MASTER'S THESIS

Green nanotechnology for the application of tellurium nanowires in biomedicine

A Thesis Presented

By

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“Nothing important was ever achieved without someone taking a chance”

H Jackson Brown Jr

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ABSTRACT

In a world of constant duality, where the population density increases as dramatically as natural resources disappear, there are concerns that should be addressed from the chemical point of view. Traditional chemistry has undoubtedly enhanced the quality of life, but at the price of threatening the environment and human health. Chemical processes are responsible for the contamination of all the known ecosystems all over the planet, to more or less extent.

The traditional physico-chemical approaches for the synthesis of compounds and drugs that are used for healthcare concerns (such as antimicrobial resistance or the fight against cancer) are facile and straightforward. Nevertheless, there is a cost associated with these approaches that needs to be overcome, such as the production of toxic by-products and the non-biocompatibility of the products. Therefore, new methods are needed, and green chemistry offers a suitable and novel answer, achieving a safe and environmentally-friendly design, manufacture and use of chemical products.

There are also significant concerns currently facing the healthcare system, one of which is antimicrobial resistance (AMR). AMR is known as the ability of bacteria to form a resistance towards antibiotics, and it can easily occur due to mutations when they are exposed to antibiotics. This results in a cessation of efficiency and efficacy of treatments, increasing the possibility to get a bacterial infection without having an available cure. AMR is rising at an alarming rate and is projected to become the first cause of death worldwide in less than 50 years.

Cancer – known as an incontrollable growth of mutated cells ending with the creation of spreadable tumors – is also a significant concern, affecting more than 2 million people per year. Therefore, there is an urgent need to find an affordable and effective treatment that avoids all the side effects that current treatments offer.

In both cases, nanotechnology can be the answer to these challenges, mainly because of their high surface area-to-volume ratio, which enables an extraordinarily high reactivity, together with the possibility of functionalization to penetrate bacteria and cancer cells.

Therefore, there is a high necessity to develop new antimicrobial, anticancer and biocompatible agents without the use of traditional chemistry, for which green chemistry may provide the solution. In the present thesis, different types of

nanostructures – focusing on tellurium nanowires – were synthesized, characterized and tested against bacteria and cancerous cells, and results were shown in two articles and a supporting information report.

Results showed that treatment with tellurium nanowires (TeNWs) at concentrations between 5 and 100 µg/mL improved the proliferation of healthy cells and decreased cancerous cell growth over a five-day period. Most importantly, the green chemistry synthesized TeNWs outperformed those produced by traditional synthetic chemical methods.

Moreover, the use of nanowires as a template for the growth of metallic nanoparticles was successfully achieved. The newly formed nanostructures – a combination made by TeNWs and the metallic nanoparticles called synergy – have shown a decrease on the bacterial proliferation of *Escherichia coli* and *Staphylococcus aureus* at concentrations between 5 and 100 µg/mL. These structures present an enhanced performance over the bare TeNWs structure.

In this manner, this study suggests that green chemistry approaches for producing tellurium nanostructures and metallic nanoparticles, may not only reduce adverse environmental effects resulting from traditional synthetic chemistry methods, but also may be more effective in numerous healthcare applications such as antimicrobial or anticancer agents.

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ABBREVIATIONS

3T3L1	Mouse embryonic fibroblast cells
A	Maximum specific growth of the bacteria
A. fulvum	*Arthroderma fulvum*
A. terreus	*Aspergillus terreus*
Ag	Silver
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
Au	Gold
$AuCl_3$	Gold chloride
AuNPs	Gold nanoparticles
C. albicans	*Candida albicans*
C. roseus	*Catharanthus roseus*
Cd	Cadmium
CDC	Center for Disease Control and Prevention
$CdCl_2$	Cadmium chloride
CDIFF	*Clostridium difficile*
CdS	Cadmium sulfide
CdTe	Cadmium tellurite
CFU mL^{-1}	Colony forming units per milliliter
CHEM-TeNWs	Chemically-synthetized tellurium nanowires
CO_2	Carbon dioxide
CRE	*Carbapenem-Resistant Enterobacteriaceae*
CuNPs	Copper nanoparticles
DMEM	Dulbecco’s Modified Eagle Medium

DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDX	Energy-dispersive X-ray spectroscopy
FDA	United States Food and Drug Administration
g	Gram
GREEN-TeNWs	Green-synthetized tellurium nanowires
h	Hour
H_2TeO_4	Telluric acid
HDF	Human dermal fibroblasts
HeLa	Henrietta Lack cells
Hg	Mercury
$HgCl_2$	Mercury (II) chloride
ICP-MS	Inductively coupled plasma mass spectrometry
K_2PtCl_4	Potassium tetrachloropalladate
kV	Kilovolt
mg	Milligram
min	Minute
mL	Milliliter
MRI	Magnetic Resonance Imaging
MRSA	*Methicillin-Resistant S. aureus*
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
N_2H_4	Hydrazine hydrate
Na_2SeO_3	Sodium selenite
Na_2TeO_3	Sodium tellurite

NH_3	Ammonia
NiNPs	Nickel nanoparticles
nm	Nanometer
NP	Nanoparticle
NPs-NWs	Synergy of metallic nanoparticles and the tellurium nanowires
NW	Nanowire
O_2^{-2}	Peroxides
O^{2-}	Superoxides
ºC	Celsius degree
OD_{600}	Optical density measurements at 600 nm
OH	Hydroxyl radicals
P. aeruginosa	*Pseudomona aeruginosa*
P. falciparum	*Plasmodium falciparum*
Pb	Lead
$Pb(NO_3)_2$	Lead (II) nitrate
PBS	Phosphate buffered saline
Pd	Palladium
$PdCl_2$	Palladium chloride
PdNPs	Palladium nanoparticles
PdNP-TeNWs	Synergy palladium nanoparticles and tellurium nanowires
Pt	Platinum
PtNP-TeNWs	Synergy platinum nanoparticles and tellurium nanowires
PVP	Polyvinylpyrrolidone
QD	Quantum dots

ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature
S. aureus	*Staphylococcus aureus*
S. typhi	*Salmonella typhi*
SD	Standard deviation
Se	Selenium
SEM	Scanning electron microscopy
SeNPs	Selenium nanoparticles
T. thioparus	*Thiobacillus thioparus*
Te^{2-}	Tellurides
TeO_3^{2-}	Tellurites
Te	Tellurium
TEM	Transmission electron microscopy
TeNPs	Tellurium nanoparticles
TeNWs	Tellurium nanowires
Ti	Titanium
TSB	Tryptic soy broth
USA	United States of America
USD	United States Dollars
VRE	*Vancomycin-Resistant Enterococcus*
Zn	Zinc
$ZnCl_2$	Zinc chloride
λ	Lag time in the bacterial growth
μ	Speed of the bacterial growth
µg	Microgram

μL	Microliter

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1. Introduction

Nanotechnology is comprised of materials in the scale of nanometers, with a size less than 100 nanometer (nm) [1,2]. These materials present unique properties [3] that allow them to be used in different fields from chemistry [4] to physics [5] and biology [6]. In recent years, study in this field has been gaining in interest due to its potential application in solving potential global concerns in the healthcare system such as antimicrobial resistance [7], and improving cancer treatments [8], imaging [9] or drug delivery [10]. Different kinds of nanostructures with different sizes and morphologies have been developed using metallic elements such as gold (Au), palladium (Pd), platinum (Pt) or silver (Ag) for a wide range of applications. For instance, Au nanoparticles (AuNPs) [11] of different shapes – nanorods, nanospheres or nanoshells – have been used in a variety of applications such as imaging, targeting and treating several cancers such as melanoma, liver, breast and prostate due to their unique physical, chemical and optical properties. Moreover, AgNPs [12] synthesized by several methodologies, such as laser ablation or biogenic approaches using living bacteria, showed antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) and antifungal activity against *Candida albicans* (*C. albicans*). Antiviral properties were also tested against MS2 bacteriophage and anticancer properties were enhanced with glioblastoma and lung cancer.

Antimicrobial resistance is one of the most alarming in regard to the healthcare system [13, 14]. It is related with the survival ability of all kinds of known bacteria to current antibiotics, which means they are no longer the most efficient or effective solution to bacterial infections [15, 16]. Some examples – stated as urgent threats from the Centers for Disease Control and Prevention (CDC) – are that of *Clostridium difficile* (CDIFF) and *Carbapenem-Resistant Enterobacteriaceae* (CRE). CDIFF is known to cause life-threatening diarrhea. With few available, effective antibiotics, CDIFF leads to more than 250,000 infections that degenerate into 15,000 deaths in the USA per year, with an associated cost of 1 billion USD. Similarly, CRE have become resistant to all or nearly all available antibiotics, causing more than 9,000 bloodstream infections that end in more than 600 deaths per year in the USA. In addition to the urgent threats of CDIFF and CRE to the healthcare system, another 16 bacteria such as *Vancomycin-Resistant Enterococcus* (VRE) or *Methicillin-Resistant S. aureus* (MRSA), contribute to more than 2 million potential infections developed and 23,000 deaths per year, and a combined 2 billion USD expended; thus, it is necessary to find a solution. There is an increasing percentage of the bacteria becoming resistant to antibiotics (around 11% per year since 2014), which could eventually result in antimicrobial resistance being the first cause of death worldwide [17, 18]. Figure 1 shows an example of the increasing antimicrobial resistance from *Salmonella typhi* against Ciprofloxacin antibiotic [18].

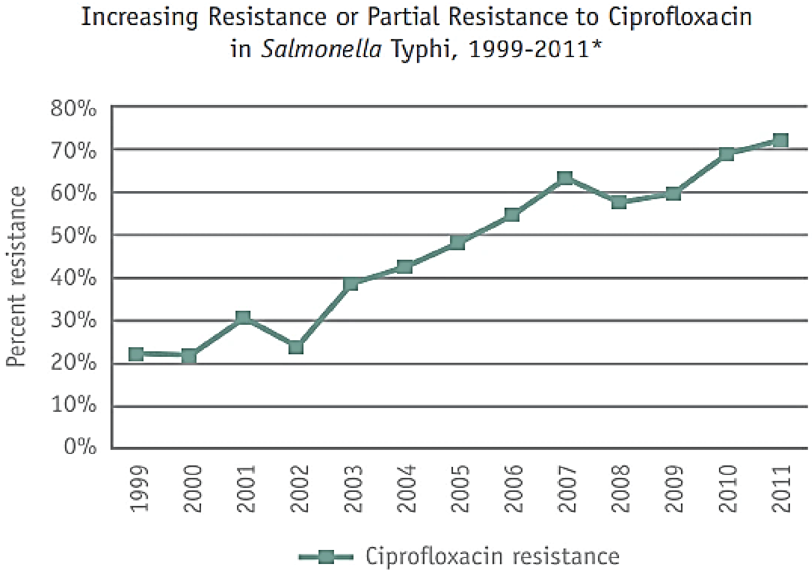

Figure 1. Increasing resistance to Ciprofloxacin in* Salmonella Typhi *from 1999 to 2011. *On average, there is an increasing tendency (around 40%) of this bacteria becoming resistant to the currently available antibiotic from 1999 to 2011.*

Metallic nanoparticles have emerged as a powerful solution to the resistance problem, as their multiple antimicrobial mechanisms require bacteria to develop simultaneous mutations, making it far less likely for the bacteria to develop any resistance [19]. It has been widely reviewed that metallic nanoparticles can eliminate bacteria by different mechanisms, such as cell membrane or DNA damage, ending in the destruction of the host cells, thus killing bacteria [20, 21].

Another worldwide concern is cancer, which is defined as the uncontrollable growth of cells and abnormal behavior of cells [22, 23, 24]. It is stated to be the second cause of death in the USA, with more than 1.7 million estimated cases each year [18,25]. There are potential known cancer treatments such as chemotherapy [26], radiotherapy [27] or surgery [28]; nevertheless, they present a huge variety of side effects including fatigue, anemia, and death [29, 30]. Therefore, there is a need to find alternative treatments that avoid these limitations. Here, nanotechnology – with the synthesis of different nanostructures – can be considered a solution, as cancer cells do not present the mechanism to eliminate some metallic structures, unlike normal cells that have this ability, leading to selective cancer cell death while maintaining the viability of healthy cells [31, 32]. Figure 2 shows the rising incidence in cancer cases and the death trends in children in the USA [18].

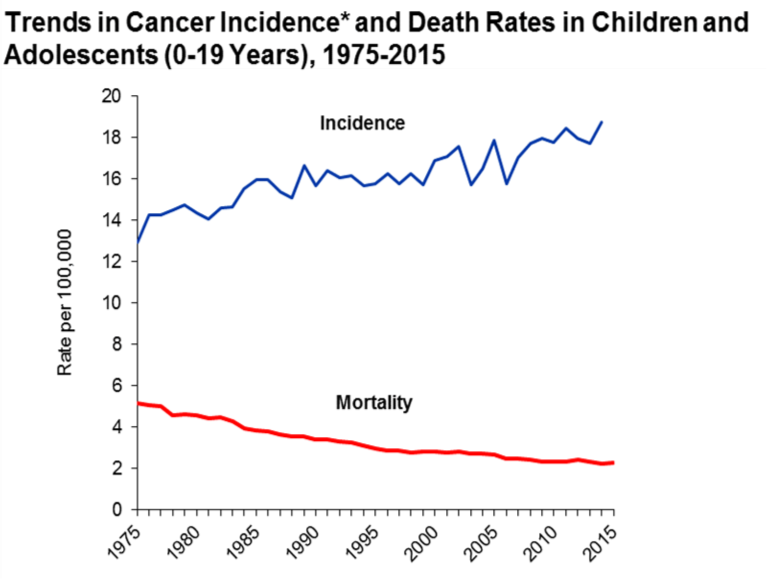

Figure 2. Trends in cancer incidence and death rates in children (0-19 years) from 1975 to 2015. *Graph shows an increasing incidence over the years with a slow decrease in the mortality due to the current existing treatments.*

Though nanotechnology has the ability to address the significant concerns mentioned above, the synthetic synthesis methods currently employed have significant limitations for practical use in biomedical applications [33]. In the past years, nanotechnology has taken advantage of traditional chemistry synthesis methods [34]. Different kinds of metallic nanoparticles have been produced by a variety of methodologies including laser ablation [35], redox processes [36], and chemical vapor deposition [37]. All these processes are well-known and established, with easy and straightforward protocols that are used in fields such as medicine, electronics, and industrial. For instance, nanomaterials have had a huge impact on the medical field, with a growing rate of almost 15 % per year in both drug delivery and *in vitro* diagnostics (the main fields of application) [38]. As an example, iron oxide nanoparticles, synthesized by a coprecipitation methodology, have been approved by the United States Food and Drug Administration (FDA) and used as a Magnetic Resonance Imaging (MRI) contrast agent for liver imaging [39].

Nevertheless, the use of what is known as "physico-chemical" approaches presents major drawbacks. The vast majority are related to environmental effects, as producing these materials results, in some manner, in a change and damage in all ecosystems [40, 41]. The use of hazardous reagents in these reactions is known to produce toxic by-products that could threaten the environment [42]. For example, the production of silver nanoparticles requires toxic reagents such as citrate, borohydride or 2-mercaptoethanol [12]. In addition, traditional chemistry presents mainly harsh reaction conditions which demand lots of energy and specific instrumentation that increase the cost of the process [43]. Aggregation is also considered a problem upon the formation of nanoparticles and translates to a lack of biocompatibility as accumulation and increases their toxicity, thereby compromising their potential biomedical applications [44, 45]. In the example of AuNPs, they have been shown to produce a reduction in the cell uptake due to their accumulation inside the cell– tested in HeLa epithelial cells – in comparison to disperse NPs [46].

Figure 3. Advantages and drawbacks of both physico-chemical and green synthesis approaches

Therefore, there is a need to overcome these drawbacks, finding an eco-friendly and cost-effective methodology that produces nanoparticles with potential bio-applications. During the past years, several reports have presented the use of living organisms and biological systems to produce nanoparticles using the same raw materials as traditional chemistry approaches [47, 48].

The use of natural products as a source to produce nanomaterials, known as "green chemistry", is an alternative approach to physico-chemical approaches [49]. It is considered an environmentally safe method to synthesize metallic nanoparticles by avoiding the use of hazardous or toxic reagents [50, 51]. Different materials such as living organisms – like bacteria [52], fungi [53], cells [54] or plants [55] – dietary compounds [56] or waste materials [57] have been used as raw materials to produce different nanostructures (Figure 4).

For instance, thermophilic *Bacillus Sp*. AZ1 were used to synthesize AgNPs with antibacterial activity against *Salmonella typhi* (*S. typhi*), *E. coli, S. epidermis,* and *S. aureus* [58]. Moreover, a variety of Antarctic bacteria belonging to the genera *Pseudomonas, Psychrobacter* and *Shewanella* have synthesized cadmium sulfide (CdS) and cadmium tellurite (CdTe) fluorescent NPs or quantum dots (QD) [59]

Yeast and fungi produce extracellular and intracellular enzymes that give them the ability to reduce metals and produce nanostructures. For example, *Aspergillus terreus* (*A. terreus*) synthesized AgNPs with antimicrobial resistance to different bacteria such as *E. coli* and *P. aeruginosa* [60]. In addition, AgNPs were synthesized by *Arthroderma fulvum* (*A. fulvum*) and presented antifungal activity against *Candida, Aspergillus* and *Fusarium* [61].

Biogenic synthesis using human cells, such as that presented by El-Said, Waleed A. et al [54] can produce metal NPs with different shapes and morphologies using cell processes such as cell lysis. Furthermore, human epithelial cells have synthesized AuNPs with applications in the biomedical field such as cell targeting [62].

Plants are also considered a good choice to synthesize nanoparticles. The leaves from *Catharanthus roseus (C. roseus)* have been able to produce AgNPs with potential antiplasmodial activity against *Plasmodium falciparum (P. falciparum)* [63]. In addition to leaves, other plant parts such as the rhizome of *Acorus calamus* have also been used for the synthesis of AgNPs with anticancer effects [64].

The use of dietary compounds has also been on the rise recently. Cow milk was used to synthesize AgNPs with antibacterial properties against different resistant bacteria as *Aeromonas sp*. and *Acinetobacter sp.* [65]. Other compounds such as honey was also reported to be useful for the synthesis of AuNPs [66].

Food waste, such as orange peels, have been used to synthesize PdNPs with antibacterial properties [67]. In addition, banana peels were also useful for the synthesis of AuNPs with antifungal and antibacterial properties [68].

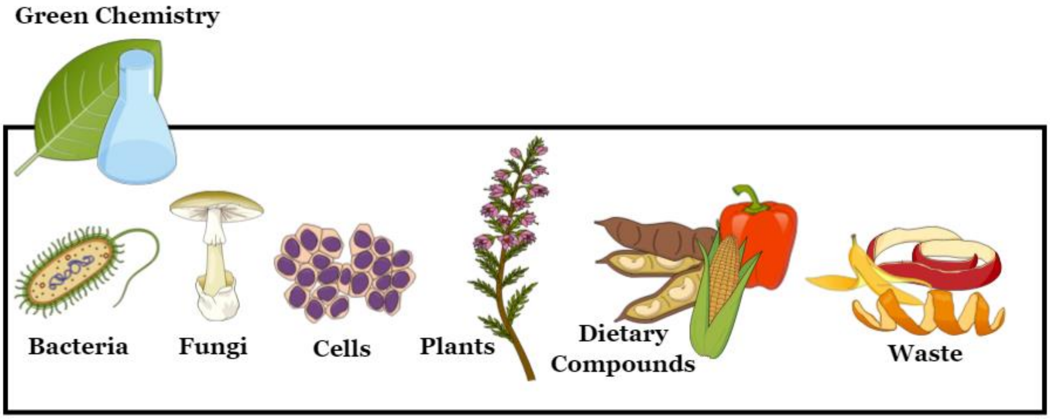

Figure 4. Green synthesis approaches. *There are eight different raw materials that can be used for the synthesis of nanoparticles: bacteria, fungi, cells, plants, dietary compounds and waste products.*

It has been reported many times that green-synthesized NPs, in comparison to traditional chemical methods, presents enhanced biomedical properties such as anticancer, antibacterial ability and biocompatibility. Moreover, these reactions were achieved in a minimum time-scale and using inexpensive reagents such as plants or organic waste, hence solving the main drawbacks of traditional synthesis [69]. As an example, nickel nanoparticles (NiNPs) made from root extracts were non-toxic to mammalian cells compared to the same ones made using conventional chemistry [70]. AgNPs where also synthesized by traditional chemistry (using glucose), and a green approach (using a plant, *Rhizophora apiculata*) showing enhanced antibacterial activity with eco-friendly methodology. [71]

Regarding aggregation, the natural coating presented on green nanostructures prevents them from aggregating, subsequently preventing deposition and accumulation in the body, which decreases their potential toxic effects [72]. In addition, this organic coating, made of biological compounds such as proteins and lipids, enhances biocompatibility, which gives green synthetic NPs high potential for use in the biomedical field to combat challenges like antibiotic resistance and cancer [73]. As an example is iron oxide NPs synthesized by the bacteria *Thiobacillus thioparus* (*T. thioparus*), the organic coating was made of proteins coming from the bacteria strain [74]. Furthermore, no functionalization is necessary, so nanoparticles obtained by these methodologies are ready to be used after a process of purification [75]. For instance, copper nanoparticles (CuNPs) showed low cytotoxicity on mouse embryonic fibroblast (3T3L1) cells when they were coated with starch in comparison to non-coated ones [76]. In addition, AgNPs with an organic coating made of polysaccharides also showed enhanced biocompatibility toward mouse embryonic fibroblasts [77].

During the past years, as mentioned previously, different methodologies have been studied to produce NPs. One of these is via hydrothermal reactions, which use high pressure and temperature in addition to water and the organic compound to obtain the metallic NPs [55, 78]. Different materials have been reported to work, such as platinum [79] or titanium oxide [80] NPs for the catalytic industry, and tellurium, a rare metalloid, is also considered a good candidate [81, 82]. Tellurium nanowires (TeNWs) have been synthesized employing hydrothermal reactions mainly for the semiconductor and catalytic applications [83, 84]; however, their biomedical applications are largely unknown, as tellurium – even the similarities with oxygen, selenium or sulfur - is not a trace element it has not been considered from biochemical interest. Nevertheless, some compounds of tellurium including tellurides (Te^{2-}) and tellurites (TeO_3^{2-}), are known to inhibit different proteins and enzymes and being capable of killing microorganisms such as bacteria or plasmodia. Besides, they can induce apoptosis in certain cancer cells [85]. As a consequence, tellurium has been considered a good candidate for biological applications. There are only a few reports on their antibacterial properties against *E. coli* and *S. aureus* showing much promise, using tellurium nanowires made from the fungi *Aspergillus welwitschiae* [86] and a combination of Te and Au nanowires over a carbon surface using a chemical approach with hydrazine [87]. Low cytotoxicity on mammalian epithelial cells was reported for the latter.

This thesis has been organized in two main objectives. In the first one, TeNWs were synthesized using both traditional synthetic and green approaches, and their properties were compared in terms of biocompatibility, antibacterial and anticancer properties.

The second objective seeks to take advantage of the potential of these structures for biomedical applications. GREEN-TeNWs have been used as a template for the growth of the metallic NPs. A new methodology for the synthesis of NPs has been developed using the NWs and different metals, such as palladium or platinum, for biomedical applications. After the reaction, both NPs and the synergy of NWs and NPs were purified and characterized, and their biocompatibility, anticancer properties and antibacterial properties were analyzed and compared.

A third objective has been set along with an aim focused on future projects and ongoing research. The use of GREEN – TeNWs as a coating for metallic surfaces in order to enhance their biomedical applications and their profit as environmental remediation agents to eliminate the presence of heavy metals such as mercury, cadmium, zinc or lead in water. As similarly than in the second aim, the coating presence over the nanowire structure would reduce the metals and allow their removal from water. Besides, tellurium present on the structure, would act as an anchor for the fixation of this heavy metals due to electrochemical effects.

2. First aim: A comparison of cytocompatibility and anticancer properties of traditional- and green-chemistry synthesized tellurium nanowires

2.1. Objective

Tellurium nanowires were synthesized using both traditional synthetic and green approaches and their properties were compared in terms of biocompatibility, antibacterial and anticancer properties. The two ways to synthesize such nanowires were as follows: (a) a clean (green) and cost-effective hydrothermal synthesis employing tellurium salt and starch and (b) a traditional approach using metallic salt and chemical synthetic reducing agents such as ammonia and hydrazine (Figure 5). After purification, the nanostructures were characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) regarding morphology, size, and composition. Cytocompatibility and anticancer properties of the chemically-synthesized tellurium nanowires (CHEM-TeNWs) and green-synthesized tellurium nanowires (GREEN-TeNWs) were compared. Cytotoxicity assays were performed over five days with both human dermal fibroblasts (HDF) cells and human melanoma cancer cells. Results showed much promise for the further study of GREEN-TeNWs compared to CHEM-TeNWs for numerous medical applications.

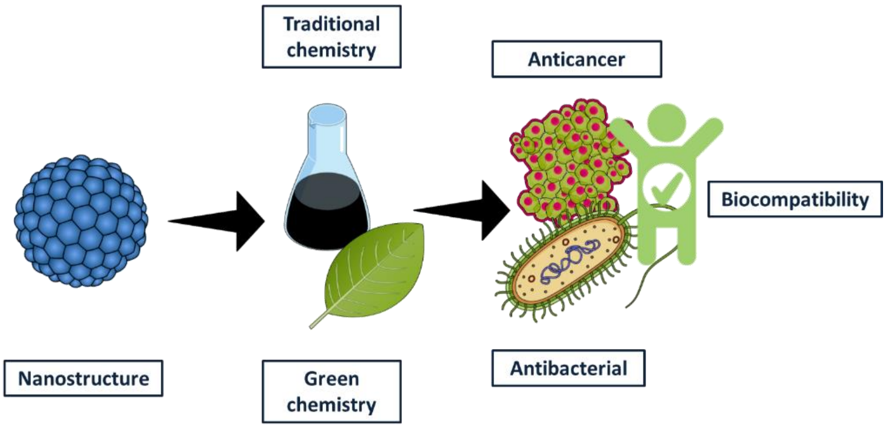

Figure 5. First Aim of the project. *Synthesis of CHEM-TeNWs and GREEN-TeNWs and their comparison in terms of biomedical applications.*

2.2. Material and methods

2.2.1. Instruments and characterization

A Heratherm™ General Protocol Oven (Thermo Scientific™) was used to produce the hydrothermal reaction in both chemical and green methodologies. An Eppendorf™ Model 5804-R Centrifuge was used for the centrifugation of samples. A FreeZone Plus 2.5 Liter Cascade Console Freeze Dry System was used to purify the samples and obtain the final tellurium nanowire structures.

TeNWs prepared by both chemical synthetic and green approaches were properly characterized via a JEM-1010 transmission electron microscope (TEM) (JEOL USA Inc., Peabody, MA). For sample preparation, purified nanostructures were air-dried on 300-mesh copper-coated carbon grids (Electron Microscopy Sciences, Hatfield, PA). The samples were then imaged up to an 80,000x magnification with an accelerating voltage of 80.0 kV.

Energy dispersive X-ray spectroscopy analysis was performed using a dedicated EDS detector coupled with a Hitachi S-4800 SEM. TeNW samples were affixed to 300-mesh copper coated carbon grids and placed into an aluminum pin mount. An accelerating voltage of 10.0 kV was used to obtain an elemental spectrum for the nanoparticles.

2.2.2. Tellurium nanowire synthesis and purification

Following a variation of the protocol described by Hong et al. [80], in a typical process of integration, sodium tellurite (Na_2TeO_3) (Sigma Aldrich, St. Louis, MO) was mixed with 1 g of polyvinylpyrrolidone (PVP) (Sigma Aldrich, St. Louis, MO) and dissolved in 30 mL of deionized water. Next, 1.5 mL of hydrazine hydrate (Sigma Aldrich, St. Louis, MO) and 3 mL of an ammonia (NH_3) (Sigma Aldrich, St. Louis, MO) solution

(25% w/w) were added. The solution was stirred at room temperature and then transferred into a Teflon-lined stainless-steel reactor and placed into an oven at 180°C for 4 h. Right after the reaction, the mixture was allowed to cool down to room temperature.

For the green synthesis route, the process was followed as described by Lu et al. [81] with some modifications. Briefly, telluric acid (H_2TeO_4) (Sigma Aldrich, St. Louis, MO) was mixed with 0.15 g of a starch (Sigma Aldrich, St. Louis, MO) solution in deionized water. Then, the mixture was transferred into a Teflon-lined stainless-steel reactor and placed into an oven at 160°C for 15 hours. The mixture was allowed to cool down at room temperature. Equation 1 shows the equation of both synthetic approaches.

$$Na_2TeO_3 + PVP + NH_3 / N_2H_4 \xrightarrow{4h/180^\circ C} TeNWs \qquad (1)$$

$$H_2TeO_4 + (C_6H_{10}O_5)_n \ (H_2O) \xrightarrow{15h/160^\circ C} TeNWs \qquad (2)$$

Equation 1. Reaction for the synthesis of TeNWs by chemical synthesis (1) and green synthesis (2).

Once at room temperature, the final products from both synthesis methods were purified using the same protocol. The nanowire solutions were centrifuged at 10,000 rpm for 20 min, and the pellet was subsequently washed twice with water and centrifuged again at the same rate and time. Finally, the precipitate was resuspended in 35mL of deionized water. The resulting solution containing the nanowires was transferred into a 20 mL glass vial, which was then placed in a freezer at -80ºC for 4 h and lyophilized overnight. The final powder was weighed and re-suspended in a suitable amount of deionized and autoclaved water to reach the final concentration needed for further experiments. Figure 6 shows a summary of the process.

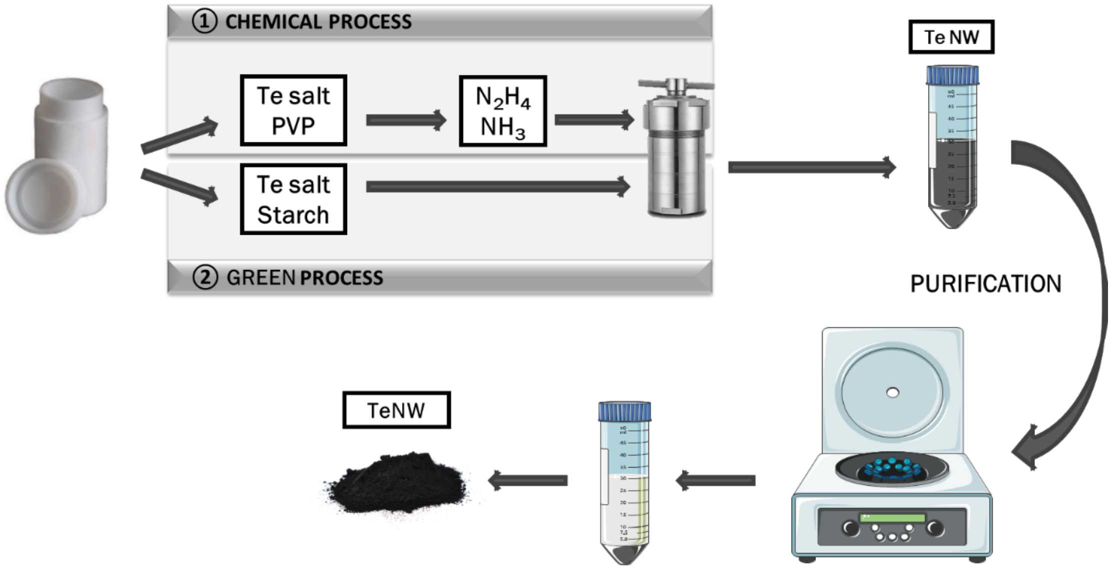

Figure 6. Methodology for the synthesis and purification of both CHEM-TeNWS and GREEN-TeNWs.

2.2.3. *In vitro* cytotoxicity assays with TeNWs

Cytotoxicity assays were performed with primary human dermal fibroblast cells (Lonza, CC-2509, AMP) and human melanoma cells (ATCC® CRL-1619, Manassas, VA). The cells were used until a passage number of 8, all materials were sterilized using ethanol. Besides, NWs solutions were prepared in deionized and autoclaved water. 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 5000 cells per well in 100 µL of cell culture medium. An incubation period of 24 h at 37°C in a humidified incubator with 5% carbon dioxide (CO_2) was employed. The culture medium was then replaced with 100 µL of fresh cell culture medium containing concentrations from 5 to 100 µg/mL of either CHEM-TeNWs or GREEN-TeNWs.

The cells were cultured for three different periods of time to compare the effects of the nanostructures on the cells after the first, third and fifth day following exposure. The cells were washed with PBS, and the original media was replaced with 100 µL of the 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 in the incubator to allow for a color change. Then, the absorbance was measured at 490 nm on an absorbance plate reader (SpectraMAX M3, Molecular Devices) for cell viability after exposure to the TeNWs. Cell viability was calculated by dividing the average absorbance obtained for each sample by that of the control sample and then multiplied by 100. Controls containing cells and media, just media and nanowires in media, were also included in the 96-well plate to identify the healthy growth of cells without nanowires and determine the absorbance of both the media and the nanowires.

2.2.4. Statistical analysis

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

2.3. Results and discussion

2.3.1. Synthesis of green biogenic TeNWs

The hydrothermal synthesis of TeNWs was successfully completed using both chemical and green synthesis protocols. The methods are compared below in Table 1.

Table 1. Synthesis conditions of the protocols used to make TeNWs. *Summary of the differences in between the two approaches regarding the reducing agent and reaction conditions.*

Reaction features	**CHEM-TeNWs**	**GREEN-TeNWs**
Reducing agents	$PVP/NH_3/N_2H_4$	Starch
Conditions	180°C/4h	160°C/15h

One of the most remarkable differences between the two synthesis methods is the relative cost and quantity of the reducing agents needed to reduce ionic tellurium to elemental tellurium. While CHEM-TeNWs were generated in an aqueous solution with the presence of three reducing agents (PVP, hydrazine, and ammonia), the GREEN-TeNWs were reduced using only starch, which is both cheaper and much more environmentally friendly.

In the chemical synthesis, using hydrazine and ammonia raise a huge safety concern when performing this process, both are flammable, extremely toxic and hazardous. Hydrazine in particular produces explosive vapors/air mixtures at about 38ºC. Besides, it is corrosive to metals and skin and a real threaten to the environment. Face shields and full-face respirators are needed to work with this chemical. PVP is however a water-soluble polymer that contributes to the formation of the nanostructures and it is FDA approved.

On the other hand, the green synthetic approach only needs starch which is clearly a safe, naturally-occurring reducing and stabilizing agent. This polymeric carbohydrate consists of a large number of glucose units that can reduce the tellurium ions to their elemental form. Therefore, because no additional agent is needed, this method holds advantages over the chemical synthesis method in both cost and safety. Another concern to note is the production of toxic by-products. While waste produced from CHEM-

TeNWs synthesis should be considered hazardous, the generation of reaction-derived products from the green synthesis process developed should not be viewed as an environmental threat.

Regarding the time for the reactions, the chemical synthesis of TeNWs took 4 h at 160ºC while the green process took 15 h at 180ºC. This extra time can be explained by the reducing power of starch, which is lower than using hydrazine, ammonia, and PVP; however, taking into account the hazards of the chemicals and by-products of the chemical synthesis approach, the green methodology still has plenty of advantages such as being more economical, easy, and following a straightforward hydrothermal synthesis method. Nevertheless, the green-synthetic approach employed can be made quicker since the time used for the growth of the nanowires can be reduced to a couple of hours. It was hypothesized that the density of wires would be less than that using 15 hours, however, this is a hypothesis that needs further development.

2.3.2. Characterization

Morphological characterization was completed for both synthesis approaches with size and coating characteristics reported in Table 2.

Table 2. Data from Transmission Electron Microscopy (TEM) for size and coating.

Structures	**CHEM-TeNWs**	**GREEN-TeNWs**
Length	Several microns	Several microns
Width	32±11 nm	25±8 nm
Coating	No	Yes

Specifically, CHEM-TeNWs (Figure 7, A-B) had an average diameter of 30 nm with a length of several micro-meters. The wires showed a low degree of aggregation. On the other hand, GREEN-TeNWs (Figure 7, C-D) showed an average diameter about 20 nm with similar length. The peculiarity of these structures came from their morphology: it seemed that nanowires started growing from a particular point and they extended as far as they could. The main trunk is divided into several smaller sections at the end of the structure as ramifications, which produced branches that grew alongside one another.

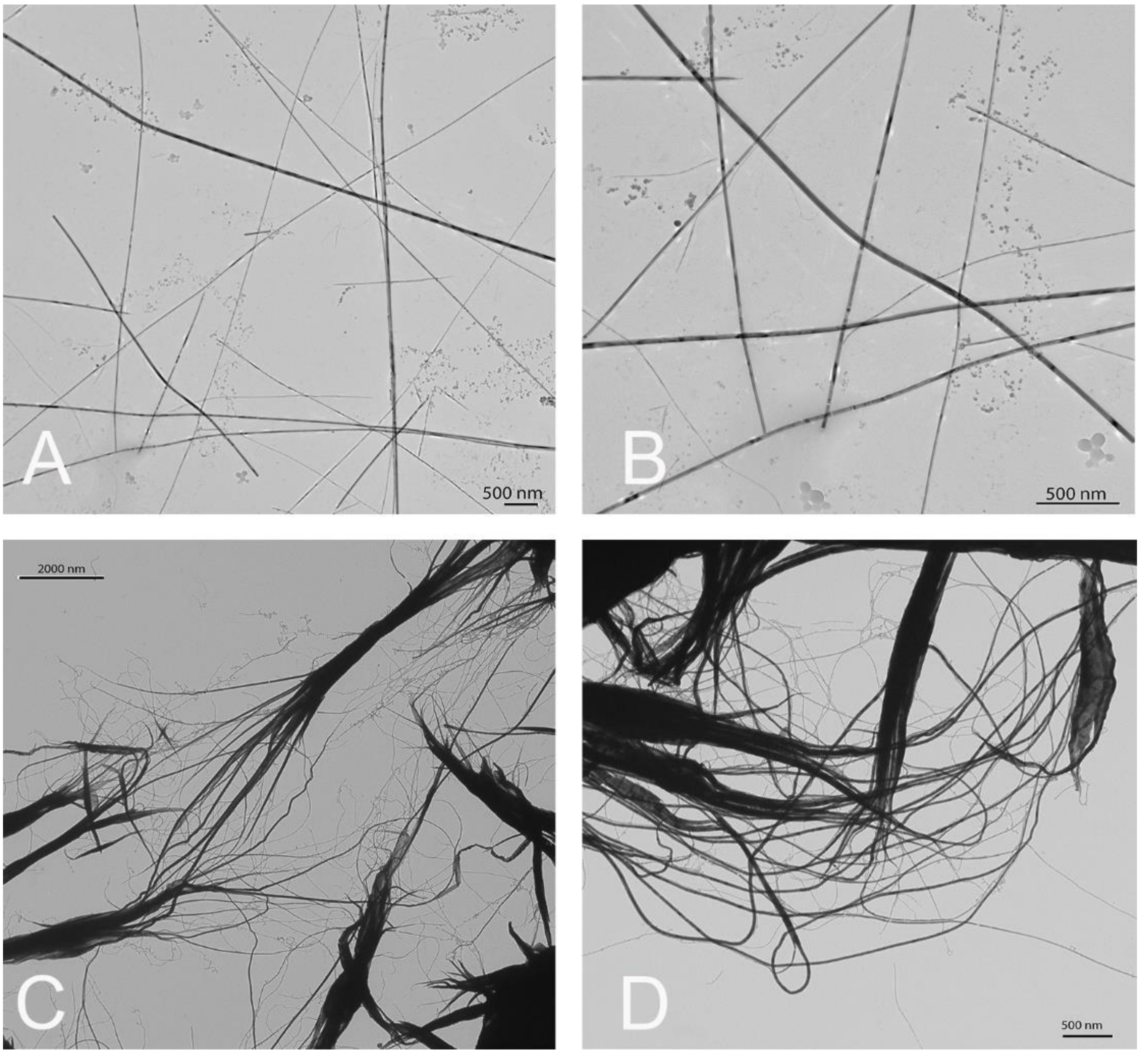

Figure 7. Transmission electron microscopy (TEM) images of TeNWs. *CHEM-TeNWs with a low degree of aggregation were found dispersed within the solution (A and B). GREEN-TeNWs (C and D) showed a higher degree of aggregation and the presence of an organic-coating was noticed.*

SEM characterization was done on the GREEN-TeNWs (Figure 8). The wires started growing from a cluster and extended for several micrometers, with a star-shaped structure that was difficult to notice when the clusters were close to one another.

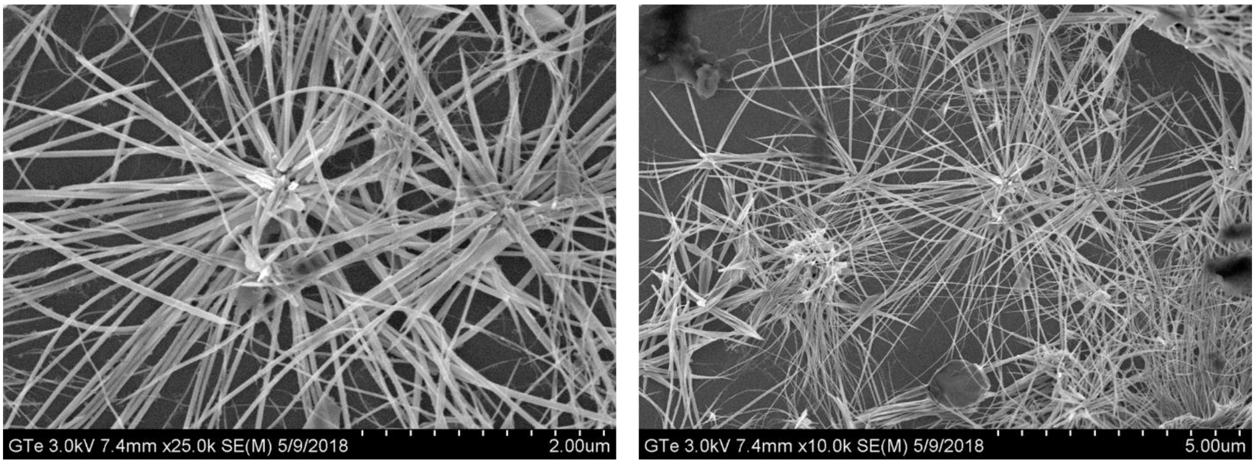

Figure 8. Scanning Electron Microscopy (SEM) images of GREEN-TeNWs. *GREEN-TeNWs grew with a star-shaped structure.*

EDX measurements were completed for both the chemically and green-synthesized nanostructures. For CHEM-TeNWs (Figure 9A), EDX analysis showed that the electron-dense nanowires had specific tellurium absorption peaks. For GREEN-TeNWs, the peaks for tellurium were higher due to the higher concentration of tellurium within the sample (Figure 9B). When the analysis focused on the surrounding areas of the nanostructures, the carbon peak was significantly raised compared with the measurement of the tellurium metallic core. This, aside from the absence of a tellurium peak, indicated an organic composition of the coating (Figure 9C).

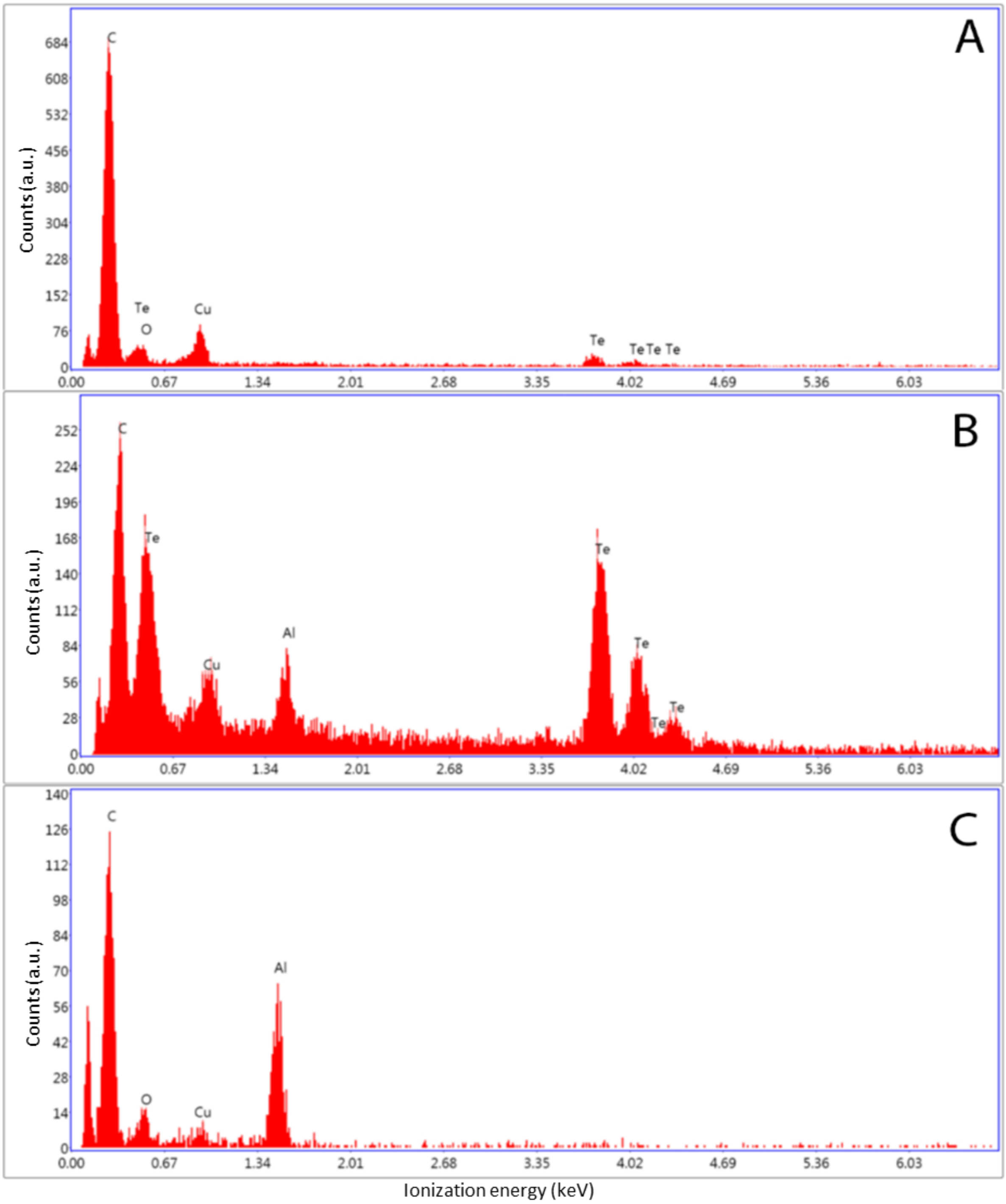

Figure 9. Energy Dispersive X-Ray (EDX) of TeNWs. *CHEM-TeNWs were characterized (A), as well as GREEN-TeNWs in both the metallic core (B) and the surrounding coating (C).*

2.3.3. *In vitro* cytotoxicity of TeNWs

In vitro cytotoxicity assays were performed with human dermal fibroblast (HDF) cells and melanoma cells (ATCC® CRL-1619, Manassas, VA). Data from the nanowire treatment were compared with a control that contained just cells and media. Further comparison was made between HDF and melanoma cells with the aim to determine potential anticancer activity. The same experiments were performed using chemical and green-synthesized nanowires to determine the effect of the natural coating present on GREEN-TeNWs on the proliferation of the cells.

For HDF experiments, nanowires with a concentration between 5 and 100 µg/mL were tested. For CHEM-TeNWs, the same proliferation trend was observed within the 3rd and 5th day for concentrations up to 15 µg/mL compared to the control (Figure 10A). However, the number of cells were less than those for the control in all the cases. Larger concentrations showed degeneration of the cell proliferation. When GREEN-TeNWs were tested on the cells, a similar proliferation trend was observed for each of the concentrations (Figure 10B). An especially unusual behavior was shown at levels between 15 and 75 µg/mL, with a higher number of cells growing within the 3rd and the 5th day compared with the first 24 h. When both nanostructures were compared, it can be concluded that the green-synthesized structures enhanced cell proliferation over a broader range of concentrations compared to the chemical synthetic ones.

The cytotoxic effect of tellurium nanostructures is the result of active physico-chemical interactions of elemental tellurium with the functional groups of intracellular proteins and the bases and phosphate groups in DNA [40]. While cytotoxicity was apparent for the CHEM-TeNWs (Figure 10A), we hypothesized that the enhanced biocompatibility in GREEN-TeNWs (Figure 10B) is most likely due to the presence of a natural, organic coating that encompasses the tellurium core. The presence of a carbon layer as the natural coating introduces a biodegradable material that can enhance cell proliferation.

When melanoma cells were treated with CHEM-TeNWs, cell proliferation showed a similar trend as that of the control at concentrations up to 25 μg/mL (Figure 10C). GREEN-TeNWs showed similar behavior (Figure 10D). Nonetheless, levels between 10 and 100 μg/mL showed a delay in the cell proliferation compared to the control over the tested time period. It has been hypothesized that TeNWs have the potential ability to slow down the signaling processes present in cancerous cells [88, 89]. Therefore, we propose that both synthesis methods produce nanostructures with anticancer properties; but, specifically, this behavior was particularly enhanced in the case of the green-synthetic structures.

In this report, the cytocompatibility and anticancer activity of tellurium nanostructures were observed with a potentially improved performance using GREEN-TeNWs. Thus, not only were the GREEN-TeNWs produced with significantly less toxic materials, but their properties towards enhancing healthy cell proliferation and decreasing cancer cell proliferation were greater.

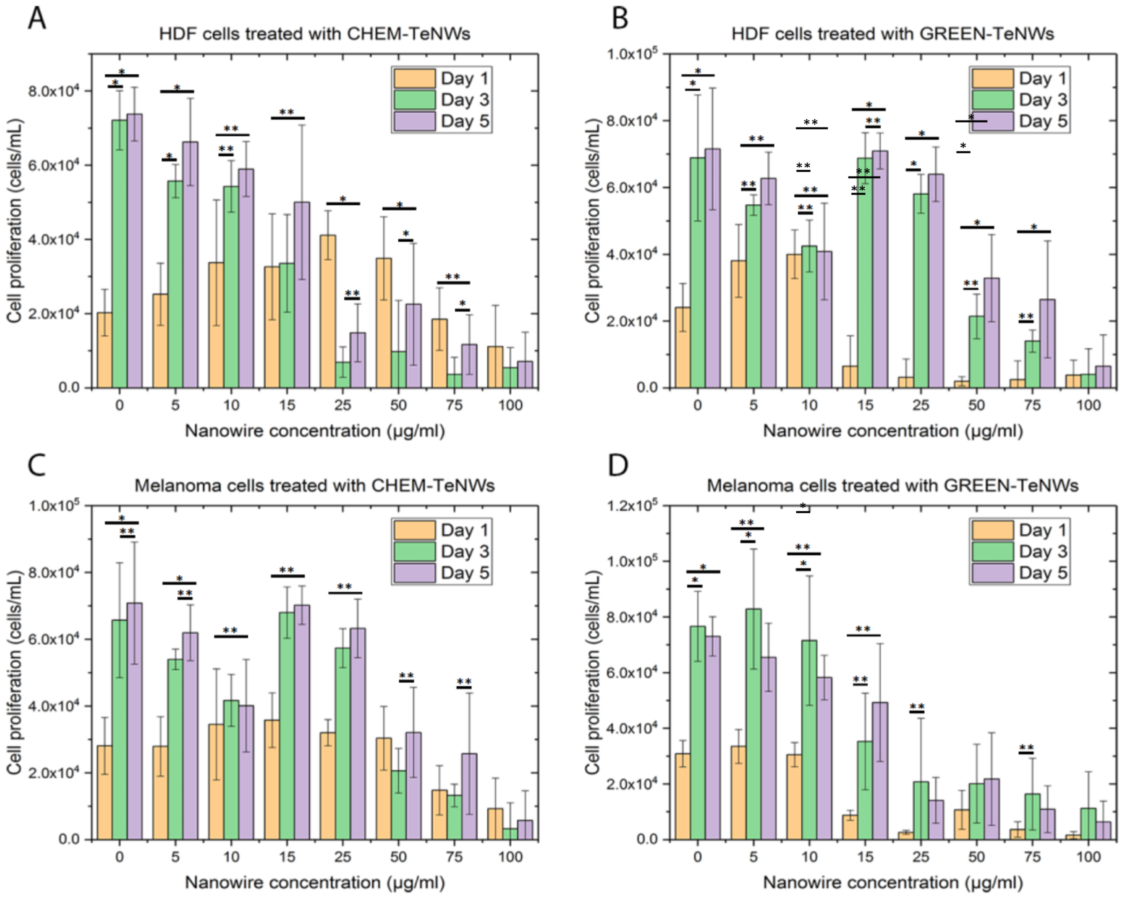

***Figure 10**. MTS assays on human dermal fibroblasts (A,B) and melanoma (C,D) cells in the presence of chemically synthesized (A,C) and green-synthesized (B,D) Te nanowires at concentrations ranging from 5-100 µg/mL.* *N=3. Data is represented as mean ± SD; *p<0.01, **p<0.005.*

Comparison was done at the fifth day of the experiments between samples to show the tendency of both cell compatibilities with HDF cells (Figure 11, A) and cytotoxic effect with melanoma cells (Figure 11, B).

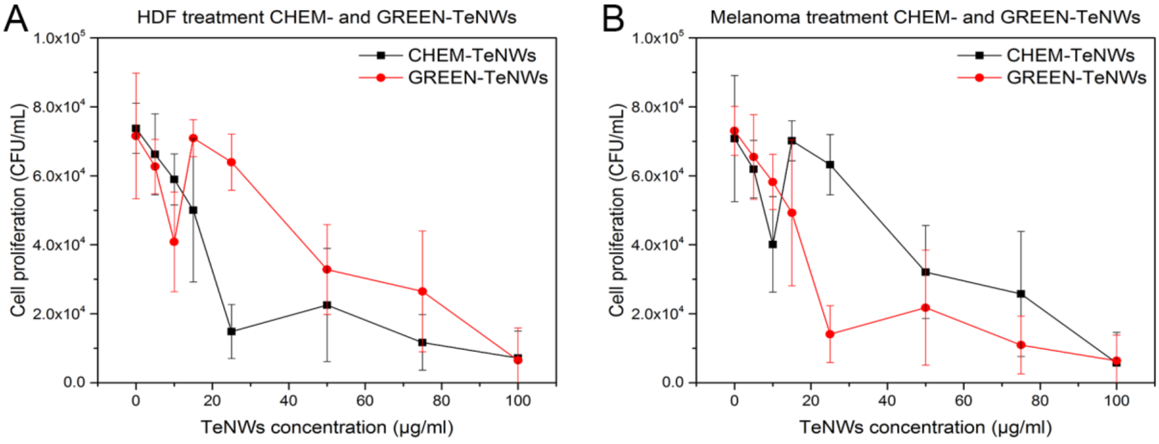

***Figure 11. Comparison between CHEM- and GREEN-TeNWs for HDF (A) and melanoma (B) cells at the fifth day of experiments**. Data from MTS assays on human dermal fibroblasts and melanoma) cells in the presence of TeNWs. N=3. Data is represented as mean ± SD; *p<0.01, **p<0.005.*

The low cytotoxicity for HDF cells can be appreciated especially at high concentrations of TeNWs, with a noticeable difference between CHEM-TeNWs –with higher cytotoxicity for HDF- and GREEN-TeNWs, that allow a high cell proliferation. At low concentrations –between 5 and 15 µg/mL- the difference in terms of biocompatibility was almost inappreciable. The biggest difference happened at a concentration of 25 µg/mL. For experiments with cancerous cells, the opposite behavior took place. GREEN-TeNWs did not allow the proliferation of melanoma cells at concentrations ranging between 15 and 100 µg/mL. As happened with HDF cells, the bigger difference happened at the concentration of 25 µg/mL. Therefore, it was possible to hypothesize that at a GREEN-TeNWs concentration of 25 µg/mL, both the maximum biocompatibility for HDF and the maximum cytotoxic effect for melanoma cells was achieved in comparison with the same concentration of CHEM-TeNWs. As a general view, the behavior of GREEN-TeNWs after 5 days in terms of low and high cytotoxicity for HDF and melanoma cells respectively was enhanced in comparison with CHEM-TeNWs.

IC_{50} values were calculated for all the experiments, with the aim to show the minimum inhibitory concentration for both HDF and melanoma cells. This value was obtained after 5 days of experiment, measuring the potency of the nanowires to inhibit the normal biological functioning of the cells.

Table 3. IC_{50} values for both CHEM- and GREEN-TeNWs in experimental assays with HDF and melanoma cells after 5 days of experiment.

Cell assay	CHEM-TeNWs	GREEN-TeNWs
HDF	60.22±15.25 µg/mL	70.05±10.58 µg/mL
Melanoma	63.14±6.078 µg/mL	16.46±1.96 µg/mL

2.4. Conclusions

Nanotechnology has taken advantage of traditional chemical synthesis methods, both physical and chemical, to solve many of the biomedical and technological challenges that face society today. Nevertheless, the cost regarding efficiency and to the environmental are high. Concerns such as the production of toxic by-products and harsh reaction conditions should be addressed. Therefore, new and alternative approaches are on the rise. One of the most promising comes from the use of green chemistry.

In this work, we successfully explored and compared two different synthesis approaches (a traditional chemical method and a green chemistry method) to elucidate the differences between the cytocompatibility and anticancer behavior of the respective nanoparticles produced from each process. Experiments with healthy fibroblasts and cancerous melanoma cells were completed over a range of concentrations between 5 and 100 µg/mL, and showed both an improvement in the cell proliferation of HDF cells and a decrease in the proliferation of cancerous cells when GREEN-TeNWs were compared to CHEM-TeNWs. An enhancement of healthy fibroblast proliferation was observed for the green synthesis of TeNWs compared to the CHEM-TeNWs, which suggests that these should be explored for a wide range of medical applications. Therefore, we propose that the present green synthetic approach offers important improvements in terms of safety, economical, efficiency and biocompatibility for numerous biomedical applications, overcoming the main drawbacks of traditional Te nanowire chemical approaches.

3. Second aim: Novel method of synthesis of metallic nanoparticles on the top of tellurium nanowires using a green synthetic approach

3.1. Objective

As probed beforehand, GREEN-TeNWs are known to have potential biomedical applications with enhanced biocompatibility, anticancer and antimicrobial properties in comparison to the traditional synthetic approach. Moreover, they present an organic coating made of starch which is known to be a reducing agent. Therefore, the second objective seeks to take advantage of the potential of these structures for producing metallic nanoparticles. GREEN-TeNWs have been used as a template for the growth of metallic NPs. A new methodology of synthesis of NPs has been developed using the GREEN-TeNWs and different metals, such as palladium (Pd) or platinum (Pt), for biomedical applications (Figure 12). After the rapid, cost-effective and simple reaction – it took one minute at room temperature without the use of any extra reducing agent – both NPs and the synergy of NWs and NPs were purified and characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) regarding morphology, size, and composition. Biocompatibility, anticancer and antibacterial properties of the NWs and the NW-NP synergies have been evaluated and compared using human dermal fibroblasts (HDF) cells, melanoma cells, and *E. coli* and *S. aureus*, respectively, with the aim of developing a better understanding of how these entities could improve the biomedical field.

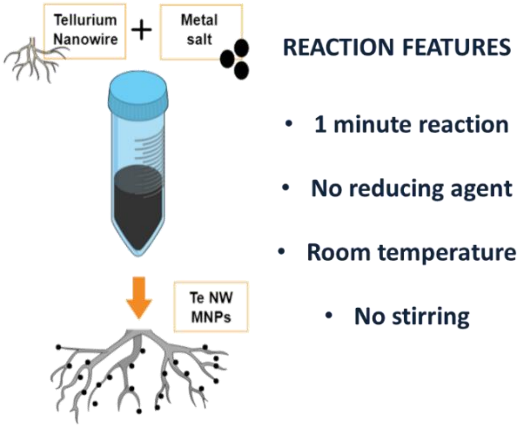

Figure 12. Second aim of the project. *Synthesis of metallic NPs on the top of the GREEN-TeNWs*

3.2. Material and methods

3.2.1. Instruments and characterization

A sonicator (Brandon M1800 Ultrasonic) was used to allow the release of NPs from the NW structure. An Eppendorf™ Model 5804-R Centrifuge was used for the centrifugation of samples. A FreeZone Plus 2.5 Liter Cascade Console Freeze Dry System was used to purify the samples and obtain the final solid nanostructures.

For the characterization of both NPs and the synergy NP–NW, a JEM-1010 TEM (JEOL USA Inc., Peabody, MA) was used. In order to prepare the samples for imaging, the purified nanostructures were air-dried on 300-mesh copper-coated carbon grids (Electron Microscopy Sciences, Hatfield, PA). Finally, the samples were imaged up to an 80,000x magnification with an accelerating voltage of 80.0 kV.

A SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA) was used to measure the optical density in bacteria and absorbance in cells. Growth curves and other bacterial assays were performed in a plate reader SpectraMax® Paradigm® Multi-Mode Detection Platform.

3.2.2. NP synthesis and purification

The process of synthesis was carried out using different metallic salts in order to produce different metallic NPs over the TeNWs. The metals were chosen due to their potential for use in the biomedical industry.

On the one hand, noble metals were used: palladium (Pd), platinum (Pt), gold (Au) and silver (Ag). Palladium is a noble metal known to be used in catalytic and optical industries, however, in the recent years it has been gaining interest for its potential as an antimicrobial agent against bacteria like *S. aureus* and *E. coli* and also as an anticancer agent for pancreatic and colorectal cancers with barely no cytotoxicity associated [90]. Platinum has been used for years as a potential anticancer agent in many forms like carboplatinum or cisplatinum, nevertheless, the small and spherical-shaped nanoparticles of this metal are nowadays largely used as a more efficient and effective treatment against different cancers such as breast or melanoma [91]. Besides, the excellent biocompatibility and optical properties – they can absorb infrared light – of gold nanoparticles make them perfect to be used in many applications such as drug delivery, imaging and especially, for cancer treatment as breast or pancreatic ones [92]. Lastly, it has largely been reported that silver nanoparticles present strong antimicrobial properties against various genera of bacteria such as *E. coli* and *S. aureus.* In addition, they are also known to present antifungal to *C. albicans* and anticancer activity towards glioblastoma or breast cancer [12].

On the other hand, rare metalloids as tellurium (Te) and selenium (Se) were also used for the synthesis of nanoparticles. As said beforehand, the biological role of tellurium is largely unknown but after demonstrating their potential biomedical applications – regarding biocompatibility, anticancer and antibacterial properties –of GREEN-TeNWs we considered this metal a good candidate. Similarly, selenium nanoparticles have been reported to present unique antimicrobial properties against different bacteria such as *P. aeruginosa* and antiviral properties against virus like H1N1 influenza, due to their role in enzyme modulation and reactive oxygen species (ROS) elimination [93].

Briefly, in a typical process of synthesis, 1 mL of a 5 mg/mL metallic salt solution was mixed with 1 mL of a GREEN-TeNWs solution after the reaction – GREEN-TeNWs were synthesized following the procedure described in section 2.2 – and rinsed with 15 mL of distilled water. The reaction took place at room temperature for 1 min without any need for stirring. Conditions can be found on table 4 were the summary of the variety of reagents used are listed depending on the desired NP.

Table 4. Reagents used on the synthesis of Pd, Pt, Au, Ag, Se and TeNPs. *All chemicals were purchased at Sigma Aldrich, St. Louis, MO.*

Type of NP	Metallic salt
Palladium NP	Palladium chloride ($PdCl_2$)
Platinum NP	Potassium tetrachloropalladate (K_2PtCl_4)
Gold NP	Gold chloride ($AuCl_3$)
Silver NP	Silver nitrate ($AgNO_3$)
Selenium NP	Sodium selenite (Na_2SeO_3)
Tellurium NP	Sodium tellurite (Na_2TeO_3)

Right after the reaction, the mixture was purified, NPs and the synergy – composed by the NPs that have grown on the top of the NWs and remained on it and the GREEN-TeNWs structure – were purified separately using different methodologies to ensure better throughput of each part.

On the other hand, so as to obtain the synergy NPs – GREEN-TeNWs, the resulting solution after reaction was centrifuged at 10,000 rpm for 20 min. The subsequent pellet was washed twice with water and centrifuged again at the same rate and time. The final solution containing the synergy NPs – NWs, was stored on a 20 mL glass vial which was located on the freezer 80ºC for 4 h and lyophilized for 48 h.

To obtain the NPs separately from the GREEN-TeNWs, the resulting solution after reaction was sonicated for 30 min at 60ºC. After that, the product was centrifugated at 10,000 rpm for 30 min, the resulting supernatant was transferred into a 20 mL glass vial – which contained the NPs – then placed in a freezer at -80ºC for 4 h and lyophilized for 48 h.

In both cases, the final powders obtained on the freeze dryer, were weighed and re-suspended in a suitable amount of deionized and autoclaved water to reach the final concentration needed for further experiments.

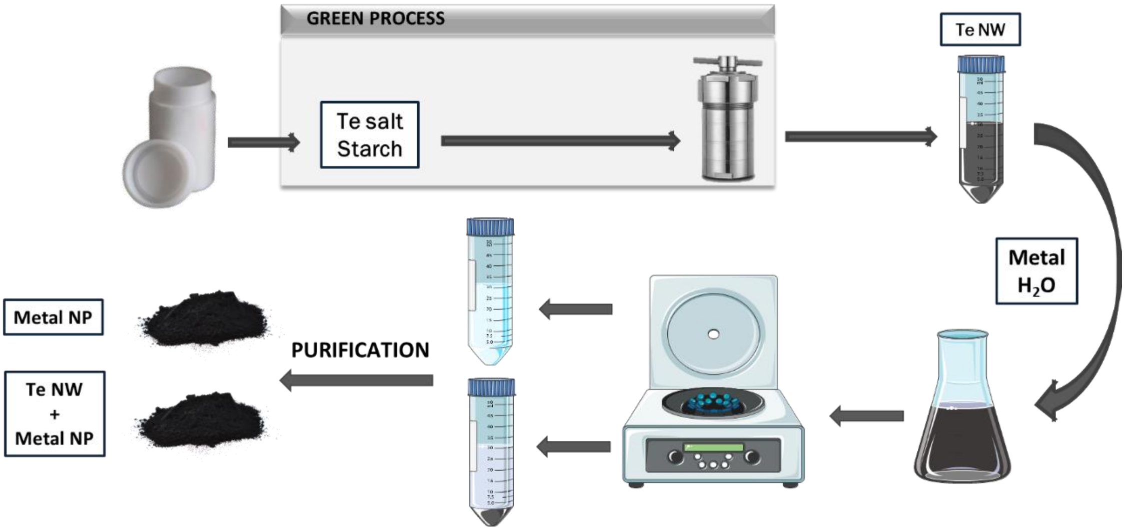

Figure 13. Methodology for the synthesis and purification of both NPs and the synergy NPs – NW.

3.2.3. Bacterial assays

Escherichia coli (strain K-12 HB101; Bio-Rad, Hercules, CA) and *Staphylococcus aureus* subsp. Aureus Rosenbach (ATCC® 12600™) were used to determine the antimicrobial activity of the synergy NP–NW using palladium (Pd) and platinum (Pt), hence, the samples were named PdNP-TeNWs and PtNP-TeNWs, respectively. A colony of each bacteria was re-suspended in tryptic soy broth (TSB) media, and the resulting bacterial solution was placed then in a shaking incubator overnight at 200 rpm and 37°C to enable the bacteria to grow.

Subsequently, the overnight suspension was diluted to a concentration of 10^6 colony forming units per milliliter (CFU mL^{-1}), using optical density measurements at 600 nm (OD_{600}) in a spectrophotometer.

Antimicrobial tests

Bacterial assays were prepared in order to elucidate the antimicrobial activity of the two systems in this study: nanoparticles produced on top of GREEN-TeNWs, the synergy GREEN-TeNWs (system 1) and GREEN-TeNWs (system 2). For the antimicrobial assays, different concentrations of the synergy were mixed with 100 μL of bacteria in TSB media and was added to each well of a 96-well plate (Thermo Fisher Scientific, Waltham, MA). No nanostructures were added on the controls. The final volume per well was 200 μL.

Then, the absorbance was measured at 600nm on a plate reader every 2 min for 24h. Controls containing bacteria and media, without the nanostructures, were used to identify the absorbance caused by the nanostructures.

The resulting bacterial growth curves were shifted to start at the origin by subtracting the initial value from the entire curve and fitted with the modified Gompertz model. The Gompertz distribution could be applied to antimicrobial assays by a change of parameters in the growth curve. The original equation (Equation 2) contains mathematical parameters (*a, b c…*) which need to be transformed into biological ones

(A, μ, and λ). Moreover, 95% confidence intervals would not be possible to calculate in biological parameters if there is no re-parametrization. The Gompertz equation was rewritten (Equation 3) by deriving an expression of the biological parameters as a function of the mathematical ones and then substituting them into the original formula, thus the new equation contains mathematical parameters with biological meaning: y, the optical density meaning, which describes the amount of bacteria, A is the maximum value of y, μ corresponds to the maximal growth rate, and λ is the lag time.

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

Equation 2. Gompertz equation in terms of mathematical parameters

$$y = A \cdot e^{\left(-e^{\left(\frac{\mu \cdot e}{A}(\lambda - t)+1\right)}\right)}$$

Equation 3. Gompertz equation in terms of biological parameters

3.2.4. *In vitro* cytotoxicity assays with the synergy

Cytotoxicity assays were prepared to determine the biocompatibility and anticancer activity of the two systems to study: nanoparticles produced on top of GREEN-TeNWs, the synergy GREEN-TeNWs (system 1) and GREEN-TeNws (system 2) – system 2 was analyzed in section 2.3.3., thus the results were taken from there.

Primary human dermal fibroblast cells (strain CC-2509, Lonza, AMP) and human melanoma cells (strain CRL-1619, ATCC®, Manassas, VA) were used to determine the cytotoxicity of the synergy NP–NW using Pd (PdNP-TeNWs) and Pd (PtNP-TeNWs). The cells were used until a passage number of 8 and all materials were sterilized using ethanol. Besides, NPs-NWs solutions were prepared in deionized and autoclaved water. 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 5000 cells per well in 100 µL of cell culture medium and incubated for 24 h at 37°C in a humidified incubator with 5% carbon dioxide (CO_2). The culture medium was then replaced with 100 µL of fresh cell culture medium containing concentrations from 5 to 100 µg/mL of either PdNP-TeNWs or PtNP-TeNWs.

The cells were cultured for a 24h-period using the same conditions and then washed with PBS. After that, the media was replaced with 100 µL of the MTS solution (prepared using a mixing ratio of 1:5 of MTS: Medium). Then, the 96-well plate was incubated for another 4 h in the incubator in order to allow for change in coloration. The absorbance was measured at 490 nm on an absorbance plate reader (SpectraMAX M3, Molecular Devices) for cell viability after exposure to the synergy NP - TeNWs. Cell viability was calculated by dividing the average absorbance obtained for each sample by that of the control sample and then multiplied by 100. Controls which contained cells and media and just media were also included in the 96-well plate to identify the healthy growth of cells without nanostructures and determine their absorbance.

3.2.5. Statistical analysis

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

3.3. Results and discussion

3.3.1. Synthesis of NPs on top of the TeNWs

With the aim to understand the mechanism formation of the NPs on the top of the NWs and try to produce as many kinds of metallic nanostructures as possible, eight different metals were tested: Platinum (Pt), Palladium (Pd), Gold (Au), Silver (Ag), Selenium (Se) and Tellurium (Te) due to their reported biomedical applications.

It has been hypothesized that starch – present over the NWs as a natural organic coating due to the green synthetic approach (section 2.3.2) – acts as a reducing agent and allows for the formation of the metallic NPs. Besides, there is a belief that the NWs perform as a metallic anchorage for their synthesis, favoring the reduction process on their surface. Therefore, the reaction is thermodynamically favored, providing a straightforward and quick methodology for the synthesis of metallic NPs using a green synthetic approach. It can be stated that this route produces metallic NPs such as the ones mentioned beforehand, in one minute at room temperature and without the use of any reducing agent, other than the natural starch present on the NWs coating.

Right after the addition of the metallic salt, a change in coloration – slightly clear or brownish in the case of Pd – and cluster appearance occurred subjected to the use of different metallic salts. For instance, Figure 14 shows an example of the appearance of clusters and change in coloration after the reaction (Figure 14 B) in comparison to the conditions before the reaction (Figure 14 A). Pd, Au, Se and Te showed precipitants, whereas Pt and Ag did not. It is hypothesized that cluster appearance was related to the power of reduction needed to produce the metallic NPs. It is elucidated that, the less power of reduction consumed for the formation of a NPs, the less starch consumed, and thus the more remaining for the synergetic structure. As an example, Figure 15 shows PdNPs (Figure 15 A) surrounded by a huge amount of organic matter whereas PtNPs

(Figure 15 B) – which did not present cluster formation – only presented the coating that covers the NW structure. It can be interpreted that this organic coating agglomerated and formed these clusters containing more synergetic structures.

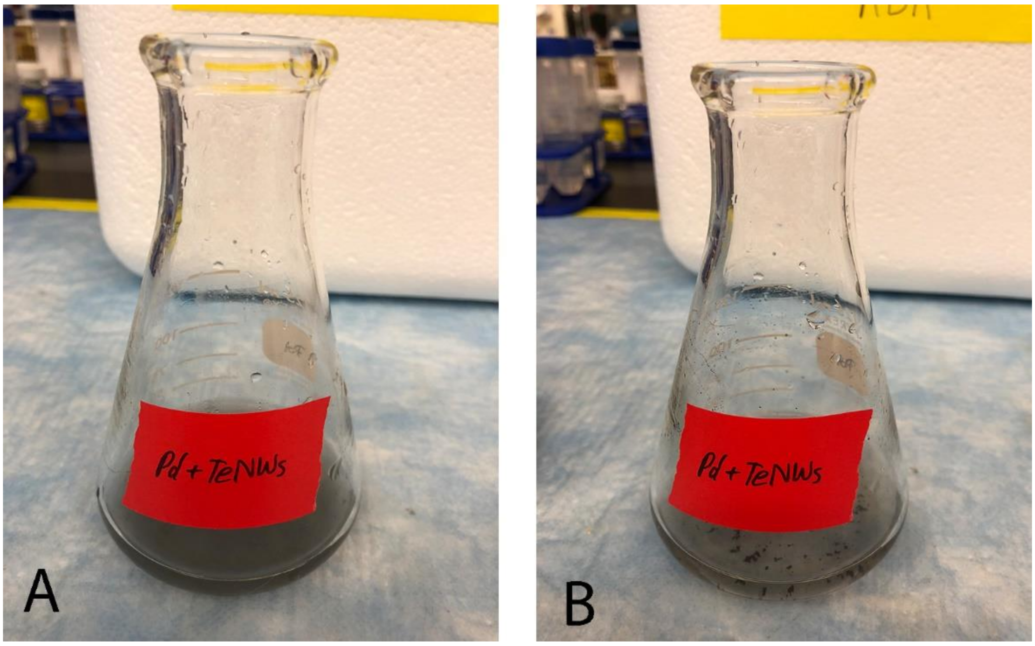

Figure 14. Palladium experiment. *When palladium chloride was mixed with an aqueous solution of GREEN-TeNWs, there was a change of coloration and appearance of clusters after 1 min reaction at RT.*

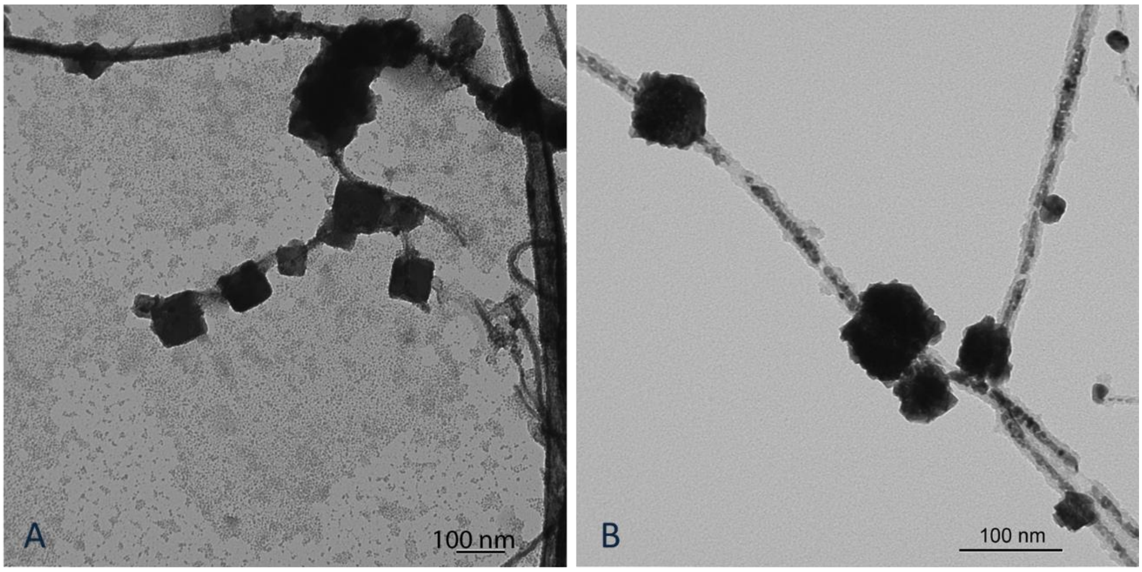

Figure 15. Organic coating present on the NPs formation*. PdNPs (A) presented a huge amount of coating, on the contrary PtNPs(B) only showed the coating which surrounded the NW structure.*

3.3.2. Characterization

TEM characterization was conducted in order to show the formation of metallic NPs on top of the TeNWs and within the surroundings right after the reaction took place. Palladium (PdNPs), platinum (PtNPs), gold (AuNPs), silver (AgNPs), selenium (SeNPs) and tellurium (TeNPs) NP – TeNWs formulations are explained and further discussed below.

Palladium nanoparticles (PdNPs)

PdNPs – small and cubic-shaped – were formed on top of the NWs, growing all over the NW without any preference (Figure 16A, B, C, D). The newly formed nanostructures were surrounded by the organic coating present on the NWs (Figure 16A and B). A high degree of release of the NPs from the NWs with no application of physical stimuli was also observed. The small NPs remained in solution with no aggregation (Figure 16, E and F). These observations suggested that the NPs remained stable and they did not form clusters over time, leading to a desired low degree of aggregation.

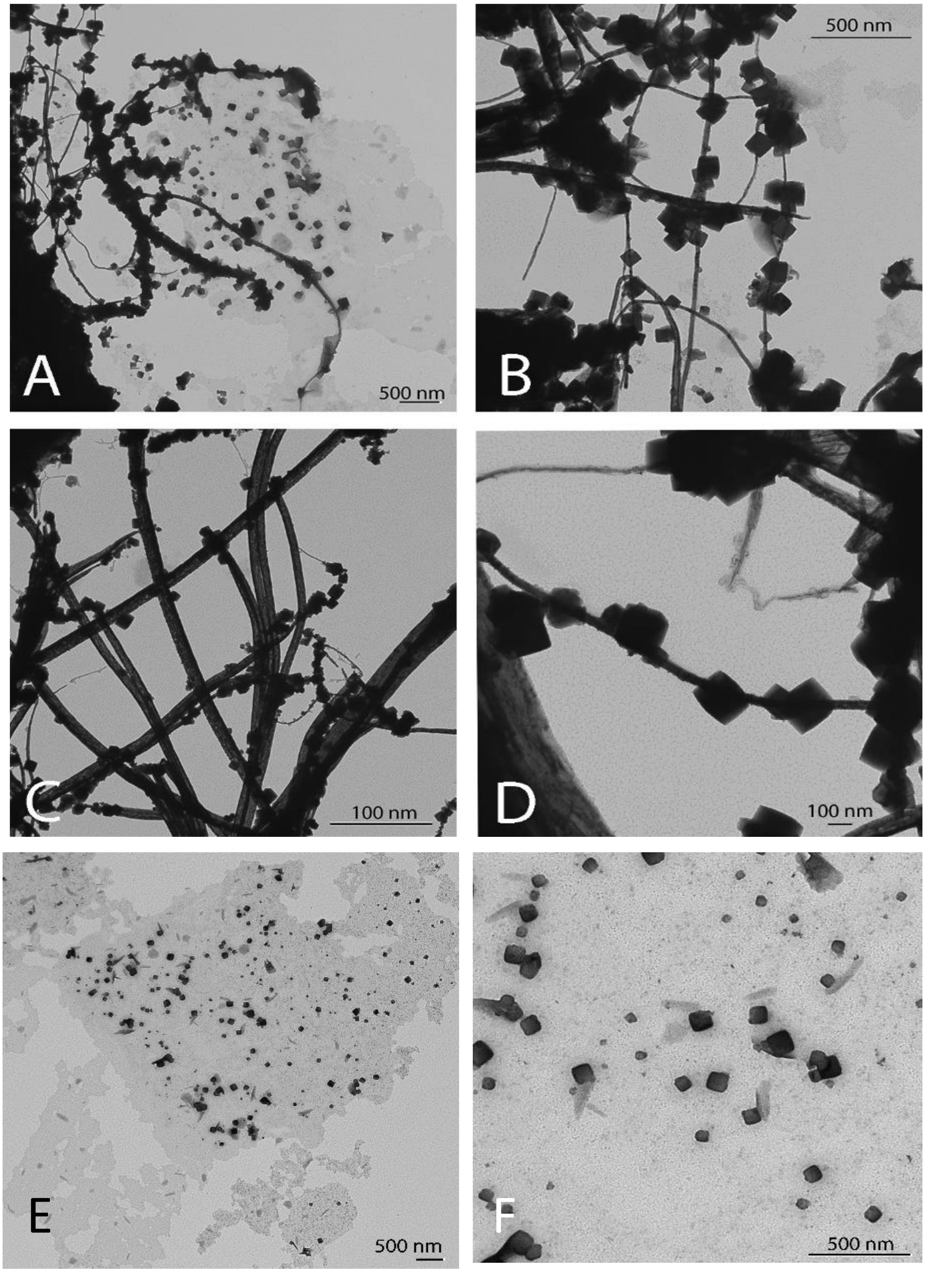

Figure 16. TEM characterization of palladium NPs. *Palladium structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

<u>Platinum nanoparticles (PtNPs).</u>

Small amorphous and spherical-shaped PtNPs were formed on top of the NWs, growing without preference on it (Figure 17A, B, C, D). The newly formed nanostructures were surrounded by the organic coating presented on the NWs (Figure 17 B). There was also observed a small degree of release without further aggregation of the NPs without physical stimuli application (Figure 17 B). NPs remained stable and did not form clusters over time as visually seen when the reaction took place.

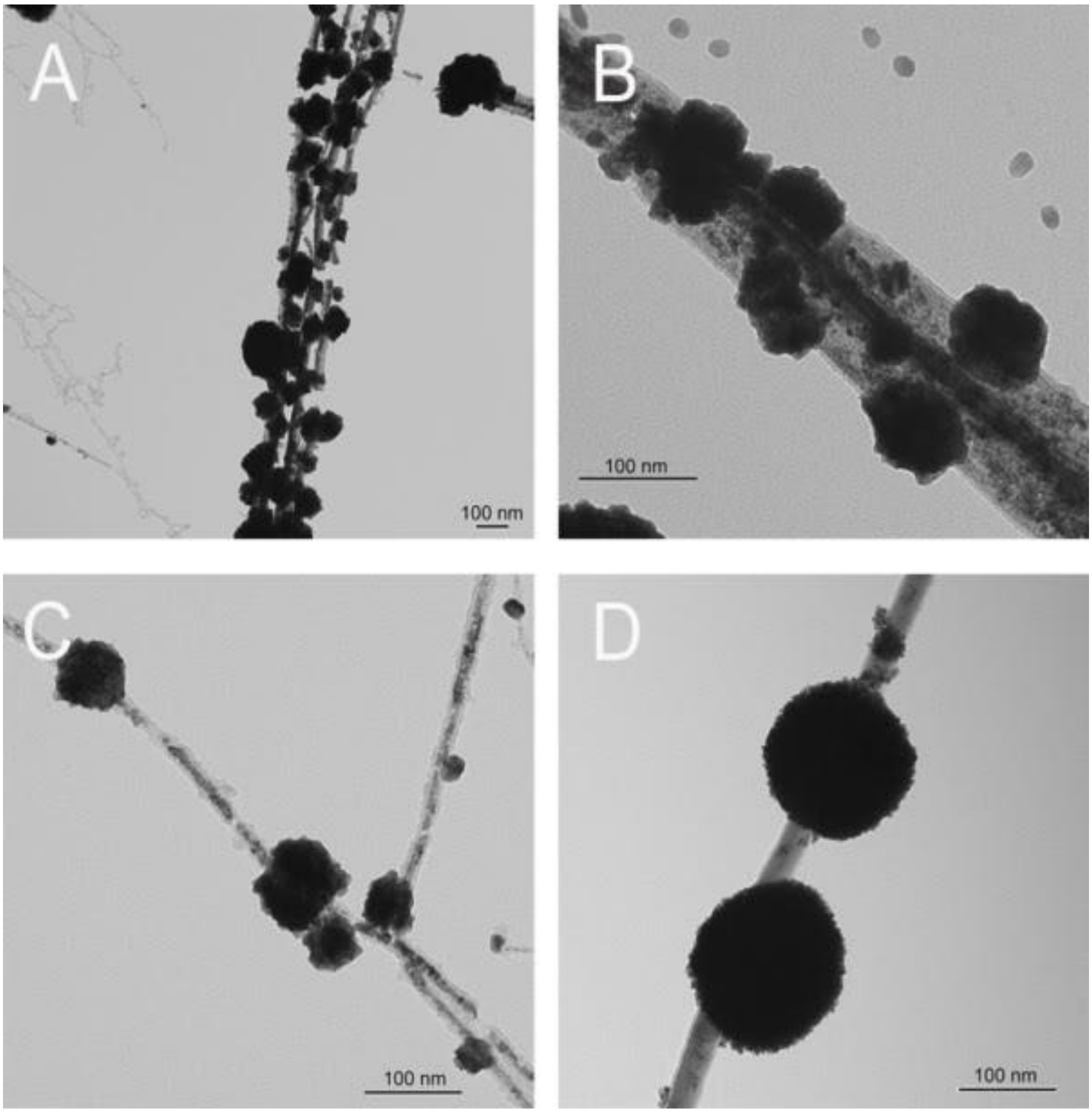

Figure 17. TEM characterization of platinum NPs*. Platinum structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

Gold nanoparticles (AuNPs)

AuNPs were formed with a spherical shape and grew with preference on the edges of the NW, similar to the AgNPs (Figure 18 C, D), but also over the NW (Figure 18 A, B). The organic coating was observed surrounding the NPs (Figure 18 C). AuNPs were found to have a great concentration over the NWs with a small degree of release; it is hypothesized that the gold may form an alloy with tellurium, as the structure of the NW seemed to disappear in the TEM image (Figure 18 B).

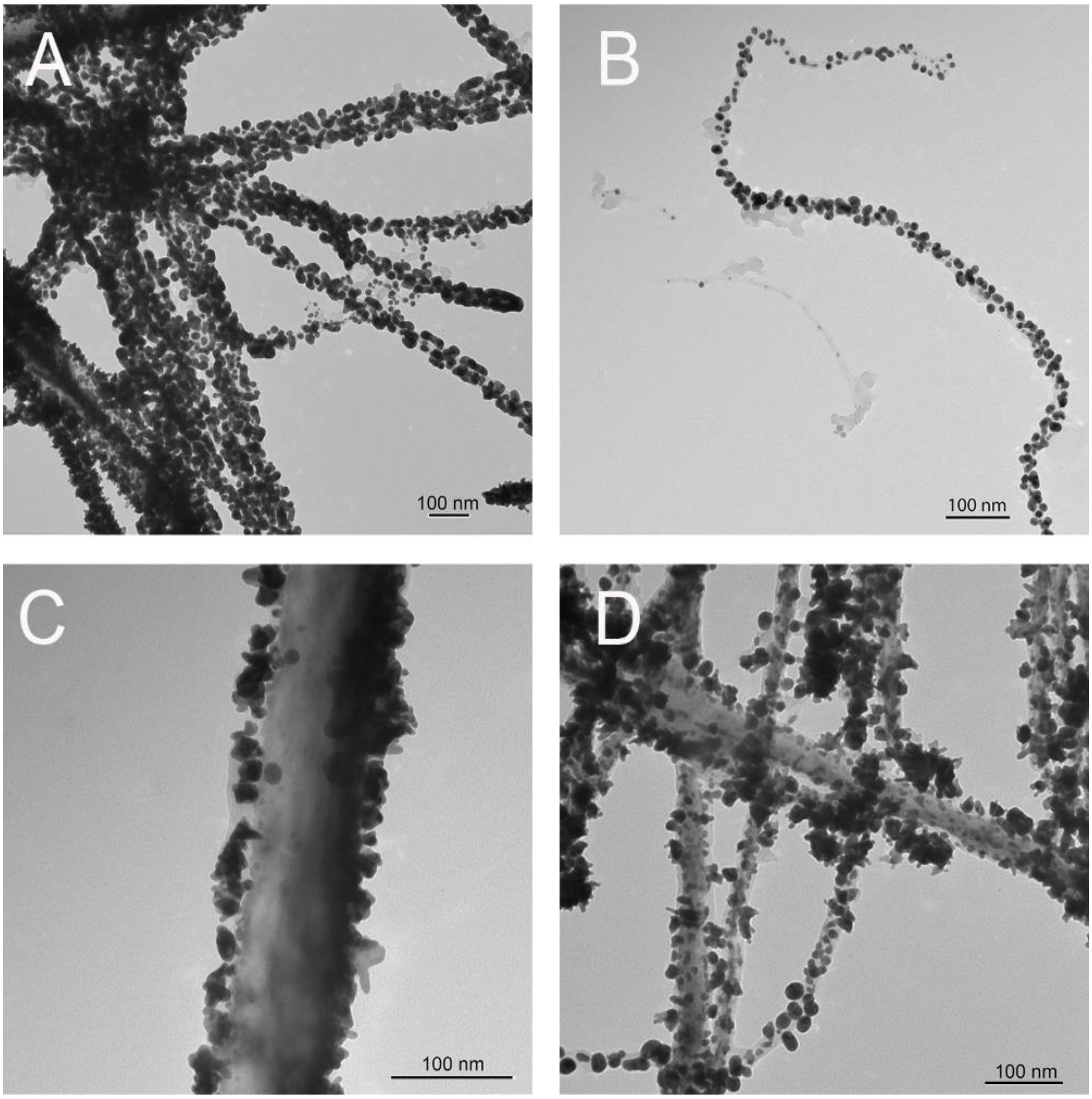

Figure 18. TEM characterization of gold NPs. *Palladium structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction*

Silver nanoparticles (AgNPs)

Figure 19 shows the formation of small and amorphously-shaped AgNPs on top of the NWs, concentrated on their sides. They seem to be more apt to grow on top of smaller wires rather than in accumulations or clusters (Figure 19, A and B). The organic coating presented on the NWs surrounded the newly formed nanostructures (Figure 19, C and D). Without any need of sonication, a huge number of NPs were released from the NWs and remained in solution without aggregation (Figure 19, E and F).

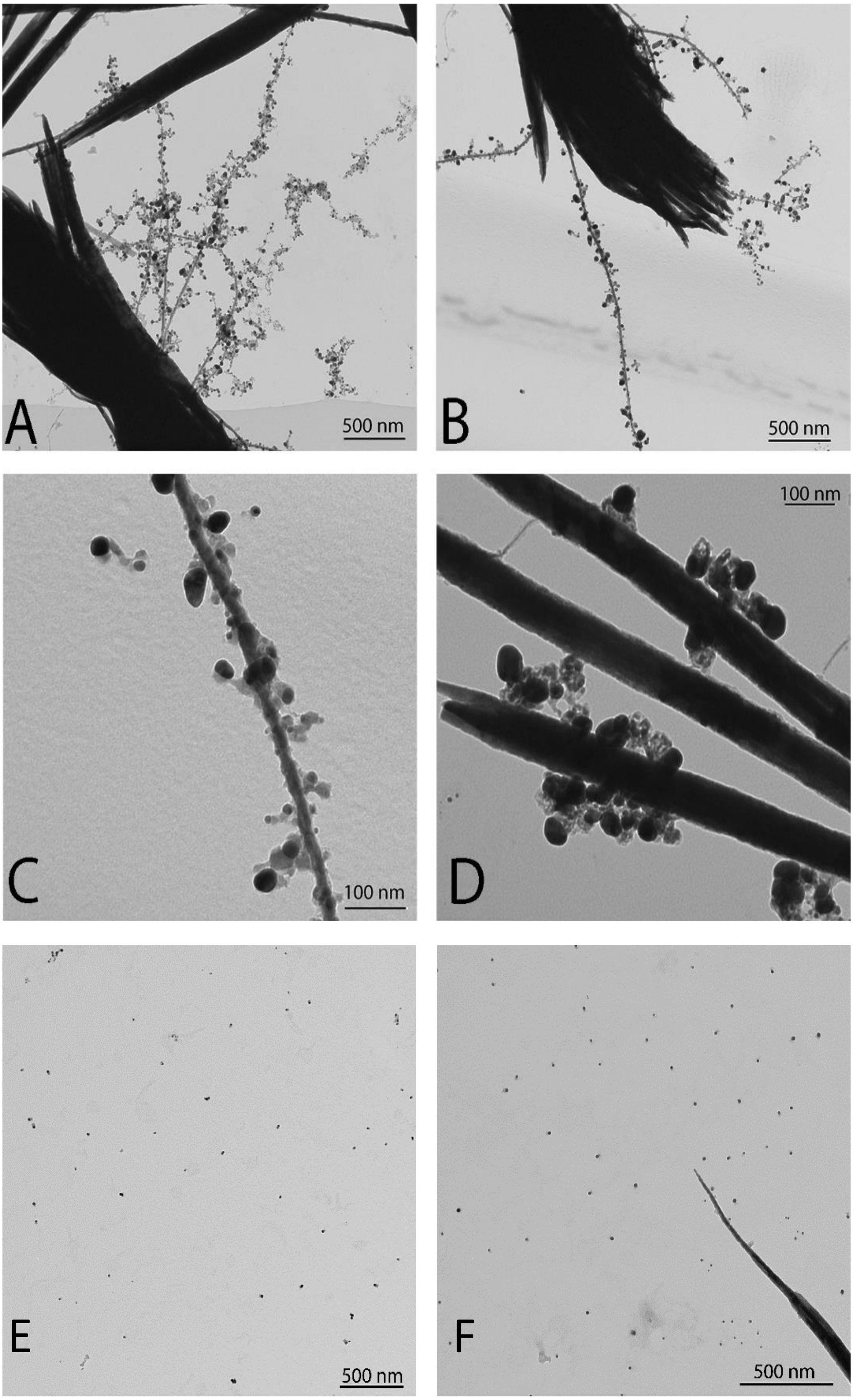

Figure 19. TEM characterization of silver NPs. *Silver structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

Selenium nanoparticles (NPs)

SeNPs were formed in a spherical-shaped on the top of the NWs, growing without any preference (Figure 20, A, B and C). These structures contained the organic coating present on the NW structure (Figure 20 A, B and C). A high number of free NPs were observed in solution without the use of any physical stimuli (Figure 20 D).

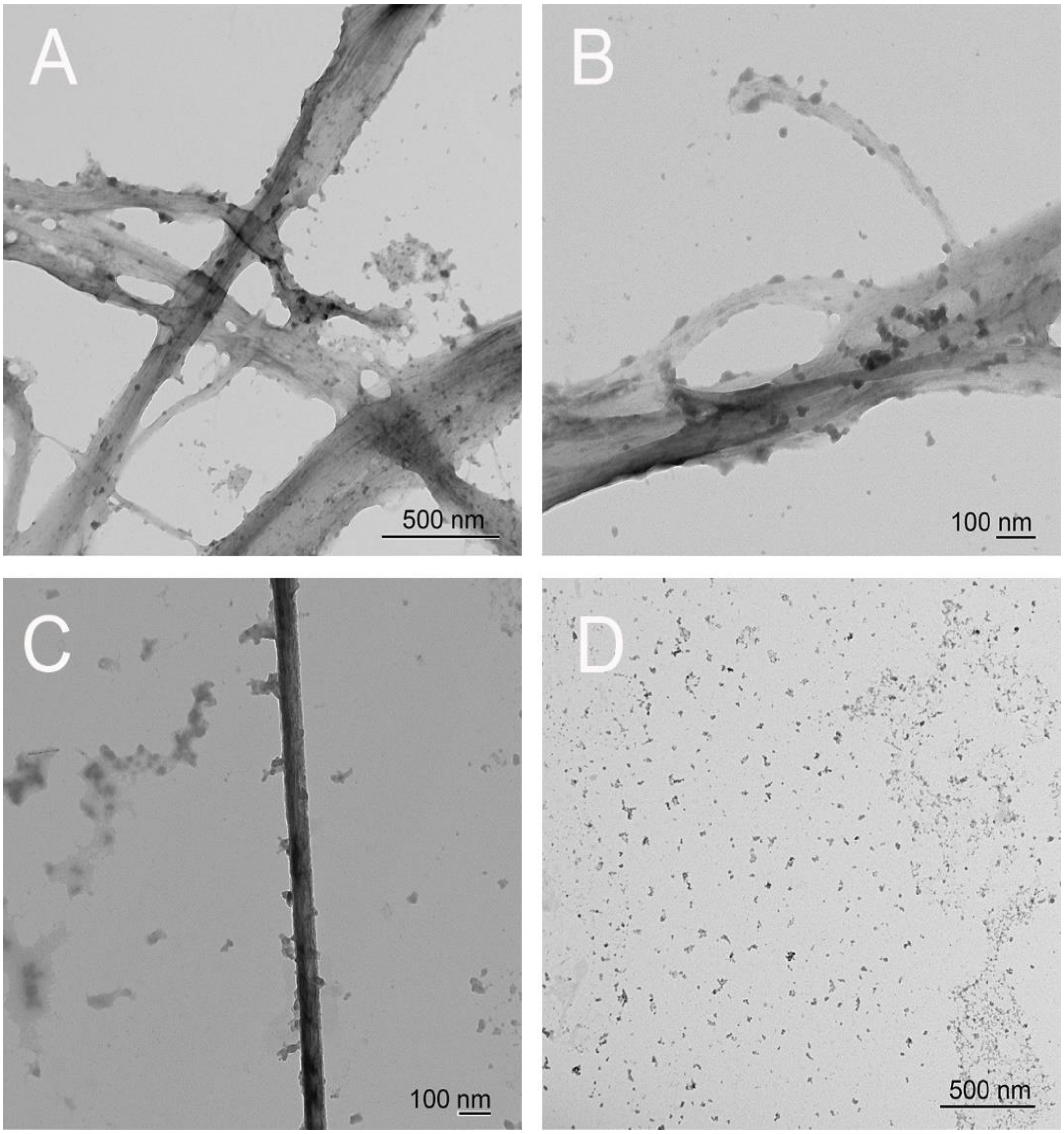

Figure 20. TEM characterization of selenium NPs. *Selenium structures growing on top of the tellurium NW were observed using TEM characterization right after the reaction.*

Tellurium nanoparticles (TeNPs)

Figure 21 shows the formation of rod-shaped TeNPs on top of the NWs, growing along the NW (Figure 21, A, B, D). The newly formed nanostructures were surrounded by the organic coating on the NWs (Figure 21 A, B, C, D). Figure 21 C shows a little degree of release of the nanorods.

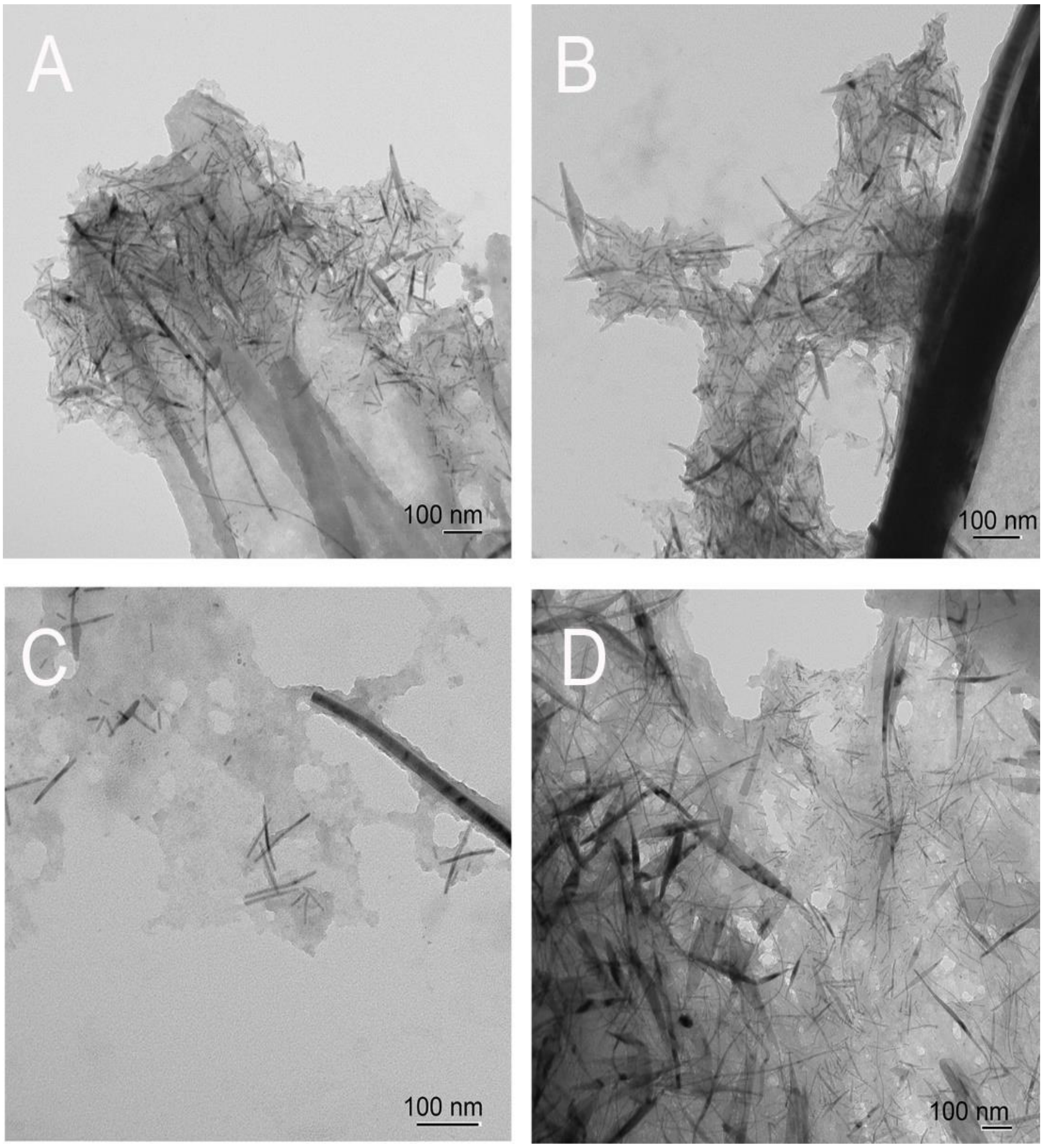

Figure 21. TEM characterization of tellurium NPs. *Tellurium structures growing on top of the tellurium NW were observed using TEM characterization right after the reaction.*

3.3.3. Bacterial assays

Antimicrobial activity was tested on both GREEN – TeNWs and the synergy NP–NWs using Pd-TeNWs and Pt-TeNWs in order to compare their potential antibacterial activity. The assays were done against two different types of bacteria, *E. Coli* and *S. aureus*.

As observed in Figure 22, the bacterial growth suffered a decay when bacteria were in contact with different concentrations of GREEN-TeNWs. Therefore, it can be concluded that the GREEN-TeNWs have antibacterial activity against these kinds of bacteria.

Interestingly, there was a bigger decay of the bacterial growth of *S. aureus* compared to that of *E. coli*. It is hypothesized that this can be explained because *E. coli* is a Gram-negative bacterium, which means it presents a double membrane, while *S. aureus* is a Gram-positive bacterium with a single membrane. In consequence, it should be easier for Gram-negative bacteria to be protected from the effects of nanostructures. Nevertheless, further research should be carried out to find an explanation for these results.

Each concentration of GREEN-TeNWs seemed to work similarly against *S. aureus*. There was not much difference between 5µg/mL and 100µg/mL. However, when they were tested against *E. coli*, concentrations larger than 25µg/mL were more efficient at killing the bacteria. Therefore, while all the concentrations (in the range of allowed concentrations by FDA) of NWs produced a delay in growth, a concentration of 25µg/mL seemed to work best against both bacteria.

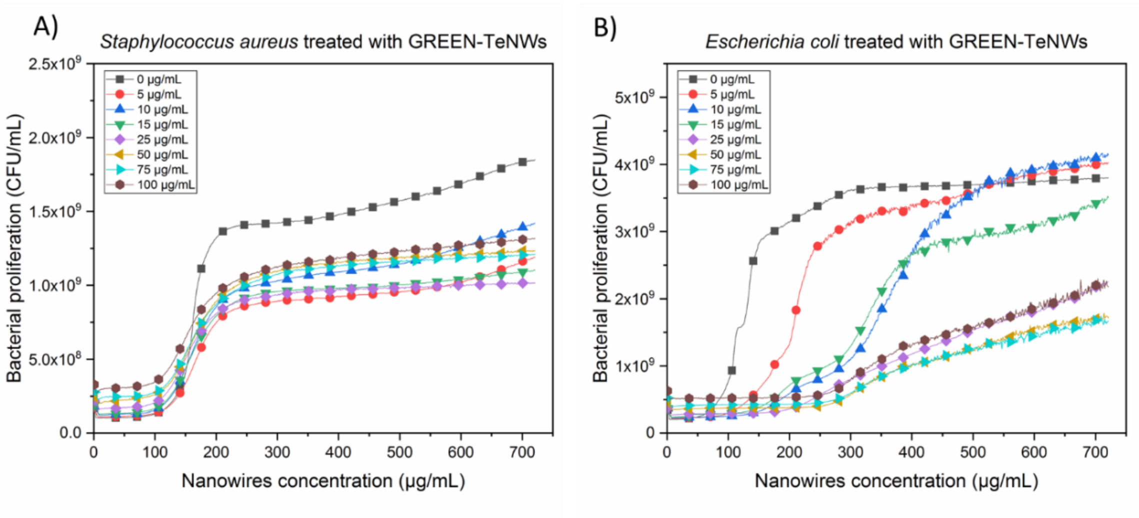

Figure 22. Effect of GREEN – TeNWs on* Staphylococcus aureus *(A) and* Escherichia coli *(B). *Growth of a 10^6 CFU mL^{-1} suspension of* S. aureus *(A) and* E. coli *(B) for 24 hours in presence of different concentrations GREEN – TeNWs. The values represent the mean ± standard deviation, N = 3.*

Oxidative stress is known as the production of reactive oxygen species (ROS) and it is one of the main mechanisms by which the NPs present antimicrobial activity. ROS are chemically reactive agents that contain oxygen free radicals such as peroxides (O_2^{-2}), hydroxyl radicals (OH) or superoxides (O^{2-}). These species can easily penetrate the membrane of the cells by diffusion and are produced naturally during the metabolism of oxygen. They are not considered harmful unless their concentration rises significantly, which is the case when bacteria get in contact with the NPs. Therefore, we hypothesize that the exposure of the cells to the NWs induces the formation of ROS, and consequently produces a systematic failure of the internal metabolism that leads to bacterial cell death.

Antimicrobial tests of the synergy were performed using PdNPs and PtNPs (Figure 23). It can be interpreted from data that the presence of different synergy concentrations – independently of the metal used – produced a reduction on the bacterial growth. Hence, it can be said that both synergetic nanostructures showed a certain degree of antimicrobial activity against *E. coli* and *S. aureus.*

Antimicrobial assays performed on both Gram-negative and positive bacteria showed similar results than those carried out with plain NWs.

In the particular case of *S. aureus*, there was a slight enhancement in the approach since the application of different concentrations of both Pt- and Pd-TeNWs resulted in a prolonged delay of the bacterial growth, which tendency will not reach the one of the control in any case. In contrast, for some of the lowest concentrations in experiments with plan NWs, the tendency showed that the bacteria would eventually reach the growth rate of the control.

When *E. coli* results are compared, it is possible to state a clear improvement in bacterial growth inhibition. When plain NWs were employed, a few of the concentrations seem to have a chaotic effect in the bacterial growth, with a break in the inhibitory tendency that leads to a non-inhibitory behavior, especially noticeable in 5 and 10µg/mL. In contrast, when the synergy system is tested against the bacteria, we can observe a perfectly grouped tendency of inhibition for almost all of the concentrations –except for 5 µg/mL, that eventually will reach the growth profile of the control bacteria- for the treatment with PdNWs-TeNWs; and this tendency was broken for some of the concentrations in the case of PtNPs-TeNWs, showing a lower inhibitory effect than PdNPs-TeNWs.

As an overall observation, there is a slight improvement in the bacterial inhibition of the NPs-TeNWs with respect to just TeNWs, while this enhancement is especially noticeable for PtNPs, that shows a better inhibition than PdNPs.

It was hypothesized that the combination of both structures – the NWs and the NPs – can trigger a higher production of ROS due to the increase in surface area compared to single NWs or single nanoparticles. Besides, and due to the presence of two metallic species, the hypothesis of a second mechanism of bacterial inhibition related to metal-ion release can be stated. In this mechanism, it is believed that metallic ions, coming from the nanostructures, can be absorbed on the cell membrane making changes on the enzymatic concentration, therefore producing modifications on different processes of the bacteria which lead to a decay on its proliferation [94]. However, further experiments should be conducted to elucidate the behavior and parameters affecting both of these mechanisms.

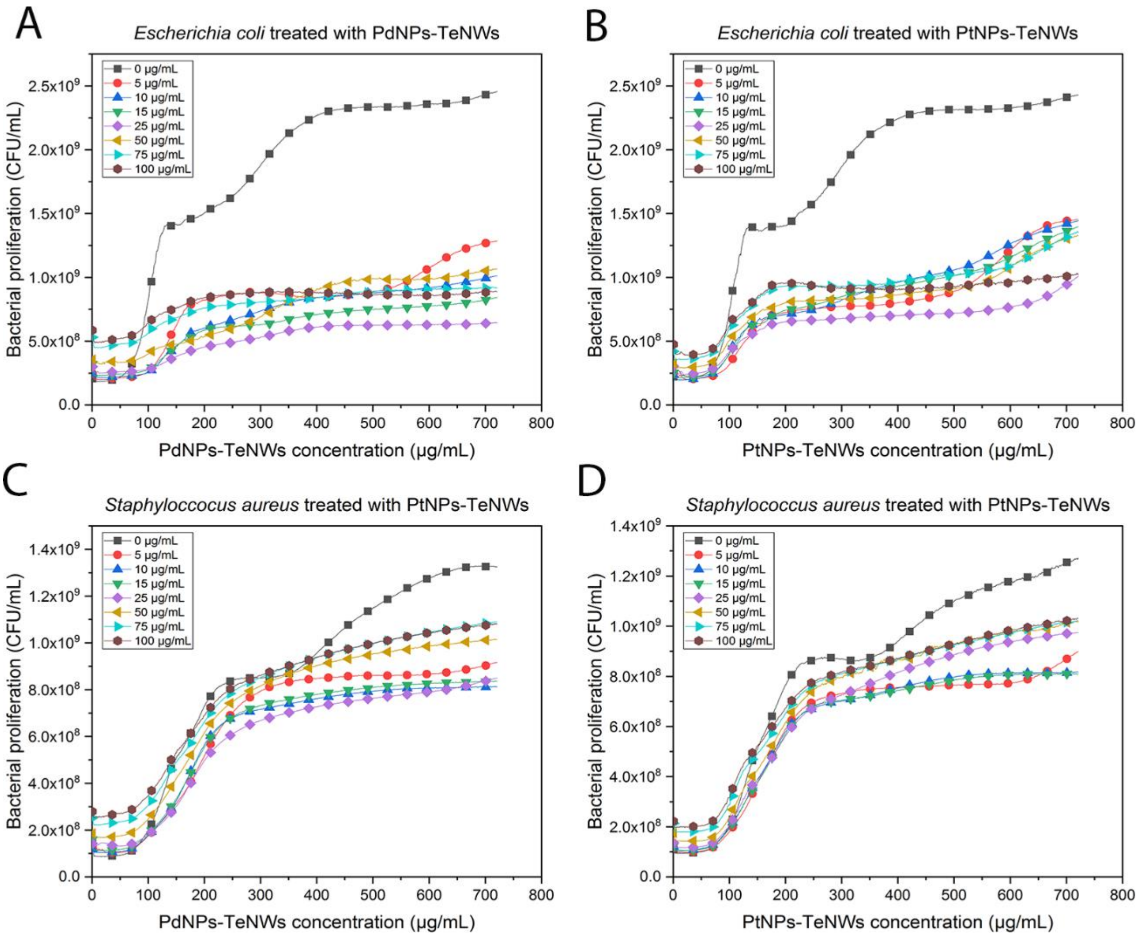

Figure 23. Effect of PdNPs-TeNWs and PtNPs-TeNWs on* Escherichia coli *(A,B) and* Staphylococcus aureus *(C, D). *Growth of a 10^6 CFU mL^{-1} suspension of* E. coli *(A, B) and* S. aureus *(C, D) for 24 hours in presence of different concentrations synergy NPs – TeNWs. The values represent the mean ± standard deviation, N = 3.*

The Gompartz equation's parameters were only calculated and plotted for analysis of the GREEN-TeNWs (Figure 24). Data came from the treatment of *E. coli* and *S. aureus* with GREEN-TeNWs.

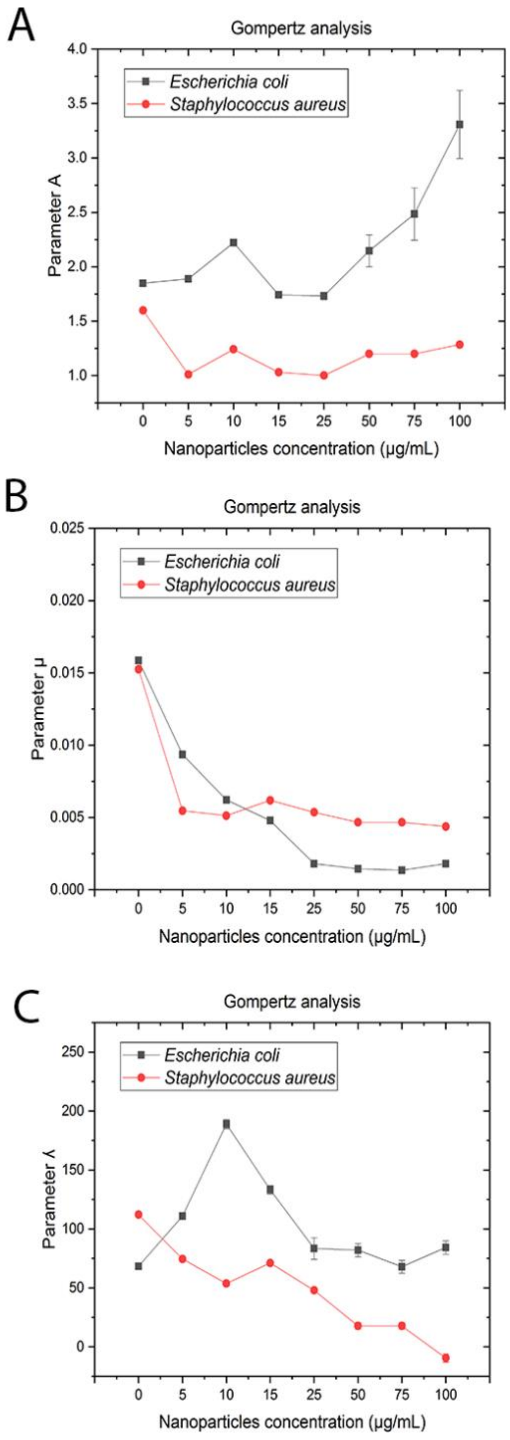

Figure 24. Analysis of parameters A, λ and µ from modified Gompertz equation. *All parameters were analyzed in relation with the GREEN – TeNWs concentration. The effect of the nanowires was analyzed, taking data from the Gompertz equation fitting of bacterial growth curves.*

The parameter *A* represents the maximum specific growth of the bacteria under experimental conditions. It was observed in *Staphylococcus aureus* (Figure 24, A) that at higher concentrations there was a decrease on the growth, on the other hand, in

Escherichia coli this tendency was only maintained up to 25 μg/ml. It seems GREEN – TeNWs had less antimicrobial properties against *Escherichia coli*.

The parameter λ represents the lag time in the bacterial growth under experimental conditions (Figure 24, B). The graphs showed that at higher concentrations of GREEN – TeNWs there was less of a lag phase, which represents the time bacteria needs to adapt to the growing conditions generated by the presence of the nanowires. Therefore, the presence of nanowires interfered in the bacterial growth as they are dividing slowly than without nanostructures in the media.

The μ parameter represents the speed of the bacterial growth (Figure 24, C). It was demonstrated that at larger concentrations of GREEN – TeNWs growth was slower. Although this tendency was more visible in *Staphylococcus aureus* and it can be applied in both cases.

3.3.4. *In vitro* cytotoxicity assays with the synergy

Human dermal fibroblasts (HDF) cells and melanoma cells were used to perform *in vitro* cytotoxicity assays. The use of HDF cells allowed determining the biocompatibility with healthy cells while melanoma cells were used with the aim to determine potential anticancer activity. The collected data was compared with a control that contained media and cells and just media to compare the normal growth of the cells without the presence of nanostructures. The experiments performed using PdNPs-TeNWs and PtNPs-TeNWs with the purpose to elucidate the differences between both metals. Further comparison with GREEN-TeNWs results – section 2.3.3 – were done in order to determine which nanosystem enhance anticancer and biocompatibility properties.

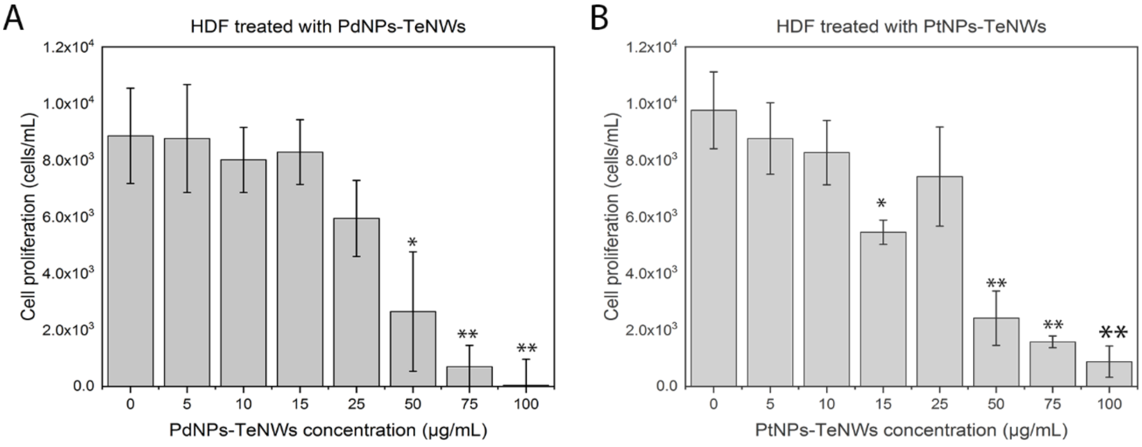

Figure 25. ***HDF assays on human dermal fibroblasts cells in the presence of PdNPs-TeNWs(A) and PtNPs-TeNWs (B), at concentrations ranging from 5-100 μg/mL.*** *N=3. Data is represented as mean ± SD; *p<0.05, **p<0.01.*

For HDF experiments, both system 1 (Figure 25 A) and 2 (Figure 25 B) were tested with a range of concentrations between 5 and 100 μg/mL. It can be deduced, that for concentrations up to 25 μg/mL , cell proliferation maintains stable thus, cells are not dying in presence of the synergy. However, larger concentrations – from 50 to 100 μg/mL – showed a depletion in the cell proliferation, especially on the case on PdNPs hence not being suitable concentrations for cell viability.

Comparing the results with GREEN-TeNWs it can be stated that the synergy presents a decrease on the biocompatibility regarding HDF cells. Although both systems allow cell viability, GREEN-TeNWs show – for smaller concentrations – an increasing on the cell proliferation which can be translated on potential applications such as tissue regeneration. It has been hypothesized that these differences came from the presence of the organic coating surrounding the NWs structure. As said beforehand, starch is a biodegradable polymer which is known to recover the metallic core of the nanoparticle so it decreases its toxicity. Nevertheless, this polymer is used – in system 1 and 2 – as a reducing agent for the metallic nanoparticles growth on the top of the nanowires meaning that, after reaction, there would be less presence of starch as can be seen in different TEM images (Figure 14). Moreover, it is elucidated that the differences between both metallic structures came from the use of this starch; as previously mentioned, Pt needs more power of reduction so it consumed more starch and it is less remaining on the structure thus, biocompatibility is decreased.

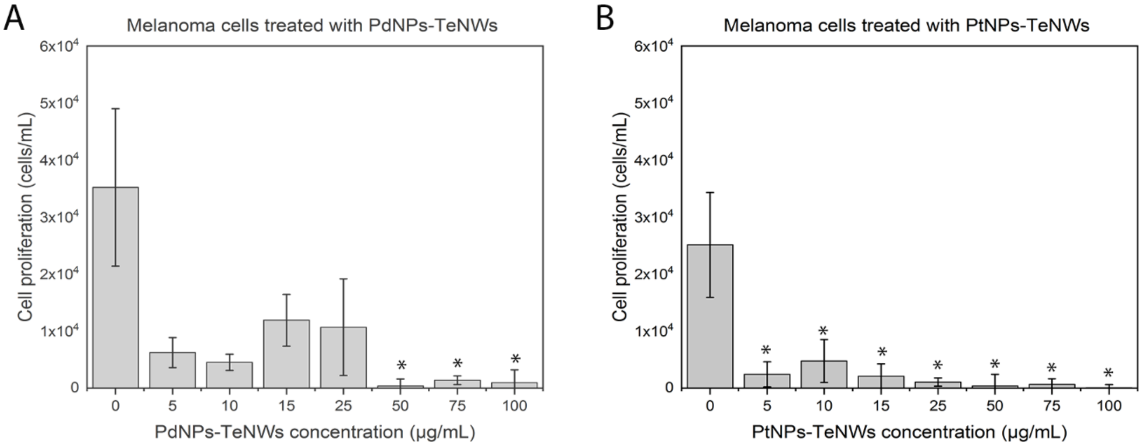

Figure 26. HDF assays on melanoma cells in the presence of PdNPs-TeNWs(A) and PtNPs-TeNWs (B), at concentrations ranging from 5-100 μg/mL. *N=3. Data is represented as mean ± SD; *p<0.05.*

For melanoma experiments, both system 1 (Figure 26 A) and 2 (Figure 26 B) were also performed with a range of concentrations between 5 and 100 µg/mL. Graphs showed a decrease on the cell proliferation in all ranges of concentration, especially using PtNPs.

The comparison between GREEN-TeNWs and the synergy system reflects enhanced anticancer properties for the last ones. It has been hypothesized that the presence of two different metallic species increases the formation of ROS, and consequently produces a systematic failure of the internal metabolism that leads to cell death.

In this report, the cytocompatibility and anticancer activity of tellurium nanostructures were observed with a potentially improved performance using the synergetic system NPs-TeNWs. Despite the enhancement on cell proliferation of GREEN-TeNWs, the synergetic system, a greater decrease on cancer cell proliferation. Hence, it can be said in overall, that the synergy present in a large range of concentrations properties towards enhancing healthy cell proliferation and decreasing cancer cell proliferation better than bare NWs structure.

3.4. Conclusions

Green chemistry is known to be an alternative for the traditional synthesis of metallic NPs; however, there is still a need to find new methodologies to synthesize nanostructures in a cost-effective, straightforward and environmentally friendly methodology. Here, a novel synthetic route has been presented, which takes advantage of previously tested green nanostructures -GREEN-TeNWs-. This route produces metallic NPs made of Pd, Pt, Au, Ag, Se and Te, in one minute, at room temperature and without the use of any additional reducing agent. The reaction is thermodynamically favored and hence, because of the presence of the NWs, provides a straightforward methodology for the in situ generation of metallic nanoparticles with potential biomedical applications.

The synthetic process presented slight differences for the different metals used. Despite that fact, microscopy characterization showed that the formation of all metallic NPs over the TeNW occurs. Thus, it was hypothesized that starch acts as a reducing agent, thereby avoiding the need for any extra reagent to help the generation of metallic structures. This synthetic approach was not only useful for generating NPs, but also for obtaining the synergy NP-NWs, which present themselves as suitable antibacterial entities.

In this work, two different green synthetic nanostructures (NWs and the synergy NP-NWs) were successfully produced, with the aim to elucidate the differences between the antibacterial, and anticancer, and biocompatibility behavior of both systems. Regarding the antimicrobial properties, it has been demonstrated that the synergy NP-NWs presented a stronger delay on the bacterial growth – against both *E. coli* and *S. aureus* – than the GREEN-TeNWs, thus becoming a slightly more powerful solution to treat microbial infections. Moreover, it has been appreciated that the synergy presents an enhancement on the anticancer properties, in the whole range of concentrations the cell growth sharply decreases. Besides, these nanostructures can be considered biocompatible as the cell proliferation is hardly reduced for concentrations up to 25 µg/mL.

No differences between different metallic nanoparticles were observed on the process; hence it is hypothesized that the potential biomedical applications do not rely on the kind metal used but on the conjunction of the structure.

4. Ongoing research

GREEN-TeNWs, as stated on the previous aims, represent an eco-friendly and cost-effective methodology to produce nanostructures with enhanced biomedical applications over the chemical synthesis. Therefore, a third objective sought to take advantage of the potential of these structures has been set along with an aim focused on future projects and ongoing research.

In the past years, the medical use of metallic surfaces has been rising in interest in the implant area, metals such titanium present antimicrobial activity, thus these surfaces avoid having problems with bacterial infections which are a significant concern on these treatments [95, 96]. In this research, we thought to introduce the GREEN-TeNWs as a coating for these titanium surfaces in order to enhance their potential biomedical applications.

Heavy metal water pollution represents a significant concern affecting the world. Metals such as mercury (Hg), cadmium (Cd), zinc (Zn) or lead (Pb) are known to be hazardous pollutants which tend to accumulate in living organisms, therefore they have become a significant threat not only for the environment but also for the healthcare system [97, 98]. For all those reasons, there is a need to find a cost-effective and eco-friendly approach to remove them from water. Thus, after observing the effective methodology to produce nanoparticles on the top of the nanowire structure – section 3 – it was considered that not only would noble metals reduce using starch but also heavy metals thus, one could take profit of the nanowire structure as environmental remediation agents to eliminate the presence of these heavy metals in water.

4.1. Functionalization of metallic surfaces

4.1.1. Objective

One of the major concerns related to bacterial infection is found in implants [95]. Although orthopedic implants have improved their quality, they are not free from the action of bacteria. Most of the infections found in implants are caused by common bacteria, such as *Staphylococcus aureus* or *Escherichia coli* [96].

In fact, one of the milestones in this research field consists of the development of implant surfaces that diminish bacterial adherence and the formation of a biofilm [99]. There have been several attempts to reduce bacterial adhesion to metallic surfaces by using a technique of corrugation at the nanoscale [100, 101]. In this way, nanocolumnar titanium coatings are considered suitable recoveries as they exhibit similar antibacterial properties against Gram positive (*Staph. aureus*) and Gram negative (*E. coli*) bacteria. These structures have been fabricated in two sputtering systems with very different characteristics (a laboratory setup and semi-industrial equipment), thus possessing different morphologies (150 nm long columns tilted 20º from the normal and 300 nm long ones tilted 40º, respectively).

When a synergic route is followed and these coatings are functionalized with tellurium nanorods, the antibacterial properties are enhanced, especially for the long nanocolumns. The biocompatibility is preserved in all of the nanostructured coatings. Therefore, having these antecedents and after observing the potential antibacterial activity of GREEN-TeNWs – section 3.3.3 – we established a third objective, to mix the NWs with metallic surfaces made of titanium (Ti) – two types of surfaces (called Short and Long Columns) synthesized by our collaborators in Spain – which present antimicrobial properties. If NWs could coat this metallic surface, it is possible they could enhance biomedical applications – as antibacterial, biocompatible agents – of the titanium surfaces (Figure 27).

Current experimentation has demonstrated that GREEN-TeNWs can homogeneously coat the titanium surfaces. Further investigations on reaction conditions to improve the coating, antimicrobial tests, and biocompatibility assays will be carried out in the future.

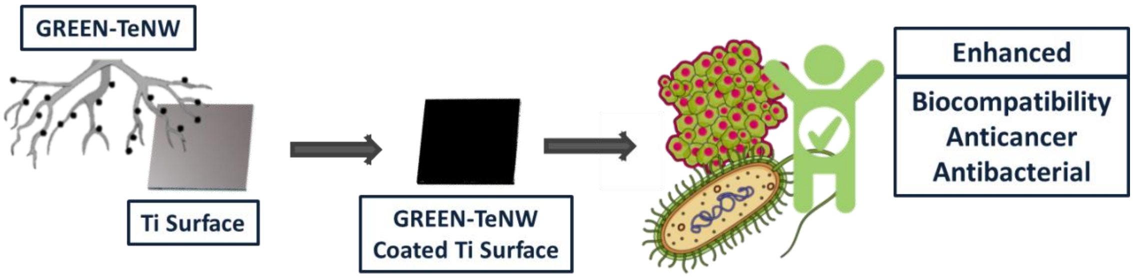

Figure 27. Third aim of the project*. Coating of metallic surfaces using GREEN-TeNWs.*

The final aim of this project was the enhancement of the previously reported antibacterial and biocompatibility properties of titanium surfaces that already showed potential ability to reduce bacterial adhesion.

4.1.2. <u>Synthesis and characterization of the metallic surface coated with GREEN-TeNWs</u>

Synthesis of GREEN-TeNWs over the metallic surface

The synthetic procedure used was the same as that for the green synthesis of the TeNWs (section 2.2.2) with the difference that we added the metallic surface on the reactor (Figure 28).

Briefly, 0.15g telluric acid (H_2TeO_4) (Sigma Aldrich, St. Louis, MO) was mixed with 0.15 g of a starch (Sigma Aldrich, St. Louis, MO) solution in 10mL deionized water.

Then, the mixture was transferred into a Teflon-lined, stainless steel reactor, onto the bottom of which the metallic surface was placed prior to mixture deposition. The solution completely covered the metallic surface, and the container was placed into an oven at 160°C for 15 h. The mixture was allowed to cool down at room temperature.

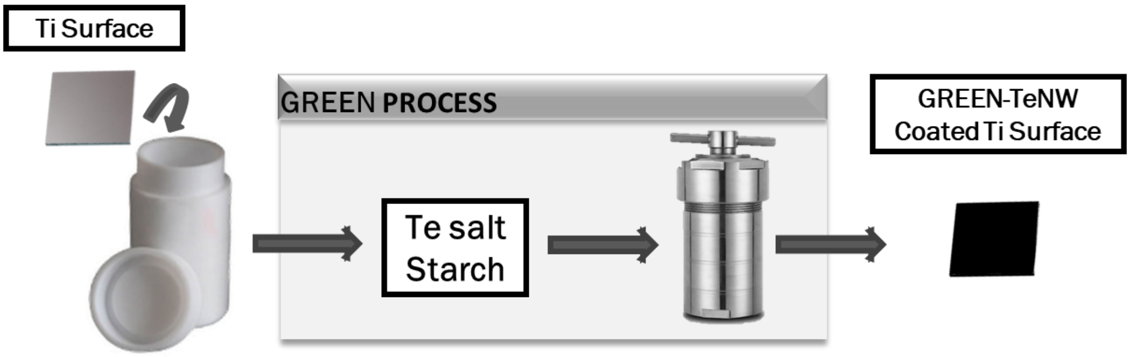

Figure 28. Methodology for the synthesis of the GREEN-TeNWs coating over the metallic surfaces.

<u>Characterization of GREEN-TeNWs over the metallic surfaces</u>

SEM imaging and EDX analysis were carried out in order to identify the morphology and composition of the coating. Figure 29 shows how the NWs grew over the metallic surface. As seen, only the structure of NWs was observed, suggesting that they homogeneously coated the surface, as the titanium metallic surface was not at all visible.

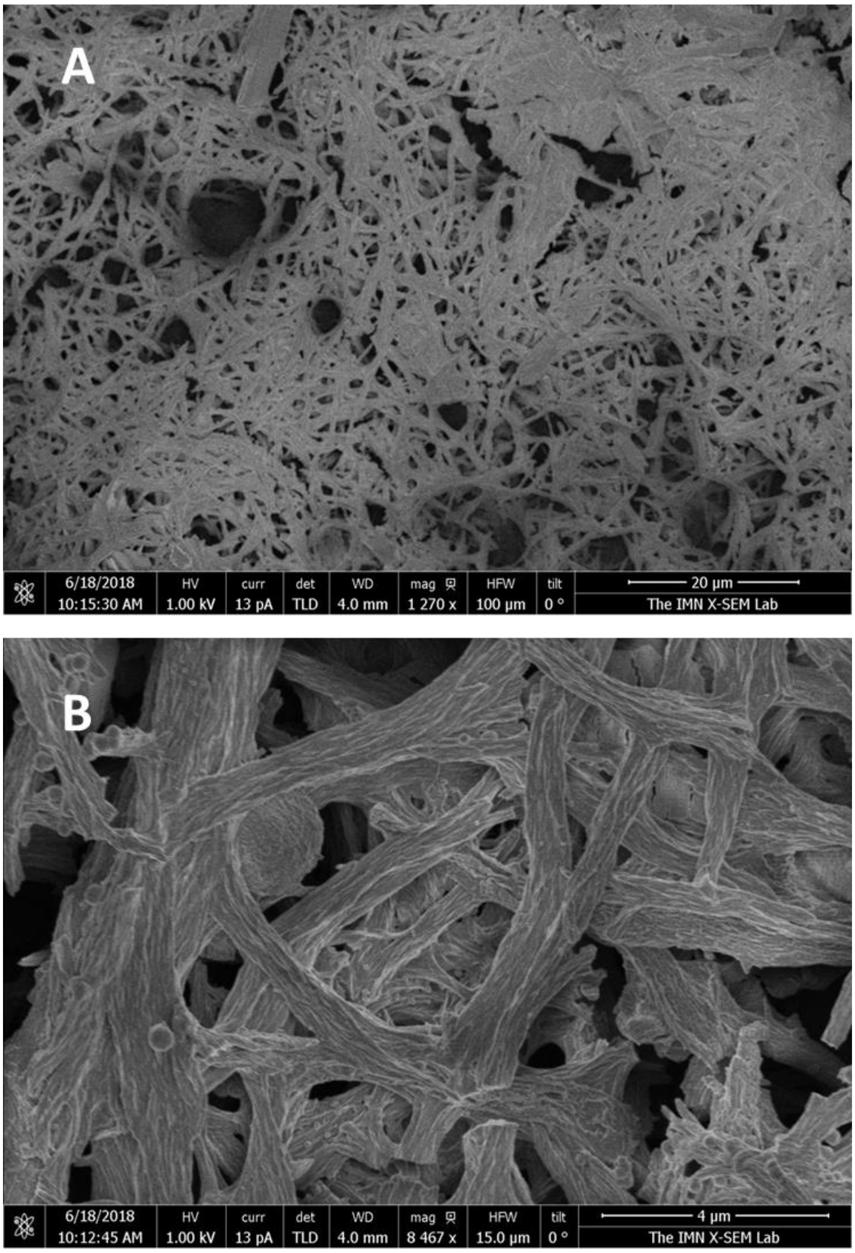

Figure 29. SEM characterization right after the reaction of the coating in Short Column samples*. Tellurium NWs cover homogeneously the metallic surface.*

Long column samples were also observed in SEM. The images obtained were similar to the ones with short column samples in that the TeNW structure completely covered the metallic surface (Figure 30, A and B).

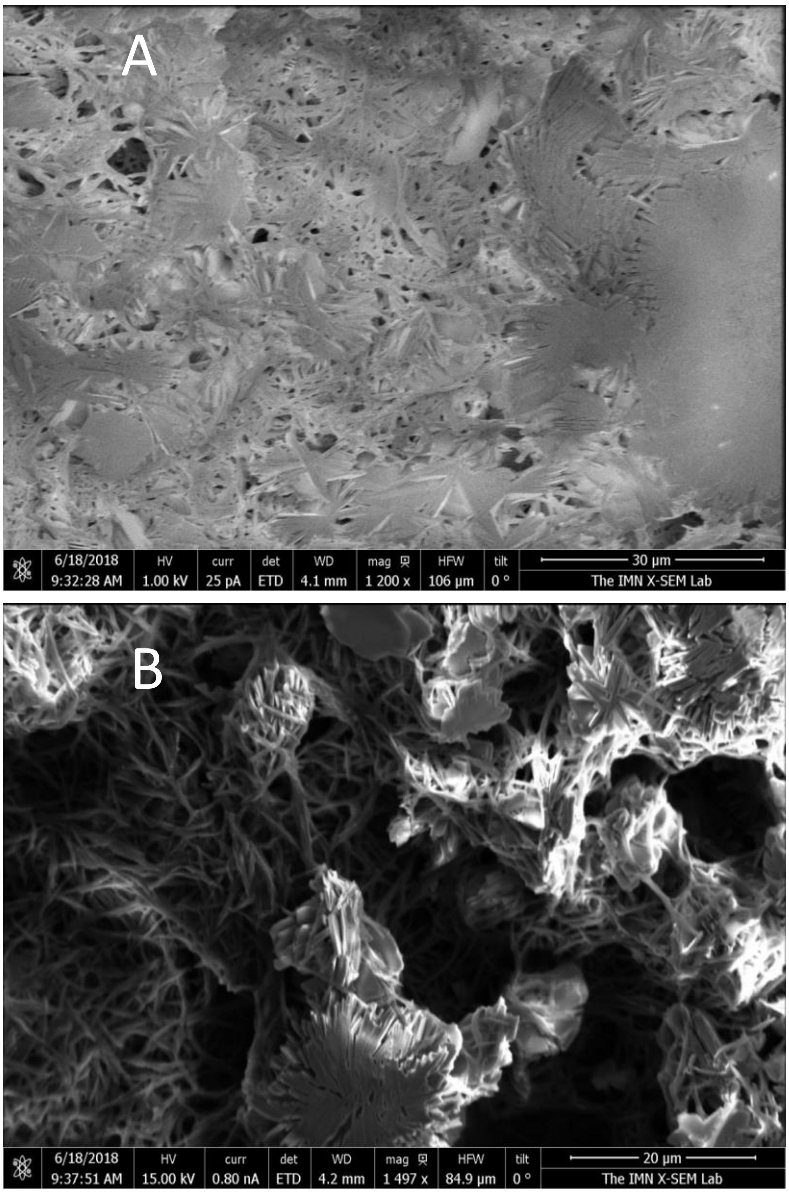

***Figure 30. SEM characterization right after the reaction of the coating in Short Column samples**. Tellurium NWs cover homogeneously the metallic surface.*

SEM images showed in both cases that there are too many TeNWs on the metallic surface, which means there is a need to change the reaction conditions. We hypothesize that happened because the titanium surface can act as a catalyst, enhancing the growth of tellurium.

EDX analysis of both samples was completed to know the composition of the sample. Figure 31 shows EDX analysis for short column samples where two different spots were analyzed. In both spots, the presence of tellurium was confirmed, showing different peaks, and carbon and oxygen species were found, which correspond to the organic coating of the NWs. Nevertheless, titanium peaks were not observed, which again suggests the coating over the metallic surface was excessive.

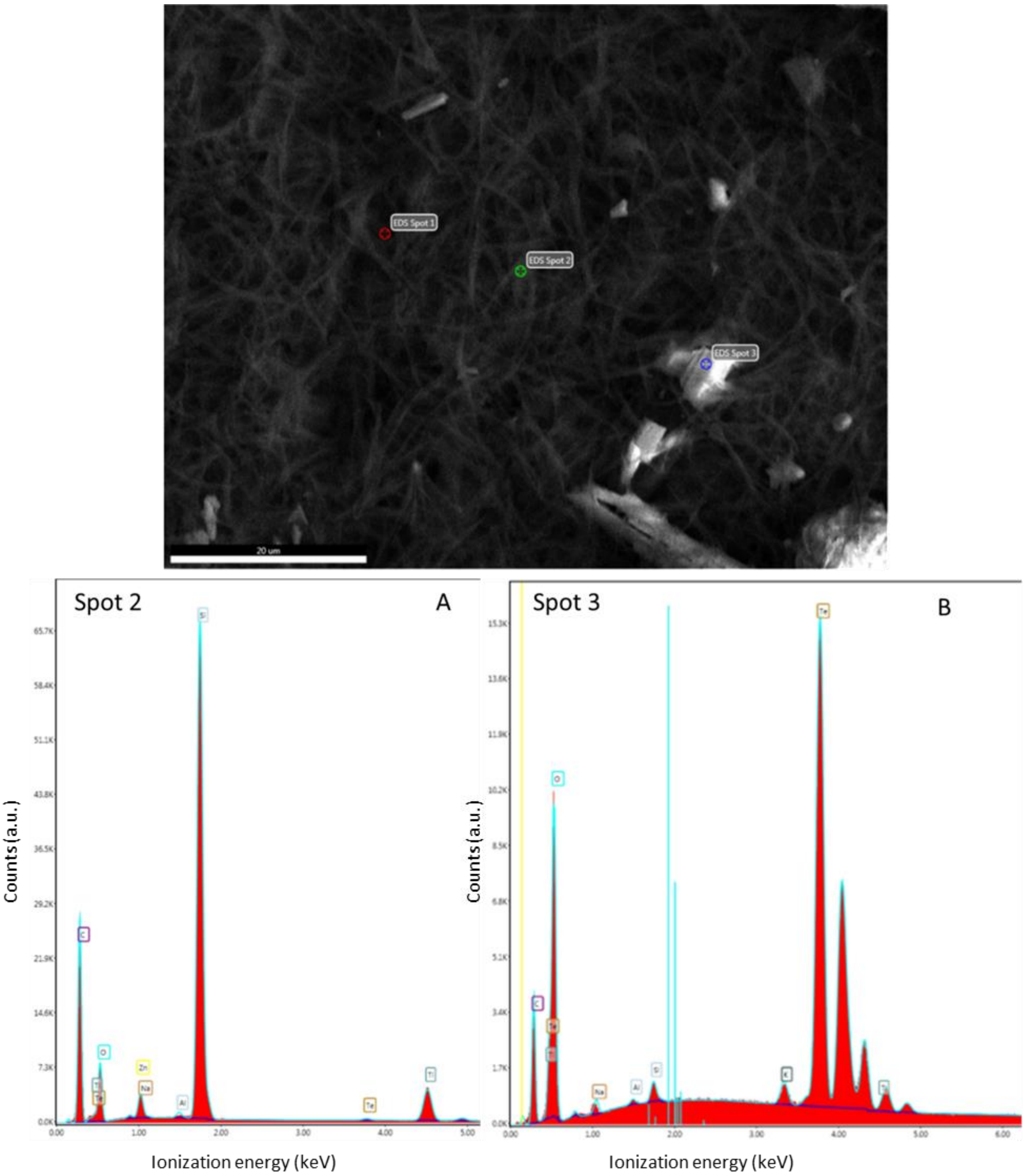

Figure 31. Energy Dispersive X-Ray (EDX) of the coating of GREEN - TeNWs over the short column sample. *Different parts of the coating were analyzed showing the presence of tellurium and its organic coating.*

Long column samples were also analyzed in three different spots (Figure 32, A, B and C). EDX analysis showed the presence of tellurium, carbon and oxygen species similar to that of the short column sample. However, in this case, titanium peaks were observed, confirming the presence of the surface; nonetheless, the peaks are relatively lower than the rest, again suggesting that the coating is too wide.

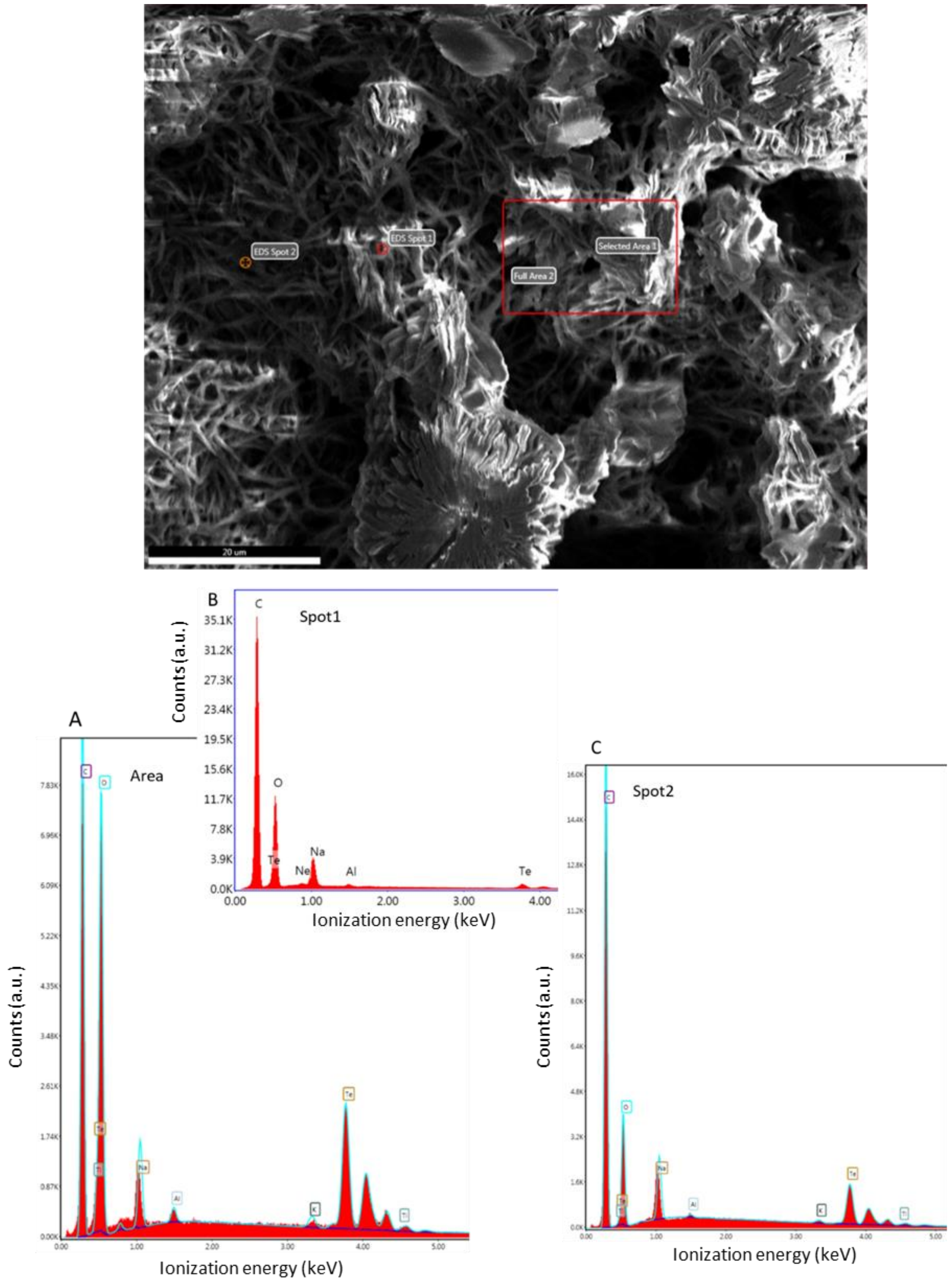

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Figure 32. Energy Dispersive X-Ray (EDX) of the coating of GREEN - TeNWs over the long column sample. *Different parts of the coating were analyzed showing the presence of tellurium and its organic coating and the titanium surface.*

For further experiments, the concentration of NWs should be decreased in the hydrothermal reaction. After that, antimicrobial and biocompatibility tests should be carried out in order to demonstrate whether TeNWs enhance these properties or not.

4.2. Heavy metal removal from contaminated water

4.2.1. Objective

Heavy metals – a group of elements composed of metals such as mercury (Hg), cadmium (Cd), lead (Pb) and zinc (Zn) – are known to be a serious concern nowadays. The rising population translates into a higher industrial activity, thus the pollution of hazardous waste – including heavy metal pollution – is dramatically increasing, specifically related with water contamination [98]. Heavy metals are stated to accumulate inside living organisms producing toxic effects. For instance, Cd – present in most batteries – can decrease bone density [102] or Hg – used in electrical and dentistry industries – is considered a neuro and nephrotoxic agent [103]. Therefore, they are considered a significant health concern and there is an urgent need find cost-effective and environmental-friendly approaches to remove them from water supplies.

As a consequence, after seeing that NPs can growth on the top of the NWs, we hypothesized that heavy metals can be grown over the NW structure (Figure 33), thereby making it possible to remove the heavy metal ions from water in a quick and inexpensive methodology in comparison with other natural processes used nowadays such as the use of living organisms as bacteria, fungi or plants [104].

Current experimentation has demonstrated the formation of such NPs, and further experimentation for the quantification of heavy metal removal from water should be carried out.

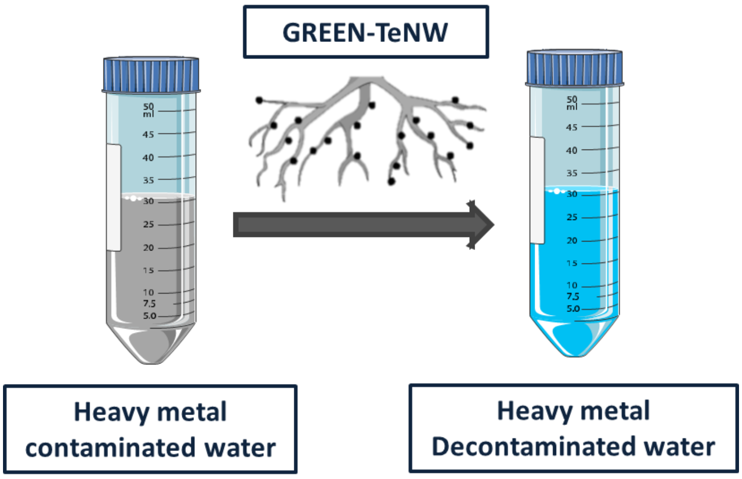

Figure 33. Third aim of the project. *Heavy metal removal from water using GREEN-TeNWs.*

4.2.2. Synthesis and characterization of heavy metals NPs

Synthesis of heavy metal NPs

The synthetic approach followed was the same used in section 3.2.2. Table 5 lists the summary of the variety of reagents used depending on the desired NP.

Table 5. Reagents used on the synthesis of Zn, Hg, Pb and CdNPs. *All chemicals were purchased at Sigma Aldrich, St. Louis, MO.*

Type of NP	Metallic salt
Zinc NP	Zinc chloride ($ZnCl_2$)
Mercury NP	Mercury (II) chloride ($HgCl_2$)
Lead NP	Lead (II) nitrate ($Pb(NO_3)_2$)
Cadmium NP	Cadmium chloride ($CdCl_2$)

Characterization of heavy metal NPs

TEM characterization was conducted in order to show the formation of metallic NPs on top of the TeNWs and within the surroundings. Zn (Figure 34), Hg (Figure 35), Pb (Figure 36) and Cd (Figure 37) were analyzed.

Figure 34 shows the formation of small and spherically-shaped Zinc NPs on top of the NWs, growing on the edges of the NW (Figure 34, A, B, C). The newly formed nanostructures were surrounded by the organic coating on the NWs (Figure 34, B and C). Zinc NPs are found in a great concentration and agglomerated in the edges on the NW (Figure 34, A and C).

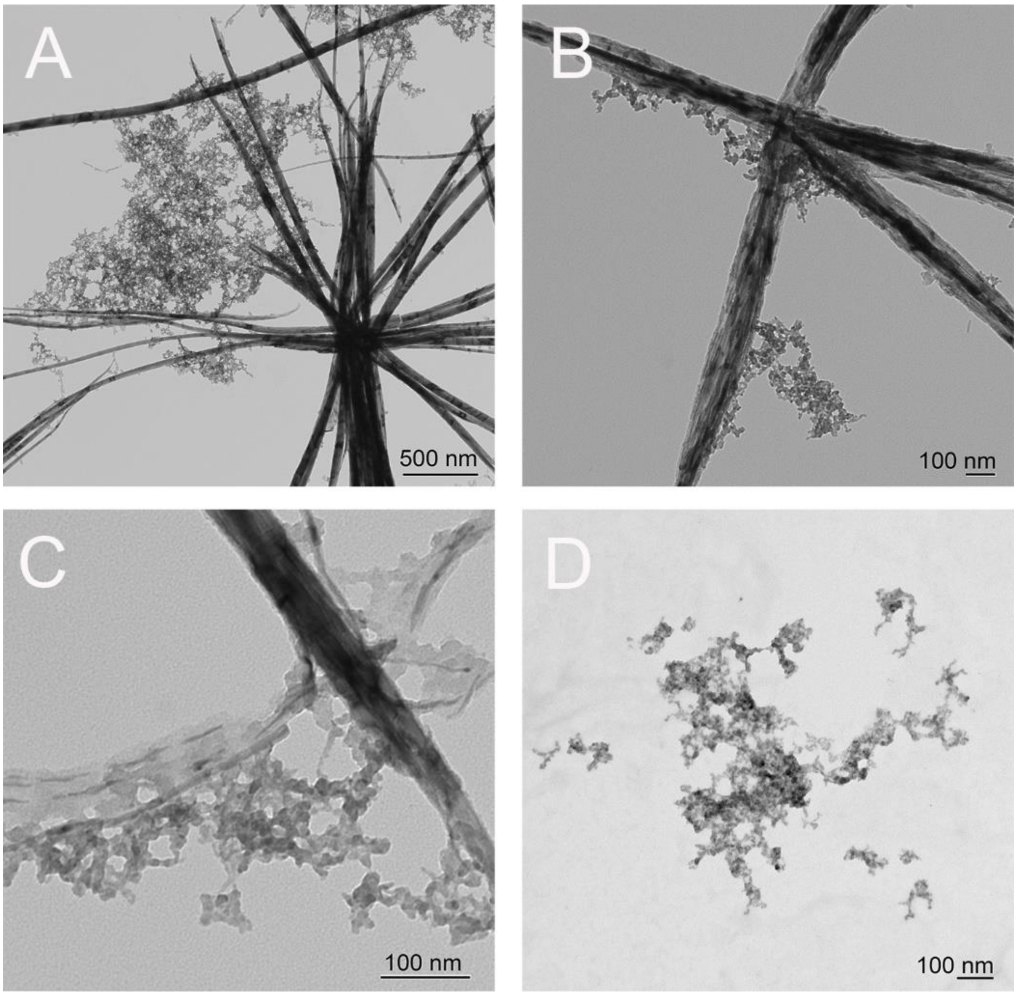

Figure 34. TEM characterization of zinc NPs. *Zinc structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

Mercury spherically-shaped NPs grew all over the NW (Figure 35, A and B). The organic coating was observed surrounding the new NPs (Figure 35, A, B, C and D). NPs had an average size of more than 100nm (Figure 35, C and D). This is probably because mercury tends to agglomerate and form bigger structures.

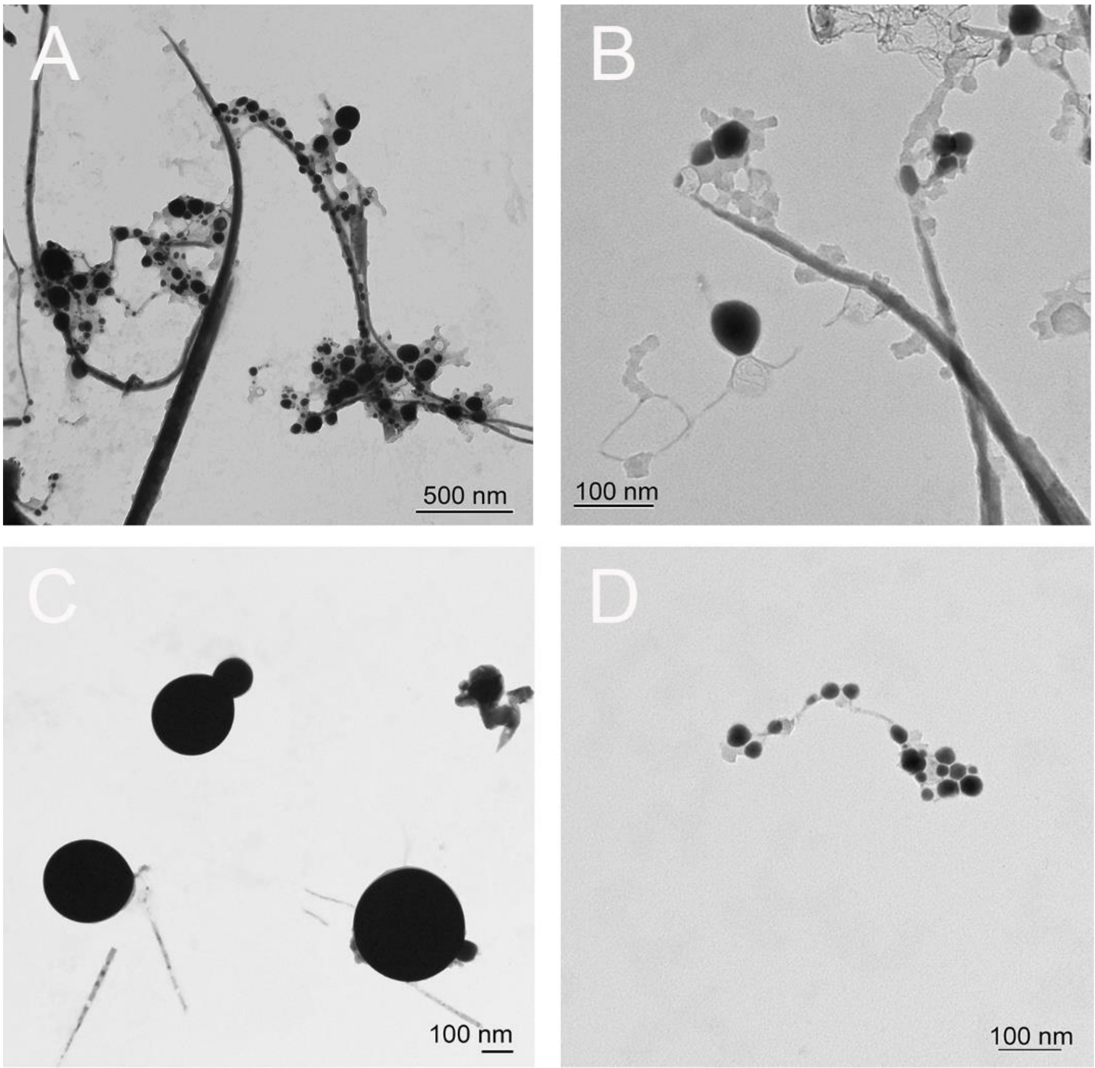

***Figure 35*.TEM characterization of mercury NPs.** *Mercury structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

Lead amorphous NPs were formed within the presence of the NW, however they were not found attached to it (Figure 36, A and B). The organic coating of the NWs was present over the lead NPs (Figure 36, A, B, C and D). Although NPs were formed, not finding them attached to the NWs suggested that they might not remain on this structure, so it would not be possible to use tellurium NWs to remove this heavy metal from water.

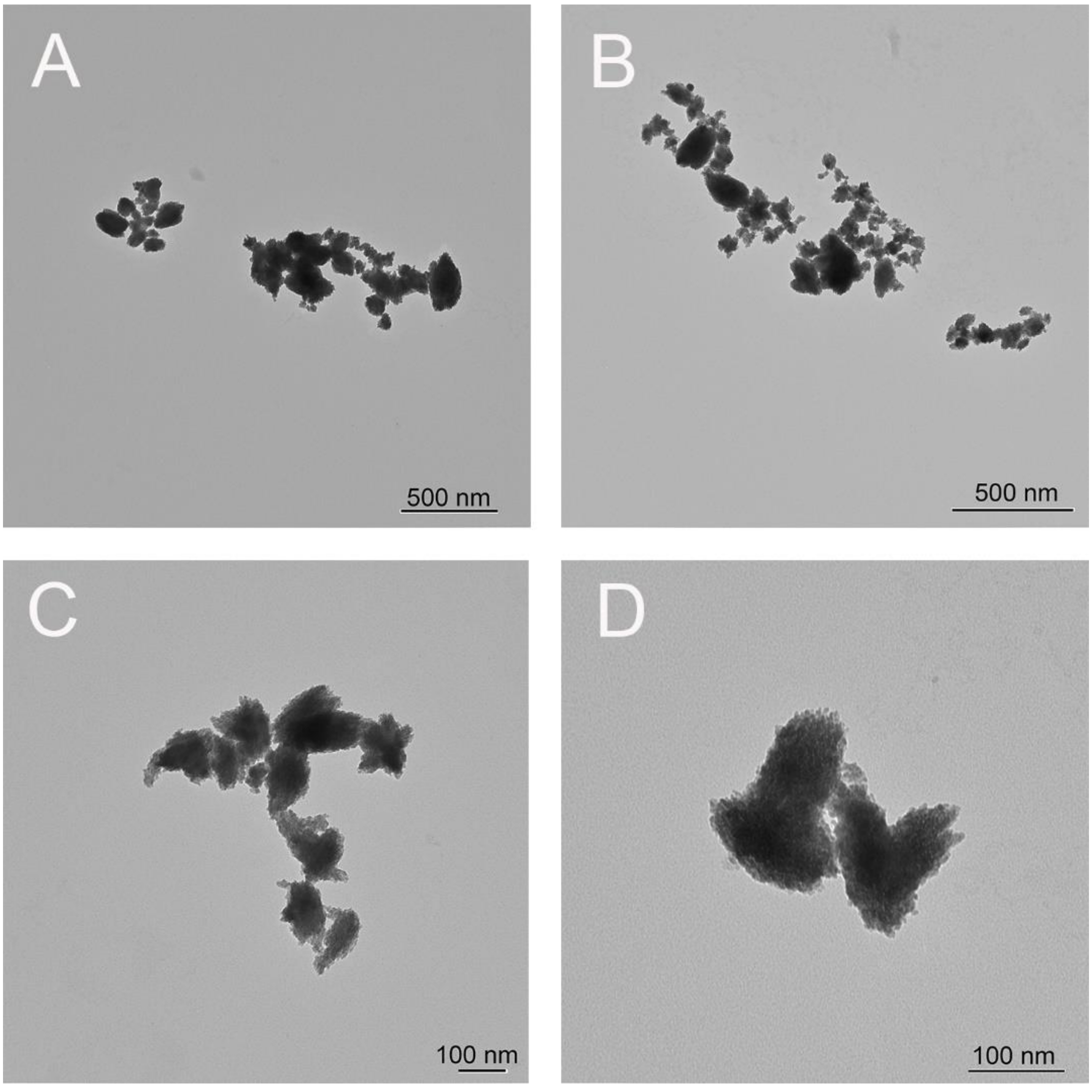

Figure 36. TEM characterization of lead NPs. *Lead structures were observed using TEM characterization right after the reaction.*

Figure 35 shows the formation of small and spherically-shaped cadmium NPs on top of the NWs, growing without any preference (Figure 37, A, B, C). The organic coating was also observed on the NPs (Figure 37, C and D). The resulting NPs were found in a small concentration and agglomerated on the edges of the NW (Figure 37, C and D). TEM characterization did not show the release of the NPs.

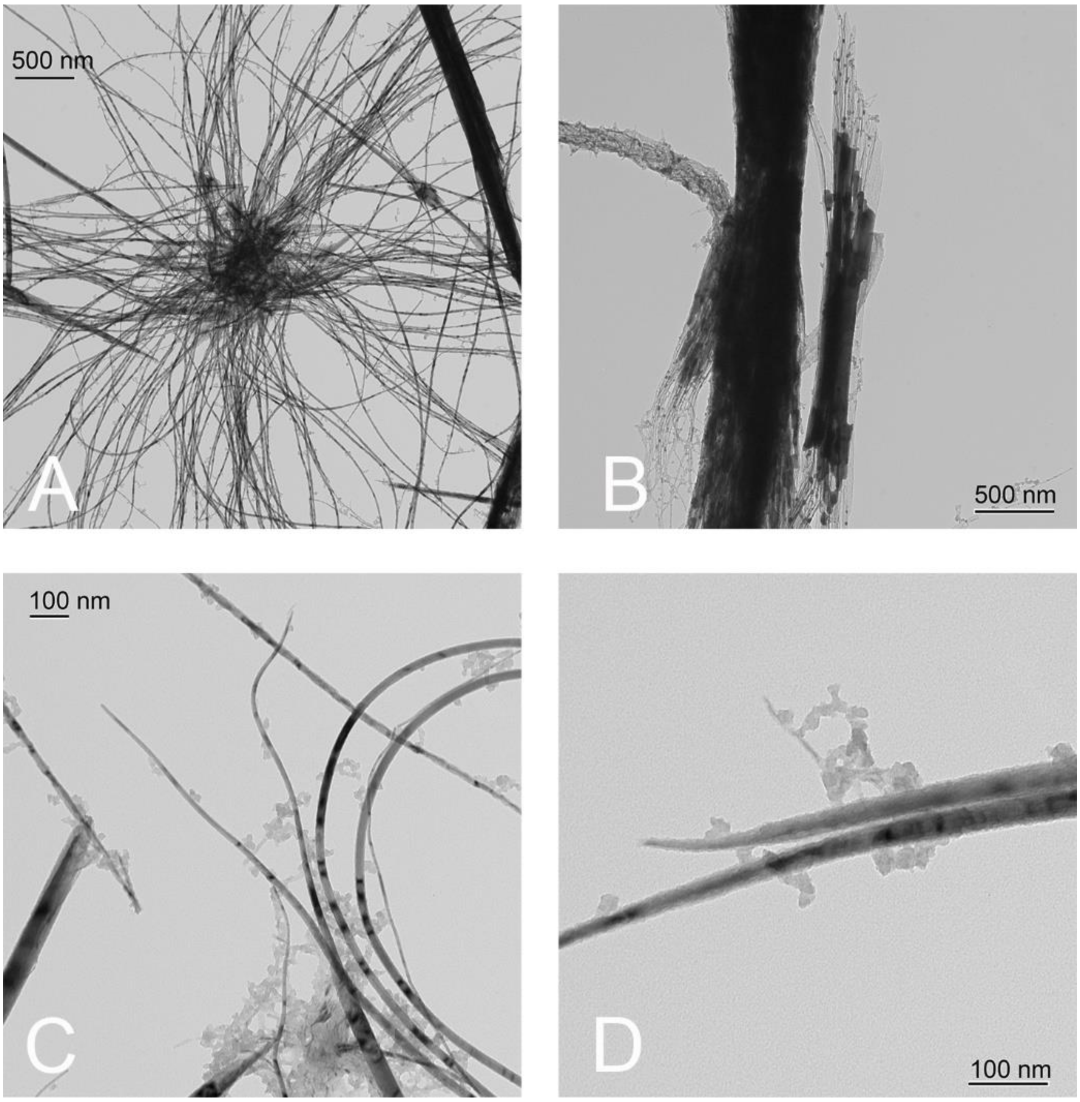

***Figure 37*. TEM characterization of cadmium NPs.** *Cadmium structures growing on top of the tellurium NW was observed using TEM characterization right after the reaction.*

In general, metal NPs are formed on the NW, indicating that heavy metals present in aqueous media can be reduced by the starch present on the NW structure. This leads to the formation of the NPs, and thus affords their removal from contaminated waters. Moreover, heavy metal NPs tend to agglomerate, so it would be easier to remove them as bigger structures would be formed. It is hypothesized that starch kepts the structure together.

4.2.3. Heavy metal removal from water

Once the NPs formed was observed, the next step consisted of the design of a methodology which allowed the elimination of heavy metals in water.

Briefly, in a typical process, 1 mL of a 5 mg/mL metallic salt solution (Table 5) was mixed with 1 mL of a GREEN-TeNWs solution after the reaction –synthesized following the procedure described in section 2.2 – and rinsed with 15 mL of distilled water. After 1 minute at room temperature the solution was centrifuged, the subsequent supernatant was kept for inductively coupled plasma mass spectrometry (ICP-MS) analysis in order to quantify heavy metal removal.

Further research should be carried in order to show results.

5. Conclusion

During the past years, green chemistry has been considered a potential alternative synthesis method to overcome the main drawbacks of traditional synthetic approaches, such as harsh reaction conditions, production of toxic by-products, expensive and time-consuming methodologies. In this research, GREEN-TeNWs were successfully synthetized by a hydrothermal reaction using starch as the raw material, hence following a green-synthetic methodology.

In addition, GREEN-TeNWs presented potential enhanced biomedical applications - over a range of concentrations between 5 and 100 μg/mL - in comparison to the CHEM-TeNWs. Anticancer properties were tested towards melanoma cells showing a reduction in the cell proliferation after 5 days of analysis. Furthermore, GREEN-TeNWs presented biocompatibility properties when treated on human dermal fibroblast (HDF) cells increasing their proliferation. Therefore, all these characteristics make GREEN-TeNWs excellent candidates for numerous therapeutic applications.

Moreover, it has been attested that GREEN-TeNWs can be used as a template for the growth of metallic NPs as a cost-effective and straightforward methodology. This process generates independent metallic nanostructures in just one minute, at room temperature and without the use of any extra reducing agent than the starch present as a coating of the NWs. Therefore, a new route to obtain NPs in aqueous media has been accomplished.

Additionally, this technique produces synergetic metallic structures composed of NP-TeNWs. The present entity is considered a structure with enhanced biomedical applications in comparison to the bare GREEN-TeNWs structure. Synergetic structures made of PdNPs and PtNPs were used to test these properties in order to elucidate the different effects caused by the use of various metallic salts.

Antimicrobial assays towards *E. coli* and *S. aureus* showed a significant delay on the bacterial growth with respect to GREEN-TeNWs. It is hypothesized that could be explained because of the large active surface area the NP-NWs present.

Anticancer properties were proved on melanoma cells. The synergy showed a decrease in the proliferation of cancerous cells when NPs-TeNWs were used compared to GREEN-TeNWs. No cytotoxicity was associated to these nanostructures when treated in concentrations up to 25 µg/mL.

Therefore, the synergetic structures obtained on this novel green-synthetic process offer important improvements on in terms of antimicrobial, anticancer and biocompatibility properties in comparison to GREEN-TeNWs previously synthetized.

Besides, ongoing research is proving that GREEN-TeNWs can be used as a coating agent for metallic surfaces in order to improve their antibacterial and biocompatibility attributes. In addition, the utilization of GREEN-TeNWs is not limited to the medical field, as ongoing researchers are demonstrating, TeNWs offer a huge potential for their use in new areas of study such as environmental remediation. These structures can be used to remove heavy metals in water by forming nanoparticles of these elements on the top of the NW thus preventing them to be found in the aqueous media.

Nonetheless, plenty of research should be done in order to elucidate the unknowns of this technology and its implantation into the biomedical field.

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