الأكاديمية العربية للعلوم والتكنولوجيا والنقل البحري
Arab Academy for Science, Technology & Maritime Transport

Development of Cellulose Nanofibers Filaments and 3D Scaffold for Bone Tissue Engineering Application

Keywords:

Cellulose nanofibers; wet Spinning; 3D Structure; Antimicrobial activity; Hydroxyapatite; Biomedical applications

- **Funding and Duration:**

  Total cost: 500,000 pounds for 12 Months.

  Research Theme: Biomedical applications

Proposal Summary in English

There is a high demand to develop a multifunctional material with adequate mechanical properties, adapted electrical properties, and thermal conductivity to fulfil the function of sensing and actuation, as well as antimicrobial activity. Multifunctional materials can be used in various applications, including artificial muscles, bone tissue engineering, sensors, and textiles. Cellulose nanofibrils (CNF) have a variety of advantages, which render them suitable for use in different applications. The present project is aiming to investigate and develop modified multifunctional cellulose nanofiber filaments and 3D scaffolds for bone tissue engineering applications. This can be achieved by incorporating different additives such as biopolymers and silver nanoparticles. In addition, effect of the ion-exchange mechanism of silver nanoparticles on the lattice stability and growth of nano-hydroxyapatite (HAp) particles in simulated body fluid will be studied. Furthermore, selected composite 3D scaffolds will be loaded with an active agent (drug) and subjected to in-vitro studies and biological evaluation aiming to optimize its medicinal performance. As a result, particular interest will be paid to the implementation of green processes and the use of non-toxic reagents/solvents at the different stages.

Proposal Summary in Arabic

ان المواد متعددة الوظائف تتطلب أن تكون ذات خواص ميكانيكية جيدة وذات موصلية كهربائية وحرارية ملائمة للتطبيق المستهدف ، بالإضافة إلى النشاط المضاد للميكروبات. يمكن استخدام المواد متعددة الوظائف في تطبيقات مختلفة وعديدة، بما في ذلك العضلات الاصطناعية وأجهزة الاستشعار والمنسوجات الإلكترونية والمكثفات الفائقة. تعتبرألياف السليلوز النانونومترية (CNF) ، وهي المواد الأكثر وفرة على الأرض، من المواد الواعدة لتطوير مواد جديدة متعددة الوظائف. تتمتع ألألياف السليلوزيه النانونومترية بمجموعة متنوعة من المزايا ، مما يجعلها أكثر ملاءمة للاستخدام في التطبيقات الطبية. يهدف هذا المشروع إلى انتاج خيوط والسقالات ثلاثية الأبعاد للتطبيقات الطبية الحيوية. سيتم ذلك من خلال دمج إضافات مختلفة مثل البوليمرات الحيوية وجسيمات الفضة النانوية. بالإضافة إلى ذلك ، سيتم دراسة تأثير آلية التبادل الأيوني لجسيمات الفضة النانوية على استقرار الشبكة ونمو جسيمات النانو هيدروكسيباتيت (HAp) في محاكاة سوائل الجسم. علاوة على ذلك ، سيتم تحميل السقالات ثلاثية الأبعاد المركبة المختارة بعامل نشط (دواء) وستخضع لدراسات في المختبر وتقييم بيولوجي بهدف تحسين أدائها الطبي. سيتم اعطاء اهتمام خاص لتنفيذ التجارب المعملية بطرق جديدة وغير ضارة واستخدام الكواشف / المذيبات غير السامة في المراحل المختلفة من إعداد المواد.

Introduction/Background

Development of biomaterials from natural resources is currently the subject of intense research during the past two decades. Special attention has been paid to cellulose nanofibers (CNF), due to their renewable origin as well as their high surface area and excellent mechanical strength. CNF which are derived from cellulose, the most abundant structural elements on the Earth, is very promising bio-based material for the elaboration of new multifunctional materials for different application as shown in **Fig.1**. CNF are also considered as biocompatible and non-toxic biomaterial. In the same context, cellulose nanofiber hydrogels and their related nanocomposites have gained increasing interest, in particular in modern medicine, for biomedical and pharmaceutical specially tissue engineering [1]. The use of CNF hydrogels as starting biomedical materials for scaffolds has a role in promoting three-dimensional cell culture or drug-releasing matrixes [2-4]. Similarity of CNF and collagen, easily regarding functionalization, high affinity with water and pseudo-plastic and thixotropic properties was investigated [4-6]. Substitution of collagen with CNF is a promising trend for more efficient and lower cost scaffolds. Blending of CNF and other biopolymers such as alginate, gelatine and collagen can enhance the performance of CNF for cell encapsulation, while providing immune protection and allowing good metabolic functionality in a 3D culturing environment is an interesting topic. Spinning of CNF into one-dimensional filaments has received a large attention recently. This technique is a suitable way to produce cellulosic filaments material through valorisation of the distinguished benefit of CNF mechanical strength via alignment along the filament axis [7-10]. Several factors influence the filament formation and its properties, such as formulation of the hydrogel as well as the coagulation bath, shear rate during spinning, and drawing ratio. The type of coagulation reagent, bath concentration and temperature play important roles on filaments spun from CNF and their characteristics, such as morphology, mechanical and viscoelastic properties [11-12]. Furthermore, spinning can be combined with functionalization routes that impart new properties to CNF and their filaments. The incorporation of silver nanoparticles (Ag NPs) has enhanced surface catalytic activities, thermal conductivity, and antimicrobial activities during the continuous production of CNF filaments which gives them new potential applications as antimicrobial materials in medical applications [13].

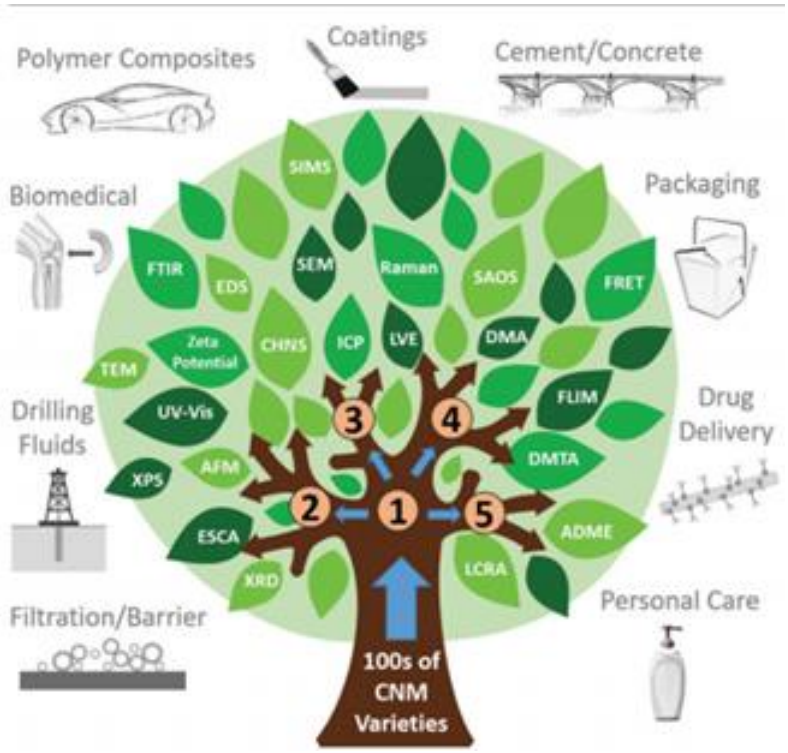

Fig.1 CNF various applications in industry in biomedical field. (adapted from [1])

The utilization of 3D printing, especially in biomedical applications has many advantages towards the personalized products and mass customization which can be used for the manufacturing and fabrication of the models, surgical parts, and various types of implants. In addition, CNF hydrogels have been used in bio-printing, where it was reported that the addition of CNF prevents of the collapse of printed shapes. The shear thinning characteristics of CNF and alginate suspensions are particularly desirable features, as they enable hydrogel inject ability, and thus, the fabrication of nanocomposite threads or textile yarns [5, 6]. The improvement of biocompatible frameworks in 3D printing has been particularly encouraged for tissue engineering applications. The fabrication of biomimetic bone scaffold is one of the most important applications of the biomaterials to replace the damaged hard tissue and repair bone defects. Hydroxyapatite is the primary inorganic component of solid biological tissues such as bones and teeth [5, 14, 15]. The optimized hydrogels will be used as a carrier of a selected drug to prepare composite scaffolds having the potential to be used as a drug delivery system.

To the best of our knowledge, there are only few studies reported in spinning of CNF hydrogels [8, 16, 17]. The present project comprises three main tasks as shown in **Fig.2**:

- Development of composite nanomaterials with wet spinning technique and filaments.

- Spinning to produce high-quality filaments: parameters that can affect the process and the winding mechanism will be optimized.
- Applications: in this section, different forms will be prepared (filaments and 3D devices) and their performance will be tested (structural, mechanical, and biological properties). Furthermore, the drug-loaded scaffolds will be subjected to studies related to in-vitro drug release.

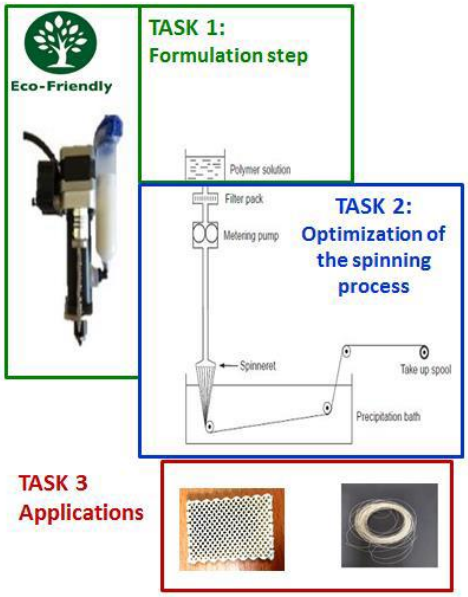

Fig.2 Project tasks to prepare CNF filament and 3D scaffold.

Objectives:

Hydrogels and nanocomposites have gained increasing interest in modern medicine, for biomedical and pharmaceutical applications as well as tissue engineering. Because of that, nanofibrillar cellulose (CNF) is the focus of study due to its versatile and interesting properties. For instance, studies deal with the use of CNF hydrogels as biomedical materials for scaffolds that promote three-dimensional cell culture or drug-releasing matrixes. CNF have similarity to collagen fibers: they can be easily functionalized, exhibit high affinity with water and pseudoplastic and thixotropic properties. CNF are also considered as biocompatible and non-toxic biomaterial. Alginate is a widely studied biopolymer, also known to be biocompatible, non-toxic, and frequently used in tissue engineering. This is a linear polysaccharide found in species of brown algae; it forms gels, particularly in the presence of divalent ions, such as calcium and barium, where the positively charged ions bind with the negatively charged glucuronic acids. Alginate is a recognized material for cell encapsulation, while providing immune protection and allowing good metabolic functionality in a 3D culturing environment. In addition, CNF/alginate hydrogels have been used in bio-printing, where it was reported that the addition of CNF prevents of the collapse of printed shapes. The shear thinning characteristics of CNF and alginate suspensions are particularly desirable features, as they enable hydrogel injectability, and thus, the fabrication of nanocomposite threads or textile yarns.

- The main objective of the present project is to develop a novel multifunctional biomaterial based on filaments and 3D scaffold via wet spinning as the main process based on cellulose nanofibers. The isolation of cellulosic nanofibers will be carried out from local agricultural wastes via chemical and enzymatic methods (green methods).
- During the current project, new formulations of CNF-based filaments and 3D scaffolds will be constructed and evaluated using alginate, gelatin, and fish collagen; green routes of preparation will be followed.
- Antimicrobial properties are extra advantage to the filaments and scaffolds which inhibit the attack of microbes while use, so silver nanoparticle will be used as antimicrobial agent and its role in the growth of HAp from SBF is to be studied.
- A detailed antimicrobial activity towards the Gram-negative and positive bacteria and the extent of this activity will be studied and correlated with the biomineralization of HAp. Likewise, the biocompatibility, cellular binding, and proliferative studies will also be carried out to confirm the cytotoxicity and bioactivity of the HAp particles precipitated in the presence of the silver nanoparticles. Finally, selected formulations will be loaded with an active moiety (drug) to enhance their pharmaceutical and medicinal effect.

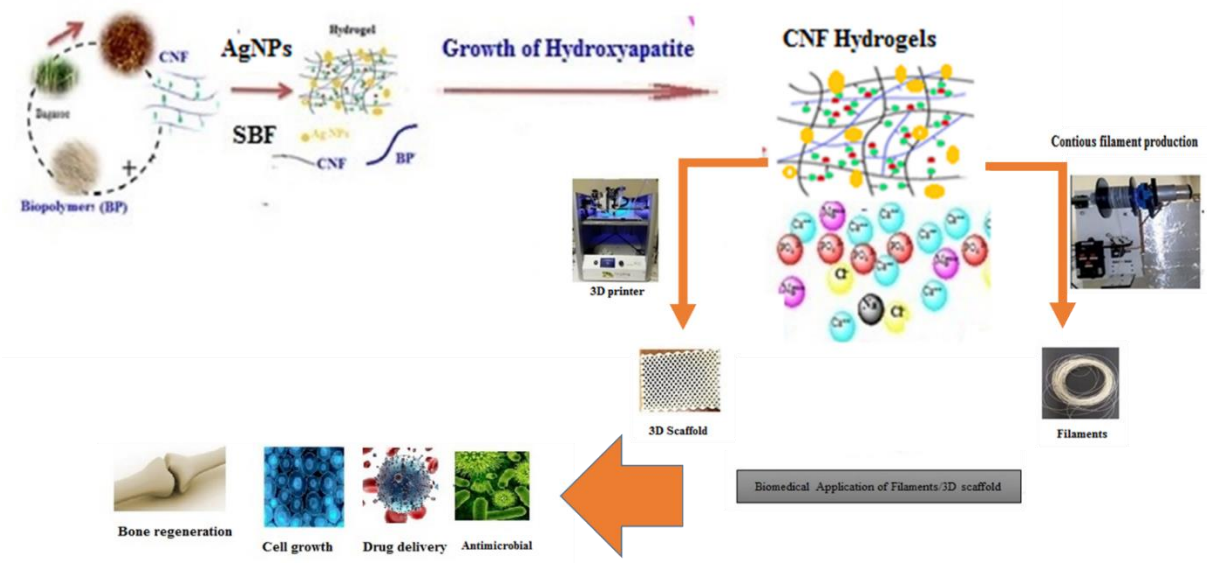

Fig.4. Fabrication process for 3D printing scaffold and continuos filament production from hydrogels.

Project Description :

Today, the high demands on multifunctional materials request them to be highly resistant, light, have adapted electrical and thermal conductivity, and even to fulfil the function of sensing and actuation. Multifunctional materials can be used in various applications, including artificial muscles sensors [6-8], electronic textiles [9-10] and supercapacitors [11-12]. Cellulose nanofibrils (CNF, according to **Fig.5**), which are the most abundant structural elements on Earth [13], are very promising bio-based multifunctional materials [14-18]. As an example, the Young's modulus of macrofibres obtained by assembling NFCs can reach 80 GPa [19]. At present, NFCs are not only able to lead to materials with excellent mechanical performance but may also be used in various domains due to their possibility to be functionalized, to act as a dispersing agent [20-21] or a rheology modifier, to improve barrier properties of films. Consequently, they can be exploited to make high-performance materials. In this context, the present project aims at developing new multifunctional materials, using cellulose nanofibres (CNF) and wet spinning as the main process, to produce filaments and 3D structured mats/tissues **(Fig.2**). Two main application domains are targeted which are the production of biocompatible filaments for medical applications and 3D structured for reinforcing scaffolds or medical patches. Despite the apparent gap between the targeted applications, they are based on the same scientific approach which relies on the elaboration of alginate/CNC based filaments /structures. The major expected breakthrough is the development of composite filaments with outstanding mechanical properties (E > 10 GPa) that can be used either as filaments or 3D structured tissues. Particular interest will be paid to the implementation of green processes and the use of non-toxic reagents/solvents at the different stages of the elaboration of the materials.

Spinning cellulose nanofibrils (CNF) into one-dimensional filaments has received a large attention recently [22-24]. This technique is a suitable way to produce cellulosic filament material and to optimally harness their intrinsic strength. In fact, this gives the possibility to fully benefit from the CNF mechanical strength through their alignment along the filament axis [22-24]. Furthermore, spinning can be combined with functionalization routes that impart new properties to CNF and their filaments. Several factors influence the filament formation and its properties, such as formulation of the hydrogel as well as the coagulation bath [25-30], shear rate during spinning [31,32], and drawing ratio [26,33,34]. The type of coagulation reagent, bath concentration and temperature play important roles on filaments spun from NFC and their characteristics, such as morphology, mechanical and viscoelastic properties [35-37].

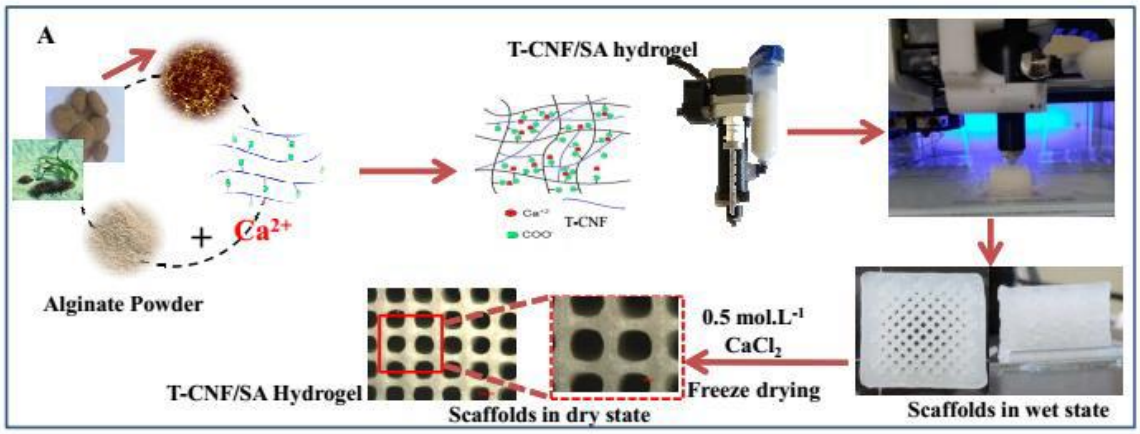

Fig.5. Fabrication process for 3D printing scaffolds.

There are a few studies dealing with the spinning of CNF. Most of them reports non ecofriendly formulation and/or not safety process, particularly for the coagulation bath often composed of toxic reagents or organic solvents [23,16,25]. In this project, a new formulation and a safety process will be studied to produce both filaments and 3D structured mats/tissues (**Fig.6**). The new formulation will be made of biocompatible and biodegradable substrates namely alginate and CNF (prepared by green routes). The use of glycerol will be tested to modify the properties of the final product. The filaments or 3D structured mats will be produced by an eco-friendly wet spinning step as the main process. In our case, coagulation will be performed by using calcium salts-based baths.

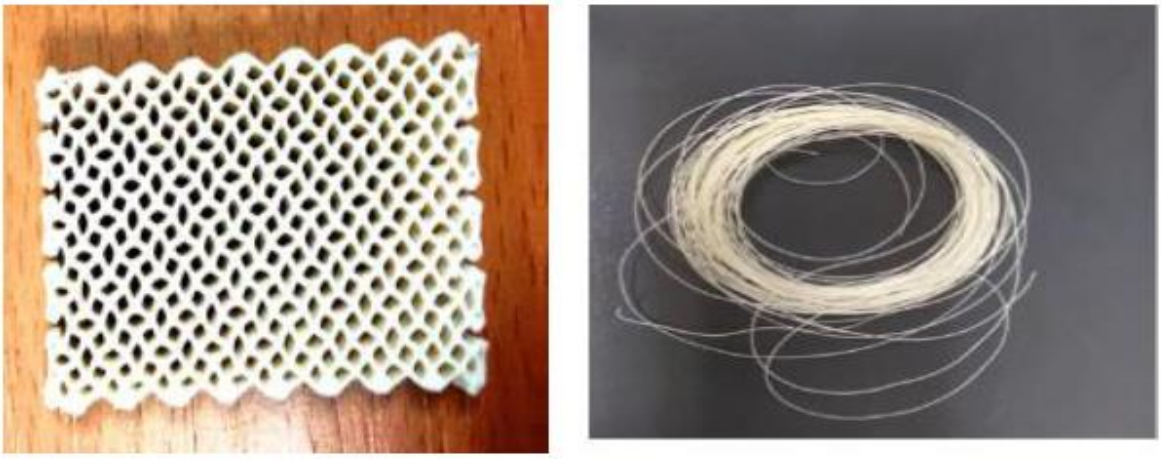

Fig.6. Preliminary study of the feasibility of preparing a CNF / Alginate.

Research Design and Methods

The project will be divided in four main tasks in detailed below:

WP1. Preparation and characterization of extracted and modified cellulose nanofibers.

Bagasse raw materials will be utilized as starting material to produce cellulose nanofibers. The obtained cellulose fibers will be fully characterized (DP, morphological properties, chemical composition…). The delignification of bagasse raw materials will be carried out in a reactor according to our reported procedure [18]. In brief, a total alkali charge of 7 % expressed in NaOH (based on w/w o.d. material), an anthraquinone concentration of 0.1% (w/w with respect to o.d. material) and a cooking time at constant temperature of 120 min. The liquor to solid ratio and the temperature are fixed at 10 and 160°C, respectively. After this stage, the obtained fibers will be bleached using sodium chlorite method to remove the residual lignin. Cellulose fibers will be first refined with a disk refiner at 2% (wt/wt) until a drainage index of 90°SR (ISO 5267-1). All the necessary equipment is available in Cellulose and Paper Department, National Research Centre.

Two ways will be investigated as pretreatment of the *cellulose fibers* namely:

Enzymatic treatment

The enzymatic hydrolysis will be performed utilizing cellulase Celluclast 1.5 L and endoglucanase FiberCare R enzyme solutions at various concentrations. The reactions will be performed under a gentle blending of 2 wt. % cellulose suspensions at a temperature of 50 °C during 2 h in acetate buffer solution of 50 mM and pH of 5, will be prepared using sodium acetate trihydrate and acetic acid. The enzymatic activity will be stopped by heating the suspension at 80 °C for 15 min. Chloroform at 0.01 wt. %will be utilized as a biocide.

- **Chemical treatment**

The synthesis of surface functionalized CNFs through TEMPO-assisted oxidation of cellulose fibers will be achieved through the procedure as described in our previous works [5, 18]. A 10g of Cellulose fiber will be suspended in 750ml of water containing 0.025g TEMPO and 0.25g sodium bromide. NaClO solution (3.84 mmol/g of cellulose) will be added to the slurry under continuous agitation. The pH of the will be kept constant at 10.5 under room temperature through the addition of 0.5M NaOH using a pH stat till no further decrease in pH will be observed. The reaction will then be brought to a stop and the pH adjusted to neutral using hydrochloric acid (HCl). The pulp obtained will be washed using deionized water and finally subjected to homogenization to produce functionalized CNFs containing carboxylic. As a result, for this treatment, a charged carboxylate groups are produced on the surface of the fiber. Thereby, C6 hydroxyl group is selectively converted into carboxylate group and consequently, the nanofibers separate from the fibers due to the repulsion between the negatively charged carboxylate groups. After oxidation pre-treatment of prepared fibers, a 1-2% fiber suspension in water will be homogenized with high-pressure homogenizer.

- **Characterization of the prepared CNF**

The obtained cellulose nanofibers will be fully characterized (DP, chemical composition, transmission electron microscopy (TEM), scanning electron microscopy (SEM), infrared spectroscopy, Rheology properties and X-ray will be measured.
Determination of degree of polymerization (DP) will be measured using the capillary viscometer method according to ISO 5351:2010 from the intrinsic viscosities (ηint) of cellulose, dissolved in copper (II) ethylenediamine (CED) solution. The viscometry average degree of polymerization (DPv) is calculated from the intrinsic viscosity using the Mark-Houwink-Sakurada equation:

$$DPv^{0.905} = 0.75\ \eta int.$$

Transmission electron microscopy (TEM): The optical microscopy images will be taken using optical microscope in a transmission mode. The cellulose suspensions are diluted to a concentration of 0.02 wt.% and a drop is placed between the glass slide and a cover slip. The images are captured by AxioCamMRc 5 digital camera.

Atomic force microscopy (AFM) will be carried out using a Multi-modal AFM (DI, Veeco, Instrumentation Group) with both tapping and conductive mode (C-AFM). The tips are Multi130 for tapping and MESP for C-AFM. Before the analysis, a drop of highly diluted microfibrillated cellulose suspension is deposited on a fresh mica substrate and left to air dry for at about 2 h.
***Scanning electron microscopy* (SEM):** The surface and cross section will be observed using scanning electron microscopy (Zeiss ULTRA55 Scanning Electron Microscope (SEM) with an acceleration voltage of 15kV). Each freeze-dried sample is precoated with gold/palladium alloy before observation.
X-ray Diffraction (XRD): Diffraction patterns will be obtained from a Phillips X-ray diffractometer using Cu-Ka radiation at 40 kV and 25 mA. Crystallinity index (CrI) will be calculated from the X-ray diffraction patterns.

WP2. Preparation of different Cellulose Nanofibers (Enzymatic and Oxidized) filaments with Silver nanoparticle.

Mechanical disintegration of the fibers

After pre-treatment of prepared fiber, a 1-2% fiber suspension in water will be homogenized with high pressure homogenizer. The disintegration process will be first carried out by passing the fibers suspension at a pressure of 300 bar for about 5 passes until the suspension turned to a gel. Then, the gel was further homogenized with about five additional passes at 600 bar, to ensure high level of disintegration. Due to the excessive shearing action during the homogenization process, the temperature of the suspension increased up to 60-70°C.

WP.3 Preparation and characterization of CNF/biopolymer/ Ag (NPs) suspensions with or without glycerol

- In this part of the project, the cellulose nanofibers will be used to prepare different hydrogels using biopolymers such as alginate, gelatin, and collagen with silver nanoparticles.

- Optimization and characterization of the formulation: As properties of the CNF and the suspensions may affect the performance of the filaments, the CNF aspect ratio, which is influenced by the pretreatment as well as its intensity, will be investigated. The effect of CNF solid content will also be studied as well as the possibility to combine aspect ratio and solid content by determining CNF crowding factor and measuring rheological properties.
- The effect of molar ratio of AgNPs will be studied.

Biomimetic mineralization

The biomimetic mineralization of hydroxyapatite will be done as described in our previous work [5]. Simulated body fluid (SBF) will be prepared and used to accelerate calcium phosphate formation. The CNF hydrogel will be prepared in the presence of silver nanoparticles. The mineralization process will be carried with stirring at room temperature. SBF will be renewed after centrifugation every 48h and the pH will be adjusted to 7.4 and checked regularly to minimize problems associated with SBF preparation and stabilization. The final hydrogels will be purified by centrifugation and washing five times with pure water and ethanol. The obtained Hydrogels will be fully characterized with (transmission electron microscopy (TEM), scanning electron microscopy (SEM), infrared spectroscopy, Rheology properties and X-ray diffraction.

Antimicrobial Assay

Antimicrobial activity of the prepared activated CNF hydrogels will be tested against bacteria such Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa),* Gram-positive (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus mutans),* fungi *(Aspergillus niger)* and yeast *(Candida albicans)* will be carried out by two approaches:

a) Qualitative determination approach (Disc and agar well diffusion method).
b) Quantitative determination approach (Turbidity or Broth inhibition method).
c) Qualitative determination approach.

1. Disc diffusion method

The prepared activated CNF hydrogels will be cut as discs with diameter of 5 mm. Disc diffusion method will be performed in a Petri dish containing Luria–Bertani (LB) media which composed of (g/l): peptone 10, yeast extract 5, sodium chloride 5 and agar 20. Cultures of model microorganisms will grow in LB broth at 37 ◦C and 200 rpm for 24 h. After incubation time, the cultured spread (100 µL) on agar plates and the disc of the activated materials will be placed on the inoculated agar plates and then incubated at 37 °C for 24 h. After time consumed, the antimicrobial activity of different active materials will be assessed by measuring the inhibition zone diameter.

2. Agar well diffusion method

The prepared activated CNF hydrogels will be prepared at different concentration. Agar well diffusion method will be performed in a Petri dish containing LB media. Cultures of model microorganisms will grow in LB broth at 37 ◦C and 200 rpm for 24 h. After incubation time, the cultured spread (100 µL) on agar plates and the agar well with diameter 5mm will fill up with the prepared solution (100 µL) and then the plates will be left in the refrigerator for 2 hs to complete diffusion of solution and temporally inhibit the growth of model organisms and then incubated at 37 °C for 24 h. After time consumed, the antimicrobial activity of different active material will be assessed by measuring the inhibition zone diameter.

d) Quantitative determination

1. Turbidity or Broth inhibition method

The prepared activated CNF hydrogels will be prepared at different concentrations. Turbidity inhibition method will be performed in flask containing 10 ml of LB media. All the model microorganisms will be grown in LB broth at 37 ◦C and 200 rpm for 24 h. After incubation time, the flasks will be inoculated with 100 µL of microbial suspension and assessed for antimicrobial by adding 100 mg or µL of the prepared activated materials then incubated in shaking incubator 200 rpm at 37 °C for 24 h. Microbial growth was measured by taking the optical density (OD) at 600 nm and the results will be expressed as growth inhibition % by the formula:

$$\text{Growth inhibition \%} = (OD_{control} - OD_{sample})/ OD_{control} \text{ X10}$$

Autoclaved all the required materials used in this experiment. Triplicate experiments will be performed for each activated material.

Cytotoxicity hydrogel materials

Evaluation of cytotoxicity of hydrogel materials will be conducted by means of MTT protocol. The MTT cytotoxicity assay is based on the reduction of 3- (4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H-tetrazoline bromide (known as MTT reagent) to form azure formazan in live cells that enables the evaluation of the enzymatic activity of succinate dehydrogenase and thus determine cell vitality. Next, the amount of formazan sufficiently formed for the number of live cells is determined by colorimetric measurements. Selected formulations will be loaded with an active agent (drug) to enhance their pharmaceutical and medicinal effect. This step will involve the preparation and characterization followed by in-vitro studies and biological evaluation.

WP4. Spinning process and characterization of the multifunctional bioactive hydrogels

4.1. After optimization of the formulation, the spinning conditions will be adjusted mainly by studying the effects of the shear rate and drying.

4.2. Evaluation of mechanical performance (in wet and dry conditions) will be performed by mechanical tests (DMA, tensile test,.....). The fibril orientation in the filaments will be also evaluated.

4.3. A trial at pilot scale will be carried out at the end of this project using the best formulation and spinning conditions.

Optimization of the spinning process

Spinning process: Continuous spinning of the hydrogels will be studied to prepare high strength filaments and/or 3D structured mats/tissues manufacturing. The optimization of the spinning process to produce high-quality fibers will be done by testing the parameters that can affect the spinning process and the winding mechanism. Based on a previous study [5, 6], the reference experimental conditions will be as follows: the prepared hydrogels will be extruded at room temperature using a needle 0.5 mm in diameter, a pressure of 0.5 bar at a steady dispensing head speed of 1000 mm.min-1. The width of the filament and the layer height will be set at 0.6 mm and 0.35 mm, respectively. These parameters will be used initially, before optimization. The preformed scaffolds and/or filament will be soaked in 0.5 mol. L-1 CaCl2 aqueous solution for 20 min to achieve the full crosslinking, and then washed with deionized water. Finally, the samples will be dried and tested in further experiments. 3D Printing device will be used for preparation of 3D scaffold and a screw-pump paste extruder will be used for wet spinning.

Anticipated Results and Evaluation Criteria:

Wider objectives and anticipated outcomes

- Create a new technology with enormous benefit and potential for future growth of medical devices and tissue engineering industries, especially in Egypt.
- Development the research areas at Arab Academy for Science, Technology & Maritime Transport in Aswan, and surrounding environment in upper Egypt.
- Initiating treatment of bone tissue engineering which maybe applying for Egyptian people with low cost and high adequate performance.
- Scientific collaboration between various fields such as Mechanical, production engineering, veterinary medicine, Physics, chemistry, and materials sciences.
- Scientific collaboration between AASST in Egypt and national and international institutions (please, see below, the training program).

Expected Project Outcomes and Impact to AASTMT:

AASTMT, nowadays, has attention for opening a medical science centre at El Alamein Branch to support Egyptian government's new directions. We must bring our understanding of multidisciplinary sciences to a higher level. As you know, environmental and biomedical engineering are becoming increasingly hot topics, and there's a tendency to join these two topics. With its low cost and multiple applications, varied uses, agriculture waste is preferable to other resources, including Cellulose. The main goal of the proposed project is to develop a multifunctional material for bone tissue engineering applications. Besides the scientific and economic rewards from this proposed research project to Egypt and worldwide. In particular of interest, this project is potential and essential for AASTMT, Aswan branch. Mechanical Engineering Department has been recently founded since 2018 We are strongly working on development our new faculty in a specific research area. Accordingly, this is the first time for us to apply to such grant program. We strongly ask the AASTMT funding agency to support our research project that will open doors for whole the faculty members to try obtaining research projects and making a massive collaboration within different national or international universities/institutes. This is very important to surrounding environmental, whereas there is no potential laboratory focusing on biomaterials for biomedical application in AASTMT, Aswan branch.

There are several potential impacts are foreseen for this project:

- Formulation of high value-added products made from nanocellulose derived from agricultural wastes, which still constitutes an available and cheap source of the starting material which is of great interest for the scientific researchers in Egypt and abroad.
- Acceleration of the development of nanotechnologies through preparation of nanomaterials by simple method.
- Optimization of cellulose nanofibre (CNF) based hydrogel preparation for the manufacturing of both antimicrobial filaments and 3D structured mats/tissues for biomedical applications.
- Potential transfer of the obtained results to industry due to the use of a simple process combined with CNF: several studies report that CNF based materials do not exhibit the classical drawbacks of nanoparticles for human-being health and can therefore be used as suture yarn and/or as crossed ligaments.
- Development of green processes and competitive green products meeting the societal requirements of the customers and reducing the environmental impacts.
- Cooperation between the partners from different background that will strength the research outcomes and promote the results to be international journals and applying to national and international patents.
- Mutual exchange of experience as well as easier access to experimental devices and pilots.
- Training of young scientists on biomaterials fabrication, tissue engineering, biocompatibility, clinical treatment, and biomechanical engineering.

Resources (humans & laboratories and facilities)

Professor. Mohamed Shehadeh has a good experience in the materials engineering and its applications also he has more than 15 years in the European projects and funded projects from Stakeholders.

Facilities: the infrastructure of the lab

Ragab Abouzeid has good expertise in projects management through his previous experience as Co-PI for several local and international projects. He finished a postdoctoral fellowship in France in 2018 with professors Alain Dufresne and Davide Benevente entitled Cellulose nanofiber as a bio-ink for biomedical applications using 3D printing technology. These international collaborations provided him a solid experience in the use of 3D printing techniques with cellulosic nanomaterials and professional experiences with highly advanced and sophisticated characterization tools. He has two articles published in high impact factors in 3D printing of nanocellulose for biomedical applications.

Facilities: cellulose and nano cellulose preparation in the laboratory of Cellulose and paper department national research centre to use it in 3D printer and biomedical applications

Hamouda M. Mousa: earned his B.Sc. of mechanical engineering from Assuit university in Egypt and his MSc from king Saud university, Saudi Arabia. He completed his PhD from Jeonbuk national university, South Korea. During his doctoral, his research focused on surface modification of biodegradable magnesium implant for bone tissue engineering application. He has started his career as assistant professor at mechanical engineering department, south valley university, Egypt 2016. He is a former Fulbright scholar under Egyptian Fulbright JFDP program at Texas A&M university, Texas, USA, 2017. He awarded south valley encourage award in 2017. In 2019, he started his granted Fulbright Egyptian scholar program at Massachusetts Institute of Technology (MIT), Cambridge, USA. His research interest in fabrication of materials onto micro and nanoscales with electrospinning and additive manufacturing and their emerging application in biomedical field, water treatment, biomedical devices, energy applications, and biosensors.
Facilities: Electrospinning and materials preparation in the laboratory of bioengineering and nanotechnology laboratory, mechanical engineering department, south valley university. Materials preparation as well as national research centre.
Office and Computer Facilities: laboratory space and computers facilities with Origin software are available in ASST.

Team Information:

The research team involved in the project consists of researchers of different disciplines such as mechanical engineering, materials engineering, industrial engineering. cellulose and paper, polymer chemistry, microbiology, applied organic chemistry and pharmaceutical technology.

Professor. Mohamed Shehadeh is currently the Dean of Engineering and Technology College, South Valley Campus. and a professor in the Marine Engineering Department, at the Arab Academy for Science and Technology and Maritime Transport (AASTMT). He obtained his BSc and MSc from the Marine Engineering Department at AASTMT, Alexandria, Egypt. He obtained his PhD from the mechanical engineering, Heriot Watt University, UK. He has participated in many European funds. He has more than twenty years of experience in the field of research in Mechanical, Material and Marine engineering.

1. IF Zidane, G Swadener, X Ma, **M.F Shehadeh**, MH Salem, KM Saqr. Performance of a wind turbine blade in sandstorms using a CFD-BEM based neural network Journal of Renewable and Sustainable Energy 12 (5), 053310.
2. L Aboud, AH Elbatran, A Mehanna, **M.F. Shehadeh**. Experimental Study on the Effect of Impingement Angles and Velocities on Erosion-Corrosion Behavior of API 5L-X42 Carbon Steel in Eroded Flow Medium. International Review of Mechanical Engineering (IREME) 14 (8), 493-503.
3. **M. F. Shehadeh**, A. H. Elbatran, Ahmed Mehanna, J. A. Steel, R. L. Reuben Evaluation of Acoustic Emission Source Location in Long Steel Pipes for Continuous and Semi-continuous Sources. Journal of Nondestruct Evaluation 38 (2), Article 40.

Associate. Prof. Ragab Abouzeid has good expertise in projects management through his previous experience as Co-PI for several local and international projects. He finished a postdoctoral fellowship in France in 2018 with professors Alain Dufresne and Davide Benevente entitled Cellulose nanofiber as a bio-ink for biomedical applications using 3D printing technology. These international collaborations provided him a solid experience in the use of 3D printing techniques with cellulosic nanomaterials and professional experiences with highly advanced and sophisticated characterization tools. He has two articles published in high impact factors in 3D printing of nanocellulose for biomedical applications. He will be responsible for writing the technical reports, reviewing, and discussing results with the research team. Moreover, he will be responsible for preparation of the bio-based cellulose nanofiber hydrogels and filaments preparation.

1. **Ragab E. Abouzeid**, Ramzi Khiari, Davide Beneventi and Alain Dufresne. Biomimetic Mineralization of Three-Dimensional Printed Alginate/TEMPO-Oxidized Cellulose Nanofibril Scaffolds for Bone Tissue Engineering. **Biomacromolecules, 2018, 19 (11), pp 4442–4452.**
2. **Ragab E. Abouzeid**., Khiari, R., Salama, A., Diab, M., Beneventi, D., & Dufresne, A. (2020). In situ mineralization of nano-hydroxyapatite on bifunctional cellulose nanofiber/polyvinyl alcohol/sodium alginate hydrogel using 3D printing. International Journal of Biological Macromolecules, Pages 538-547, 2020.

3. A Salama, **RE Abou-Zeid**, Cruz-Maya, V Guarino. Soy protein hydrolysate grafted cellulose nanofibrils with bioactive signals for bone repair and regeneration. Carbohydrate polymers 229, 115472.

Dr. Aly Hassan Elbatran is currently Associate Professor at Marine and Mechanical Engineering Dept.,and the Head of Mechanical Engineering Deartment , Engineering and Technology College, South Valley Campus, Arab Academy for Science and Technology. He obtained BSc and MSc from Marine Engineering Dpt., AASTMT, Alexandria, Egypt and PhD from mechanical Engineering, Universiti Teknologi Malaysia (UTM), Malaysia. He had participated in many European funds as well as works for ten years in design and research of mechanical and marine engineering fields. He has an academic background and deep research in fields of renewable energy, marine hydrodynamics, CFD and material science.

1. **AHA Elbatran**, OB Yaakob, YM Ahmed, Experimental Investigation of a Hydraulic Turbine for Hydrokinetic Power Generation in Irrigation/Rainfall Channels. Journal of Marine Science and Application, 1-12.
2. **AH Elbatran**, OB Yaakob, YM Ahmed, AS Shehata. Numerical and experimental investigations on efficient design and performance of hydrokinetic Banki cross flow turbine for rural areas. Ocean Engineering 159, 437-456.
3. L Aboud, **AH Elbatran**, A Mehanna, M.F. Shehadeh. Experimental Study on the Effect of Impingement Angles and Velocities on Erosion-Corrosion Behavior of API 5L-X42 Carbon Steel in Eroded Flow Medium. International Review of Mechanical Engineering (IREME) 14 (8), 493-503.

Hamouda M. Mousa. (Senior Assistant Professor at mechanical engineering department, south Valley university) He is material and mechanical design engineer, he was PI of STDF project and work with a multidisciplinary group in biomaterials and wastewater treatment field in south Korea, Egypt, and USA. He developed a nanofiber membrane for wastewater treatment and disinfection from bacteria using photocatalytic/antibacterial (ZnO NPs embedded within polymeric nanofiber). He performed several researches works and supervised a master and doctor students in a similar research focused in developing a nanofiber scaffolds for bone tissue engineering applications using synthetic polymers and metallic biodegradable Mg alloy. He recently has publication in nanofiber membrane using electrospinning technique for oil/water separation and bone tissue engineering. Hamouda will be responsible for designing and manufacturing of electrospinning nanofiber 3D scaffold using electrospinning technique. He also will be responsible for the 3D Scaffolds characterization and investigate the biomineralization mechanism as well as selection of the different nanomaterials that has antibacterial effect such as silver nanoparticles and zinc oxide. In addition, innovation of the best applications to use the suggested novel materials as biomedical device and writing and analysis of the results. The following is the recently related publications for the team member.

1. Hamouda M. Mousa , Mahmoud A. Mahmoud, Ahmed S. Yasin, Ibrahim M.A. Mohamed, " **Polycaprolactone tridentate ligand corrosion inhibitors coated on biodegradable Mg implant, Journal of Coatings Technology and Research, 2021.**
2. **Hamouda M.Mousa**, Husain Alfadhel, MohamedAteia, G.T.Abdel-Jaber, Gomaa A.A, " Polysulfone-iron acetate/polyamide nanocomposite membrane for oil-water separation, **Environmental Nanotechnology, Monitoring & Management, 2020.**
3. Mustafa Ghazali Ali, **Hamouda M.Mousa**, FannyBlaudez, M.S.Abd El-sadek, M.A.Mohamed, G.T.Abdel-Jaber, AbdallaAbdal-hay, SasoIvanovski, " **Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2020.**

Mohamed Taha, Assistant lecturer in Mechanical Engineering Department at AASTMT, Aswan Branch. Taha has an MSc in Mechanical in Engineering and specific in biomedical applications. Among master research, he got a scholarship in Denmark as working an assistant researcher at a southern university in Denmark for six months in the research group of "Drug Transport and Delivery" supervisor prof / Martin Brandel one of the leading scientists in that field and Editor-in-Chief of European Journal of Pharmaceutical Sciences in Elsevier publisher. Moreover, he got a scholarship from the Embassy of France/French Institute of Egypt fellowships program for the year 2019. this given for the excellence of my project about "Effect of Nano-cellulose on the properties of Biocompatible Polymeric composite". supervisor prof / Alain Dufresne. In 2020 I got a highly prestigious scholarship in France "Eiffel excellence scholarship" programme for Study the doctoral. In the lab LGP2 at Grenoble INP-Pagora, the Graduate School of Engineering in Paper, Print Media and Biomaterials is one of the best labs in France and Europe and third ranked in the world in my specialization.

1. Abdalla Abdal-hay, **Mohamed Taha**, Hamouda M Mousa, Michal Bartnikowski, Mohammad L Hassan, Montasser Dewidar, Saso Ivanovski. Engineering of electrically conductive poly (ε-caprolactone)/multi-walled carbon nanotubes composite nanofibers for tissue engineering applications, Ceramics International 45 (12), 15736-15740.

Research Team Information Table

Name of Res. Team Member in English	Name of Res. Team Member in Arabic	University / Institute in English	Position / Title	% of time spent on project	No. of months	Incentive per month (LE)	Number of other projects and their IDs	Total % of time spent on other projects	Contact No
Mohamed Shehadeh	محمد فهمى شحادة	AASTMT (PI)	Professor	30%	12	1800	-	-	01006156000
Ragab Abouzeid	رجب اسماعيل ابوزيد	National Research Center (Co-PI)	Associate Professor	35%	12	1800	-	-	01224052635
Aly Hassan Elbatran	على حسن البطران	AASTMT	Associate Professor	25%	12	1650	-	-	01111228845
Hamouda M Mousa	حموده محمد دردير موسى	South Valley University	Assistant professor	25 %	12	1650	-	-	01000444958
Mohamed Taha	محمد طه عبده	AASTMT	Assistant Lecture	30%	12	1350	-	-	01148581181

Project Management

Team Members	Role
Senior Personnel:	
Mohamed Shehadeh (PI)	The PI will be responsible about samples preparation using electrospinning. The effect of different fabrication strategies on structure morphology, crystallinity, thermal properties, and phase composition of samples by means of different investigation and characterization tests. In addition, writing international per-reviewed papers will be taken into his consideration as well as preparing the reports.
Ragab Abouzeid (Co-PI)	Study the degradation performance and mechanical integrity of the proposed materials for all pre-prepared samples. In addition, writing international per-reviewed papers will be taken into his account. In addition, specification of equipment that will be purchased form AASTMT funding should be also under his consideration.
Aly Elbatran (Researcher A)	Management and guiding of the proposed research project and selection of the used materials and plaining of their preparations. In addition, writing international per-reviewed papers will be taken into his account.
Hamouda M Mousa (Researcher A)	He will focus on the electrospinning technique and filament preparation and nanofiber formation, biomineralization test, materials characterizations, and mechanical properties. In addition, writing international per-reviewed papers will be taken into his account.
Mohamed Taha (PhD student and Researcher B)	Literature survey and ordering the needed materials and equipment, Fabrication and Characterization the prepared cellulose materials with nanoparticles physically and chemically and helping in writing and revision the result to be published at international journal.

Allowable Project Costs

Device name	uses	Estimated price (EGP)
1. 3D printer	For hydrogel design and form 3D scaffold	50,000
2. Optical microscope (Olympus)	Surface morphology up to 10,000X	83,000
3. The standard muffle furnace	To prepare nanoscale particles	60,000
4. Sonication Prob	To prepare Nano materials	65,000
5. Digital Hotplate Magnetic Stirrer	To mix the composite materials	15000
6. Analytical Balance 4 digit	To measure the number of nano materials	11,000
7. Digital sonication bath	To mixing the composite materials	20,000
Total cost		**304,000**

Breakdown of Costs Other Grant(s) (Max. two pages)

Eligible costs	Break downs		AASTMT support (L.E.)
(A) Staff Cost	PI		21,600
	Co-PI		21,600
	Researcher A1		19,800
	Researcher A2		19,800
	Researcher B		16,200
	Technicians and/or Labour		1000
	Consultation fees		-
	Total		100,000
(B) Equipment	Equipment		304,000
	Spare parts		-
	Total Equipment		304,000
(C) Expendable Supplies & Materials	Stationary		5000
	Miscellaneous Laboratory, Field supplies, Materials		50,000
	Total expendable Supplies & Materials		55,000
(D) Travel	Internal Transportation		10,000
	Accommodation		15,000
	Total travel		25,000
(E) Other Direct Costs	Services	Manufacture of specimens & prototypes	10000
		Acquiring access to specialized reference sources databases or computer software	
		Computer services	
	Report preparation		2000
	Publications & patent Costs		
	Workshops organization or Training		4000
	Others (explain)		
	Total other direct costs		
(G) Total Costs			**500,000**

Plans for Disseminating Research Results / Sustainability of the action (Max. three pages):

Diffusion is a critical to this project's ultimate success. The aim of which is to increase the project's visibility and good results is to be reported on in the press creating well-structured plan and providing diverse information/creative workshops are two of the main milestones of overall effectiveness. The present study seeks to carry out appropriate measures of disseminating knowledge and data while contributing to the broader community: academics, engineers, the government, and the media in addition to the media. Finally, this will give a priority to the needs, current issues, and “hot topics” in public health would help advance the public dimension's understanding of biomedical application development cellulose nanofibers production for bone tissue engineering.
It employs various strategies to disseminate the information. a general public relations campaign will be used to share the findings, studies, conclusions, etc. AASTMT can make creative and persuasive web pages for internal and external use. everybody will have full access to all project documents on the registry, therefore accessible for every participant It will be a tool for disseminating results, as well as the ability to send and receive news and announcements. More information on the AASTMT will be posted on the website. The government wants to work with multiple universities in Egypt to improve the data base finesse of dissemination of resources Involving researchers and students from AASTMT, Aswan University, South Valley University, and the National Research Centre to form a dissemination community Offer the specialists and staff members of AASTMT. AASTMT branches in and around Aswan University exposure to new and training on these practises so they know more about and can fulfil the responsibilities they have. As part of our new campaign, flyers and handouts and video segments about the project were circulated, as well as E-news. Speakers from Grenoble-Alpes in France and other than Grenoble Alpes will be invited to the conference, and also participants from higher education institutes will be invited from EG, academia around the world. The target groups are HE institutions, undergraduate students and researchers will be one of the main dissemination tools. They will spread the idea through the outside community of AASTMT. The interaction among the project team of the AASTMT, South valley University and National Research Centre will disseminate the idea automatically through the personal contacts, beside the role of holding proposed workshops and seminars. The enterprise sector like non-profit Aswan Heart Centre and research, Magdi Yacoub Foundation is highly targeted in the dissemination strategy.

The project has various types of outlets for spreading, but it allows different ways of getting people access to information as well, like: Knowledge and instructional design workshops and seminars. Periodical publishing of hard copies (journals, pamphlets, posters) such as Facebook, Twitter, Instagram, Google+ and YouTube, among others.

The sustainability focuses on further developing cellulose nanofibers for bone tissue engineering. Different phases of the manufacturing phase will be rewarded for using non-toxic solvents and implementing green processes. Sustainability is an essential to

the design research since Egypt is increasingly needs students to work through the curriculum in this era of ever-changing educational requirements. It comprises visibility, networking, agreement, user contributions, and policy adherence. The long-term survival mechanism incorporates long-foreseeable financing and market advantages, such as competitiveness, flexibility, stability, and sustainability into its projection. The new position of fostering Global Domination in Egypt and Africa gives us access to 3D printers for artificial biomedical. so that we can print locally built devices for those locals." Many colleges, healthcare providers, entertainment facilities, and non-profit organisations.

Detailed Plan on Project's Activities (Gantt Chart):

Activity Name	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
1. WP1: Preparation and characterization of extracted and modified cellulose nanofibers.												
1.1 Pulping and bleaching of the raw materials and analysis of prepared pulps												
1.2 Enzymatic and Chemical pretreatment of cellulose pulp												
1.3 Preparation of CNF and its Characterization												
1.4 Characterization of the prepared CNF using (XRD, SEM, TEM, UV spectra, AFM, and FT-IR).												
2. **WP2: Preparation of different Cellulose Nanofibers (Enzymatic and Oxidized) filaments with Silver nanoparticle**												
2.1 In-situ preparation of Silver nanoparticles												
2.2 Preparation of CNF/biopolymers hydrogels												
2.3 In-situ biomimetic mineralization of hydroxyapatite												
2.4 Characterization of the prepared hydrogels CNF, Antimicrobial and Cytotoxicity tests												
3. **WP3: Formulation steps: Preparation and characterization of CNF/biopolymers/ Ag (NPs) suspensions**												
3.1 CNF/biopolymers/ / Ag (NPs) with Glycerol suspensions will be prepared.												
3.2 Optimization and characterization of the formulation												
WP4 : Spinning process and characterization of the multifunctional bioactive hydrogels												
4.1 Spinning process and characterization of the multifunctional materials												
4.2 Drug loading & characterization, Drug Release and Kinetic analysis												
WP5. Applications and writing the final report												
WP6: Project communication, dissemination and exploitation												
6.1: Page on AAST website												
6.2: Organization of information workshops and seminars												
6.3: Publication of periodical hard copies materials												
6.4: The communication platforms												

	Continued Activity		Sub Activity

Key Publications and references:

1- Gaharwar, AK.; Peppas, NA.; Khademhosseini, A. Nanocomposite hydrogels for biomedical applications. Biotechnol Bioeng. 2014, 111, 441–53.
2- Bhattacharya, M.; Malinen, MM.; Lauren, P.; Lou, YR.; Kuisma SW.; Kanninen, L.; et al. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. J Control Release. 2012, 164, 291–8.
3- Lou YR, Kanninen L, Kuisma T, Niklander J, Noon LA, Burks D, et al. The Use of Nanofibrillar Cellulose Hydrogel as a Flexible Three-Dimensional Model to Culture Human Pluripotent Stem Cells. Stem Cells Dev. 2014, 23, 380–92.
4- Lin, N.; Dufresne A. Nanocellulose in biomedicine: Current status and future prospect. Eur Polym J. 2014, 59, 302–325.
5- Abouzeid, R.E.; Khiari, R.; Beneventi, D.; & Dufresne, A. Biomimetic mineralization of three-dimensional printed alginate/TEMPO-oxidized cellulose nanofibril scaffolds for bone tissue. engineering. Biomacromolecules. 2018, 19(11), 4442-4452.
6- Ragab E. Abouzeid., Khiari, R., Salama, A., Diab, M., Beneventi, D., & Dufresne, A. (2020). In situ mineralization of nano-hydroxyapatite on bifunctional cellulose nanofiber/polyvinyl alcohol/sodium alginate hydrogel using 3D printing. International Journal of Biological Macromolecules, Pages 538-547, 2020.
7- Lundahl, M. J.; Klar, V.; Wang, L.; Ago, M. & Rojas, O. J. Spinning of cellulose nanofibrils into filaments: A review. Industrial and Engineering Chemistry Research 2017, 56, 8–19.
8- Kontturi, E. et al. Advanced Materials through Assembly of Nanocelluloses. Advanced Materials 2018, 30, 1703779.
9- Mittal, N. et al. Multiscale Control of Nanocellulose Assembly: Transferring Remarkable Nanoscale Fibril Mechanics to Macroscale Fibres. ACS Nano 2018, 12, 6378–6388.
10- Clemons, C. Nanocellulose in Spun Continuous Fibres: A Review and Future Outlook. Journal of Renewable Materials 2016,4, 327–339.
11- Ghasemi, S.; Tajvidi, M.; Gardner, D. J.; Bousfield, D. W. & Shaler, S. M. Effect of wettability and surface free energy of collection substrates on the structure and morphology of dry-spun cellulose nanofibril filaments. Cellulose 2018, 25, 6305–6317.
12- Zhang, K. & Liimatainen, H. Hierarchical Assembly of Nanocellulose-Based Filaments by Interfacial Complexation. Small 14, 2018, 180-193.
13- Bettaieb, F.; Nechyporchuk, O.; Khiari, R.; Mhenni, MF.; Dufresne, A.; Belgacem, MN. Effect of the oxidation treatment on the production of cellulose nanofibre suspensions from Posidonia oceanica: the rheological aspect. Carbohydrate polymers, 2015; 134, 664-672.
14- Liam C. Palmer, Christina J. Newcomb‡, Stuart R. Kalt, Erik D. Spoerke, and Samuel I. Stupp. Biomimetic Systems for Hydroxyapatite Mineralization Inspired By Bone and Enamel. Chem Rev. 2008 November ; 108(11): 4754–4783. doi:10.1021/cr8004422.
15- A Salama, RE Abou-Zeid, Cruz-Maya, V Guarino. Soy protein hydrolysate grafted cellulose nanofibrils with bioactive signals for bone repair and regeneration. Carbohydrate polymers 229, 115472.
16- Ghasemi, S.; Tajvidi, M.; Gardner, D. J.; Bousfield, D. W. & Shaler, S. M. Effect of wettability and surface free energy of collection substrates on the structure and morphology of dry-spun cellulose nanofibril filaments. Cellulose 2018, 25, 6305–6317.
17- . Zhang, K. & Liimatainen, H. Hierarchical Assembly of Nanocellulose-Based Filaments by Interfacial Complexation. Small 14, 2018, 180-193.
18- Chen, C.; Mo, M.; Chen, W.; Pan, M.; Xu, Z.; Wang, H.; Li, D. Highly conductive nanocomposites based on cellulose nanofibre networks via NaOH treatments. Compo. Sci.Technol. 2018, 156, 103-108 .
19- Mittal, N.; Ansari, F.; Gowda, V. K.; Brouzet, C.; Chen, P.; Larsson, P. T.; Roth, S. V.; Lundell, F.; Wagberg, L.; Kotov, N. A.; Soderberg, L. D. Multiscale Control of Nanocellulose Assembly: Transferring Remarkable Nanoscale Fibril Mechanics to Macroscale Fibres. ACS Nano. 2018, 12 (7), 6378-6388 .
20- Li, Y.; Zhu, H.; Shen, F.; Wan, J.; Lacey, S.; Fang, Z.; Dai, H.; Hu, L. Nanocellulose as green dispersant for two-dimensional energy materials. Nano Energy 2015, 13, 346-354 .
21- Chen, C.; Li, D.; Abe, K.; Yano, H. Formation of high strength double-network gels from cellulose nanofibre/polyacrylamide via NaOH gelation treatment. Cellulose 2018, 25 (9), 5089-5097.
22- Industrial and Engineering Chemistry Research 2017, 56, 8–19.
23- Kontturi, E. et al. Advanced Materials through Assembly of Nanocelluloses. Advanced Materials 2018, 30, 1703779.
24- Wang, L.; Lundahl,J.; Greca, L.; Papageorgiou, C.; Borghei, M.; Rojas, O. Effects of non-solvents and electrolytes on the formation and properties of cellulose I filaments. Scientific Reports, 2019, 9, 16691.
25- Tripathi, A.; Ago, M.; Khan, S. A.; Rojas, O. J. Heterogeneous Acetylation of Plant Fibres into Micro- and Nanocelluloses for the Synthesis of Highly Stretchable, Tough, and Water-Resistant Co-continuous Filaments via Wet-Spinning. ACS Applied Materials & Interfaces, 2018, 10, 44776–44786 .
26- Torres-Rendon, J. G.; Schacher, F. H.; Ifuku, S. & Walther, A. Mechanical performance of macrofibres of cellulose and chitin nanofibrils aligned by wet-stretching: A critical comparison. Biomacromolecules, 2014, 15, 2709–2717 .
27- Iwamoto, S.; Isogai, A. & Iwata, T. Structure and mechanical properties of wet-spun fibres made from natural cellulose nanofibres. Biomacromolecules, 2011, 12, 831–836 .
28- Walther, A.; Timonen, J. V. I.; Diez, I.; Laukkanen, A. & Ikkala, O. Multifunctional High-Performance Biofibres Based on Wet-Extrusion of Renewable Native Cellulose Nanofibrils. Advanced Materials, 2011, 23, 2924–2928 .
29- Mertaniemi, H. et al. Human stem cell decorated nanocellulose threads for biomedical applications. Biomaterials, 2016 82, 208–220 .
30- Brouzet, C.; Mittal, N.; Lundell, F. & Soderberg, L. D. Characterizing the Orientational and Network Dynamics of Polydisperse Nanofibres on the Nanoscale. Macromolecules, 2019, 52, 2286–2295.

31- Mohammadi, P.; Toivonen, M. S.; Ikkala, O.; Wagermaier, W. & Linder, M. B. Aligning cellulose nanofibril dispersions for tougher fibres. Scientific Reports, 2017, 7, 11860.
32- Lundahl, M. J. et al. Strength and Water Interactions of Cellulose I Filaments Wet-Spun from Cellulose Nanofibril Hydrogels. Scientific Reports, 2016, 6, 30695.
33- Hakansson, K. M. O. et al. Hydrodynamic alignment and assembly of nanofibrils resulting in strong cellulose filaments. Nature Communications 5, 2014.
34- Kafy, A. et al. Cellulose long fibres fabricated from cellulose nanofibres and its strong and tough characteristics. Scientific Reports 7, 2017.
35- Liu, C.-K.; Cuculo, J. A. & Smith, B. Diffusion competition between solvent and nonsolvent during the coagulation process of cellulose/ammonia/ammonium thiocynate fibre spinning system. Journal of Polymer Science Part B: Polymer Physics, 1990, 28, 449–465.
36- Ferguson, J. & Ibrahim, K. The wet spinning of secondary cellulose acetate: Solution rheology and filament spinnability. Polymer, 1969, 10, 135–145.
37- Mortimer, S. A; & Peguy, A. A. The formation of structure in the spinning and coagulation of lyocell fibres. Cellulose Chemistry and Technology 1996, 30, 117–132.

Declaration of original submission and Other Grant(s) (Max. one page)

LPIs should declare that their proposal did not and will not be submitted in whole or part for funding; twice within the same cycle, or to other funding programs within AASTMT, or other funding agencies. This is to avoid any possible doubel-funding.
LPIs should state information on all submitted, ongoing and previous research funds for each key investigator over the last three years; such as: project title, name of funding agency, project duration, start and end dates, the total amount of fund/year and the abstract.

We confirm that our proposal did not and will not be submitted in whole or part for funding; twice within the same cycle, or to other funding programs within AASTMT, or other funding agencies. Also, all members here have not any previous projects related to this proposal in the last three years.

Prof. Mohamed Shehadeh (PI)

Dean of Engineering and Technology College, South Valley Campus. and a professor in the Marine Engineering Department, at the Arab Academy for Science and Technology and Maritime Transport (AASTMT).
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2021/3/15

Acknowledgment Form:

Please copy this section, sign and scan it as a part of your proposal

By signing below, I acknowledge that I have read, understand, and accept to comply with all the terms of the foregoing application, mentioned in AASTMT general conditions and guidelines for submitting a research proposal, including, but not limited to:

- The total number of the application pages should not exceed **30 pages** excluding a cover page, as well as all sections of the proposal (as mentioned in AASTMT General Conditions and Guidelines for Submitting Research Proposal).
- At any time, a contracted AASTMT project team member should only be participating in a maximum of one project.
- Allowable budget maximum limit should be strictly adhered to in the project proposal. In all cases, requested budget has to be justified in detail.
- AASTMT guidelines, IPR rules, code of ethics, etc. (www.aast.edu), should be read carefully and adhered to. These are integral parts of the contract.
- All proposals – in addition to PI and other data - must be uploaded to the AASTMT website by the designated deadline. Uploaded PI data should conform to the corresponding data in the application form.

Applications will not be considered eligible and will be discarded in the following cases:

- Proposals submitted by e-mail or sent as hard copies or uploaded to the AASTMT website after the deadline.
- Proposals not conforming to the designated format.
- Proposals whose uploaded PI data does not conform to PI data in the proposal file.
- Proposals in which the allowable budget maximum limit has been exceeded.
- Proposals in which maximum allowable contracted AASTMT project participation limit has been exceeded.
- Proposal letter does not include a scanned copy of the signed and stamped PI institution endorsement letter in case of team member work outside AASTMT.
- Proposal does not include a scanned copy of the signed acknowledgment form.

Date & Signature:

Prof. Mohamed Shehadeh (PI)

Dean of Engineering and Technology College, South Valley Campus. and a professor in the Marine Engineering Department, at the Arab Academy for Science and Technology and Maritime Transport (AASTMT).

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2021/3/15