The obtained results are similar to those obtained by XPS, with the presence of Te, Na, Ca, C, and O confirmed, together with Mg, Zn, Mn, K, and Cl, minerals that can also be found in aloe vera [Hamman, J.H., 2008. Composition and Applications of Aloe vera Leaf Gel. Molecules 13, 1599–1616 https://doi.org/10.3390/molecules13081599]. Please note the good quality of the fitting (see graph below, corresponding to the full area spectrum: the full red line corresponds to experimental data, and the cyan line corresponds to fitting), which validates the presence of the materials indicated. An important aspect to be taken into account here is that the composition of the Te NPs is unknown, and they are surrounded by a different matrix. Therefore, we do not have a standard sample to calibrate the EDX and the EDX-derived composition is not fully quantitative, but it should be taken as illustrative.

Interestingly, the ratio of Te to O obtained from the full area is lower than that obtained from the spot, 0.22 versus 0.33. The spot corresponds to one of the bigger and brighter particles, and here it indicates that the Te content is higher, suggesting that the core of the NPs is made of Te and the surrounding part is the oxidized one. The much higher number of smaller particles can be the reason why XPS mainly detects the presence of Te oxide.
Figure 23. EDX characterization of AV-TeNPs on different spots.
3.3.5. FTIR characterization

The structural analysis of the AV-TeNPs was carried out by FT-IR spectroscopy. The majority of the bands observed in the FT-IR spectrum (Figure 24) correspond to the functional groups of the most representative phytochemical constituents found in the aloe vera extract, including polysaccharides (e.g., acemannan, galactan, and pectin), proteins, vitamins, enzymes, organic acids, phenolic substances, phytosterol, flavones, organic acids, and quinones.\textsuperscript{145,158,161} The broad absorption band at 3280 cm\textsuperscript{-1} is assigned to the stretching mode of the –OH group from alcohols and phenols. The small absorption band at 2920 cm\textsuperscript{-1} may be assigned to the symmetrical and asymmetrical C–H stretching of aliphatic –CH and –CH\textsubscript{2} groups.\textsuperscript{161} The absorption bands at 1580 and 1416 cm\textsuperscript{-1} are characteristic of C=C from aromatic rings and symmetrical –COO– stretching vibrations, respectively. The absorption bands in the region of 1060–1030 cm\textsuperscript{-1} may be responsible for the presence of C–O and C–N stretching vibrations of ethers and aliphatic amines, respectively. The small absorption band at 870 cm\textsuperscript{-1} may be related to the C–H out-of-plane deformation of monosaccharides.\textsuperscript{145,161} Finally, the very strong absorption band at 687 cm\textsuperscript{-1} and the shoulder at
610 cm\(^{-1}\) are due to the symmetrical and asymmetrical axial Te–O stretching vibrations.\(^{162,163}\) These results demonstrated the presence of phytochemicals from the aloe vera extract acting as capping agents of the Te-based nanoparticles.

3.3.6. XRD characterization

In Figure 25, the experimental XRD pattern of the AV-TeNPs sample is compared with the calculated XRD pattern of elemental hexagonal tellurium (\(h\)-Te).\(^{164}\) The experimental XRD pattern shows the typical profile of an amorphous material, i.e., a very broad hump at relatively low diffraction angles (10–40°).\(^{122}\) The diffraction peaks at 2\(\theta\) = 14.2 and 17.0° could not be assigned to any crystallographic phase related to an expected Te-based compound.

![Figure 25](image.png)

*Figure 25. Comparison between the experimental XRD pattern of the AV-TeNPs sample and the calculated XRD pattern of elemental hexagonal tellurium (\(h\)-Te) (Andriesh and Bertolotti 1997).*
3.3.7. XPS characterization

The chemical composition of the sample was extracted from the full scan spectrum displayed in Figure 26. The resulting concentrations of the different elements were collected in Table 7.

![Figure 26. XPS full energy scan where the essential features are indicated](image)

<table>
<thead>
<tr>
<th>C (%_{at})</th>
<th>O (%_{at})</th>
<th>Te (%_{at})</th>
<th>Ca (%_{at})</th>
<th>Na (%_{at})</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.4</td>
<td>35.8</td>
<td>20</td>
<td>4.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Different elements besides tellurium were found on the sample, principally, oxygen and carbon that were associated with the organic coating coming from the aloe vera extract. Moreover, significant amounts of calcium and sodium were detected on the sample; these compounds are naturally present in high quantity on aloe vera.\(^{157}\) No consistent presence of nitrogen was found on the sample when the N 1s core level was measured, in comparison with other green-synthesized tellurium nanoparticles where a higher concentration of nitrogen was found.\(^{100}\)
The analysis of the Te 3d core-level spectrum (Figure 27A) revealed the presence of a small Te$^0$ component that represented between 4 and 5% of the Te. The metallic and oxide Te 3d 5/2 components were found at 573.8 and 576.1 eV. These binding energies are slightly higher than expected (573 and 576 eV), indicating that charging effects on these Te nanorods are stronger than in other compounds [A. Vernet Crua et al., A comparison of cytocompatibility and anticancer properties of traditional- and green-chemistry synthesized tellurium nanowires]. The Ca 2p core-level spectrum (cf. Figure 27B) was fitted with two spin-orbit 1/2 and 3/2 components located at 350.3 and 346.8 eV, respectively. These binding energies correspond to the presence of CaCO$_3$. The Na 1s core-level spectrum (Figure 27C) was fitted with a single component located at 1071.5 eV. Additionally, the sodium Auger line KL$_{23}$L$_{23}$ was found to be at 989.7 eV. Both the binding energy of the Na 1s core level and the position of the Auger line were compatible with the presence of Na$_2$CO$_3$, indicating that sodium was also present in carbonate form.

The O 1s core-level spectrum (Figure 27) was fitted using a minimum of two components located at 530.4 eV (69.6%) and 532.5 eV (30.4%). The most intense contribution at 530.4 eV can be ascribed to the Te oxide, in agreement with the high content of Te oxides observed in the Te 3d core-level spectrum. The component at 532.5 eV corresponds to carbon–oxygen bonds in the organic compounds and is thus associated with the aloe vera extract. The BE of the oxygen related to the presence of carbonates in the sample should be around 530.6–531.5 eV, an energy range close to the first component, ascribed to the Te oxide. The inclusion of an additional component for the fitting was ruled out as the proportion of carbonates is much lower than Te oxide and organic compounds, as was mentioned in the analysis of the full scan.

The C 1s core level spectrum displayed in Figure 27 could be fitted with a minimum of three components. Nonetheless, the fitting procedure forced the third component at the highest binding energy to have a too large full width at half maximum. Thus, this indicated the need to incorporate the fourth component and the resulting fit is displayed in Figure XE. The most intense component (59% at 285 eV) corresponds to the presence of either C–C or to C–H bonds in the sp$^3$ tetrahedral configuration associated with the organic compounds on the sample.
The other components at higher binding energies 286.7 eV (17%) and 288.4 eV (12%) were related to the presence of C–O, C–N, and carbonyl C=O bonds, respectively. Although no evidence of carboxyl or carbonates (both at around 289 eV) was found, a component at a lower binding energy (280.3 eV) could be identified. It was inferred that this component corresponds to C atoms weakly bonded in the organic compound.

![Figure 27. Te 3d XPS core level spectrum and the corresponding metallic and oxide components (A); Ca 2p XPS core level spectrum (B); Na 1s XPS core level spectrum (C); O 1s XPS core level spectrum and the corresponding components used in the fitting procedure (D); and C 1s XPS core level spectrum and the corresponding components used in the fitting procedure (E).]

3.3.8. Stability analysis

To verify the stability of the nanostructures, TEM imaging was carried out on the samples after 60 days of synthesis (Figure 28). Generally, it can be stated that the samples maintained the original structure and size they presented originally (Figure 28B). For instance, the 60-day-old AV-TeNPs sample is composed of partially agglomerated nanostructures, as seen in Figure 28A.
The stability analysis through the measurement of the Z-potential of the freshly synthesized and 60-day-old Te-based nanomaterials was also carried out. In general, a colloid or suspension is considered stable if the Z-potential is above a critical value of ±30 mV. Given the measured Z-potential values for the colloids (fresh and 60-day-old samples, see Table S4), they can be considered stable, although a slight decrease in values may be ligated to the aggregation found in 60-day-old samples.

**Table 8. Zeta-potential values for fresh and 60-day old AV-TeNPs. The pH of the colloids was 7.0 ± 0.2**

<table>
<thead>
<tr>
<th>Nanostructure</th>
<th>As-synthesized</th>
<th>60 days old</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV-TeNPs</td>
<td>-24 ± 2.34</td>
<td>-17.95 ± 3.34</td>
</tr>
</tbody>
</table>
3.3.9. Determining the antimicrobial activity of biogenic AV-TeNPs.

The bacterial proliferation was studied for 24 h using a growth curve analysis. As can be seen in Figure 29, the presence of different concentrations of AV-TeNPs produces a significant decay in the bacterial proliferation for both MDR *E. coli* and MRSA, an effect that was especially visible for the first bacteria. Besides, no significant difference was found between the concentrations.

Figure 29. Effect of AV-TeNPs on MDR *E. coli* (A), AV-TeNPs on MRSA (B). The growth of a $10^6$ CFU mL$^{-1}$ suspension of *S. aureus* (A) and *E. coli* (B) for 24 hours in the presence of different concentrations AV-TeNPs. The values represent the mean ± standard deviation.

Colony counting unit assays were done as well with both bacterial strains to further explain the effect of the nanostructures in the bacterial proliferation.
A colony counting unit assay conducted over *MDR* E. coli (A) and *MRSA* (B) showed a dose-relative inhibition of the bacterial growth when exposed to different concentrations of AV-TeNPs. The nanostructures were useful toward both bacterial strains at a range of concentrations between 5 and 75 µg/mL. Therefore, a wide range of concentrations was shown to be active towards the bacterial proliferation in both antibiotic-resistant phenotypes.

**Figure 30.** Colony counting assay of A) *MDR E. coli* treated with AV-TeNWs, B) MRSA treated with AV-TeNPs. The treatment time is 8 h. N=3. *p<0.05 versus control, **p<0.01 versus control.
MIC values were calculated to further quantify the antibacterial effect.

Table 9. MIC values for different nanoparticles against MDR E. coli and MRSA.

<table>
<thead>
<tr>
<th>MIC values (ug/mL)</th>
<th>MDR E. coli</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV-TeNPs</td>
<td>30.53</td>
<td>11.61</td>
</tr>
</tbody>
</table>

These values differ from others found in the literature, showing a decrease in the MIC values for the tellurium-based system reported here. For example, Emanuele et al. have investigated the antibacterial effect of TeNPs produced by the *Stenotrophomonas maltophilia* SeITE02 and *Ochrobactrum* sp. MPV1, which were tested against *S. aureus* and *E. coli* with MIC values of 1 and 0.5 mg/mL, respectively.\(^{167}\)

### 3.3.10. Testing the effect of nanomaterials on human cells

Cytotoxicity studies were done to explain the interaction between the AV-TeNPs and both healthy and cancerous cell lines.
A dose-relative cell proliferation decay was found when the nanosystem was cultured with HDF cells over 48 h. A low cytotoxic effect was found in a range of concentrations between 5 and 50 μg/mL at 24 h, whereas the range was increased at concentrations up to 75 μg/mL at 48 h. When AV-TeNPs were present in the cell media, the optimum range of concentrations was found to be...
5 to 50 ug/mL in experiments up to 48 h. Therefore, the AV-TeNPs can be considered biocompatible in a range of concentrations up to 50 ug/mL.

Moreover, a relatively dose-dependent cell proliferation decay was found when the AV-TeNPs were cultured with melanoma cells for 48 h. The anticancer effect was found toward melanoma cells in a range of concentrations up to 50 µg/mL at 24 h with a low cytotoxic effect, whereas the range was increased to a concentration of 75 µg/mL at 48 h. Therefore, the AV-TeNPs can be considered anticancer at concentrations up to 50 µg/mL for a 2-day treatment.

IC50 values were calculated to further study the cytotoxic effect of the TeNPs.

| Table 10. IC50 values for different nanoparticles cultured with HDF and Melanoma cells. |
|-------------------------------------------|---------|---------|
| Exposed cells                             | 1 day   | 2 days  |
| HDF                                      | 74.78   | 67.70   |
| Melanoma                                 | 1.418   | 54.06   |

These values differ from others found in the literature, showing a decrease in the IC50 values for our Te nanosystems. For example, Yang et al. investigated the anticancer effect of Te nanodots (Te-NDs) synthesized using hollow albumin nanocages that were tested against 4T1 tumor cells with values of 880 µg/mL.\(^\text{168}\)

3.3.11. Cell fixation and SEM imaging for bacteria and human cells

SEM micrographs of control MDR E.coli and MRSA (A, D) and bacteria after treatment with AV-TeNPs (B, C) are shown in Figure 32. The characterization indicated that the treatment with the nanoparticles induced changes in both bacterial strains. Disruption of the other cell membranes and cell lysis was seen after the treatment. As such, visible cell damage was observed, with an abundant presence of holes and cracks all over the cell membrane, and bacterial deformation and collapse. The cell membrane damage is commonly found to be a cause of ROS. Nevertheless, other mechanisms can also be inferred, as the direct damage of the cells due to the morphology of the nanostructures. From the SEM images of the bacteria, we can see that the membrane damage
occurs and that there is the attachment of nanoparticles to bacteria, but the exact mechanism of how damage occurs could not be identified.

![SEM micrographs](image)

*Figure 32. SEM micrographs of control MDR E.coli and MRSA (A, D) and bacteria after treatment with AV-TeNWs (B, C).*

In addition, SEM micrographs were obtained of HDF and melanoma cells with no nanoparticle treatment (A, C) and after treatment with AV-TeNPs (B, D), respectively. As can be seen, HDF cells were able to successfully proliferate in the presence of the nanostructures, with no apparent disruption or alteration of the membrane or normal growth. On the other hand, the presence of the nanostructures induced a severe membrane disruption within the melanoma cell population. No swelling or bubbling of the membrane was observed; therefore, the mechanism of cell death was hypothesized to be necrosis.
Figure 33. SEM micrographs of HDF and melanoma cells with no nanoparticle treatment (A, C) and after treatment with AV-TeNPs (B, D) respectively.

3.3.12. ROS study
Dose-dependent ROS generation was found in AV-TeNPs when these were exposed to melanoma cells. The contribution of ROS should be related to the metalloid content on the nanomaterials. It is widely known that tellurium oxyanions can trigger the generation of ROS. Once released, these can react with intracellular thiols and form intermediates that cause oxidative stress as a consequence of the formation of superoxide radicals.\textsuperscript{139} In addition, alternative mechanisms related to the nanoparticle shape and the surface chemistry might be involved as well, contributing to the cell damage by disrupting the integrity of the envelope.\textsuperscript{142,143}

In summary, AV-TeNPs showed antibacterial properties in a range of concentrations up to 50 μg/mL, showing no cytotoxicity towards healthy human cells over the same period. Furthermore, this nanostructure was found to have anticancer activity toward melanoma cells in a range of concentrations up to 50 μg/mL with no alteration of the normal proliferation of healthy skin cells. Therefore, we can conclude that our systems can be successfully used at low concentrations as biomedical agents with antibacterial and anticancer properties, and they are biocompatible in the same range of concentrations.
3.4. Conclusions

Current methods to synthesize tellurium nanoparticles use approaches that employ synthetic techniques based on traditional physicochemical pathways. The weakness of these procedures is that they require extreme reaction conditions, which produces toxic byproducts and necessitates additional purification procedures. Therefore, there is a pressing need for alternative approaches that are vastly different from traditional methodologies. Plant extracts can be used to overcome these drawbacks in a straightforward way by employing green chemistry principles. Herein, biogenic or “green-synthesized” tellurium nanoparticles were synthesized through the use of aloe vera extract. Using an eco-friendly and green method, AV-TeNPs of two different shapes (amorphous spheres and nanorods) were synthesized with a relatively homogeneous size distribution (100±19 nm length and 5±2 nm width) in a stable and quick method. Nanoparticles were obtained through tellurite reduction, accomplished by the sugars within the plant extract. These nanoparticles were characterized using TEM, SEM, and EDX to determine the size, morphology, and composition. Antimicrobial activity of all nanoparticles was studied, showing antibacterial activity toward both Gram-negative and Gram-positive bacteria for a range of nanoparticle concentrations between 5 and 50 µg/mL. Fibroblast viability in the presence of nanoparticles was tested with no significant cytotoxicity in the same range of nanoparticle concentration. An anticancer effect against melanoma cells was demonstrated as well, with a consistent delay in the cell growth over all nanoparticle concentrations. In summary, for the first time to the best of our knowledge, green-synthesized tellurium nanostructures were created with aloe vera extracts and showed significant antimicrobial activity, anticancer properties, and low cytotoxicity for healthy human cells. These findings suggest that these biogenic nanoparticles can overcome many of the shortcomings of synthetically synthesized nanoparticles and provide promising evidence for future research.
4. FUTURE PROSPECTS

The use of green-synthesized tellurium nanoparticles for enhancement of biomedical devices and bactericidal surfaces for implants was first reported by our group. In that study, nanocolumnar titanium coatings were fabricated in two sputtering systems with very different characteristics, thus possessing different morphologies. These coatings exhibited similar antibacterial properties against Gram-positive and negative bacteria. However, a synergic route was followed, and these coatings were functionalized with tellurium nanorods prepared by an environmentally friendly route. As a consequence, the antibacterial properties were enhanced while the biocompatibility was preserved in all the nanostructured coatings.

Therefore, the two systems presented in the thesis, tellurium nanowires functionalized with noble metal nanoparticles and aloe-vera-mediated nanoparticles, might be useful for the further enhancement of these implantable metallic surfaces. Because tellurium is able to exert antibacterial and anticancer activity while remaining biocompatible, the bonding of these nanostructures to morphologically functionalized nanosurfaces might be interesting from a biomedical point of view. The possibility of a synergetic effect between the bacteriostatic surfaces and the tellurium nanostructures may be able to fulfill some of the limitations of the use of metallic surfaces in biological applications.

Furthermore, the nanostructures should be studied for the building of high-performance supercapacitor and non-enzymatic hydrogen peroxide sensors. For instance, Manikandan et al. showed that when the TeNPs are applied as an electrode for the supercapacitor, they exhibit good electrochemical performances. This behavior of TeNPs opens the possibility for the enhancement of the electrocatalytic properties of electrodes for H₂O₂ biosensing. Because TeNPs exhibit high sensitivity and short response time with a wide linear range of H₂O₂, a new generation of energy storage devices and biosensors might be derived from the use of the metalloid.
5. CONCLUSIONS

This thesis focuses on the preparation of nanostructures using a green synthetic approach, overcoming the main limitations of traditional synthesis in terms of production of toxic by-products and biomedical applications. The first presented projects show how a green-synthesized tellurium nanowire is able to support the in situ growth of noble metallic nanoparticles, rendering a synergetic structure that is translated in an enhancement of antibacterial and anticancer properties with respect to the plane tellurium nanowires. TeNPs are also prepared using aloe vera extract as the unique reducing and capping agent, with a broad range of concentrations in which the nanoparticles show a strong antibacterial and anticancer effect while remaining biocompatible.

The overall aim of this thesis is to explore the potential applications of green nanotechnology in biomedicine and lay the theoretical foundation of green nanotechnology through the study of new methods and new products, as well as give knowledge about the synergetic effect between the molecules coming from natural sources and the chemistry of the elements used in the synthesis. Hence, the nanomaterials are extensively characterized to understand the potential biomedical applications and the interaction with biological tissue, strongly affected by size, morphology, and surface chemistry. These data give us safety to explore the antibacterial, anticancer, and biocompatibility features of the different nanostructures. Therefore, we present the novelty of the interaction between tellurium and biological tissue, opening the doors for plenty of chances in terms of the application of the nanoparticles in medical activities.

In the future, we need to further study the mechanism of nanoparticle formation and interactions within the human body. Overall, this work still leaves some problems to be further explored and solved, for example

- How to determine the stability mechanism of nanowires and how to prove it?
- What are the surface coating chemicals of nanoparticles synthesized by aloe vera and what is their exact role?
- How can the production of these nanomaterials be scaled-up?

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