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والبحث العلمي
/ كلية العلوم
الفيزياء

تحضير الجسيمات النانوية الهجينة كمضافات وظيفية لتصميم وتطبيق المتراكبات البوليميرية الحيوية

أطروحة مقدمة الى

مجلس كلية العلوم – جامعة ديالى

وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في الفيزياء

من قبل

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1.1 Introduction

Despite surgical advancements in medical treatments and diagnostic tools, amputation of both short and long limbs was often the primary course of action for severe medical conditions for many years [1]. In 2017, a total of 60 million amputations were reported globally, with the leading causes being falls (36.2%), automotive accidents (15.7%), and other physical injuries (10.4%). Notably, developing nations exhibited a disproportionately high incidence of these amputations [2]. Prolonged contact between the prosthetic socket and the residual limb can lead to irritation and injury, potentially resulting in the development of ulcers due to the lack of utilization of the soft tissues in the residual limb to mitigate stress and strain. Protecting the soft tissue of individuals with lower limb amputations remains a challenging endeavor [3].

Prosthetic limbs are artificial replacements for missing or amputated body parts, typically limbs such as arms, hands, legs, and feet. They are designed to restore the function, appearance, and mobility of the lost limb, enabling individuals with amputations or congenital limb differences to regain their independence and improve their quality of life [4].

However, using nanocomposites, which combine nanomaterials with traditional materials, has led to the development of prosthetic limbs that are more flexible, responsive, and biomimetic (resembling natural body parts). By integrating these nanoparticles into the socket material, the proliferation of bacteria and other microorganisms may be suppressed, hence decreasing the likelihood of illnesses linked to the usage of prosthetics. Nanoparticles may improve prosthetic socket customization and fit. By adjusting nanoparticle size and surface properties, the socket may be customized to the user. Customization may improve fit, comfort, and function [5, 6].

Modern biomedical research and development has great promise for nanotechnology. Interest in the development and application of biomaterials for bone tissue engineering has increased extraordinarily. Since bone regeneration is considered the most complex and well-orchestrated physiological process, the main purpose of researchers has been to design biocompatible materials with well-defined biomechanical and physicochemical properties that can enhance bone formation and regeneration. Further, these biomaterials aim to initiate specific cellular responses at a molecular level, from cell interaction, proliferation, cell attachment, and differentiation to extracellular matrix synthesis [7].

Bone tissue engineering has attracted great interest in the last few years, as the frequency of tissue-damaging diseases has increased exponentially after the amputation of the limb. To obtain an ideal treatment solution, researchers have focused on the development of optimum biomaterials to be applied for the enhancement of bioactivity and the regeneration process, which are necessary to support the proper healing process of osseous [8].

Recently, bone tissue engineering has concentrated on studying nanostructured metals, composites, polymers, and ceramic materials. Due to their increased surface area and roughness, nanostructured materials improve osteoblast adhesion, proliferation, bone-related protein production, and calcium-containing mineral deposition, promoting osteointegration [9]. Nanomaterials are potential orthopedic materials because they approximate the size of natural bone components. Figure (1.1) outlines Nano medicine's orthopedic potential [10].

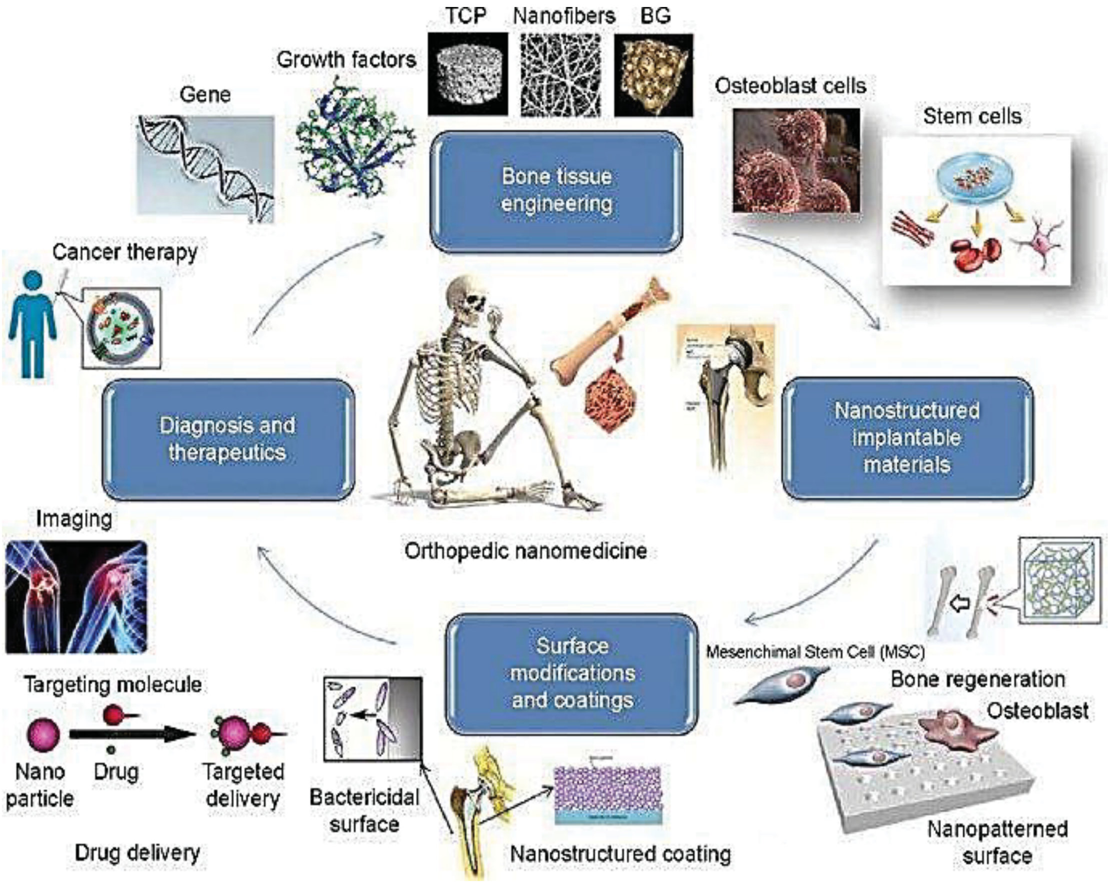

Figure (1.1): The diagram illustrates the possible uses of Nano medicine in the field of orthopedic medicine [10]**.**

In this regard, hydroxyapatite "HAP" has been the most widely used material in the biomedical field due to its great biocompatibility and similarity with the native apatite from the human bone [11]. Eggshell "Es" contains abundant calcium phosphate, collagen fiber, calcium carbonate, and hydroxyapatite "HAP". Calcium can be extracted from Es. Not only will the conversion of Es waste to CaP/HAP greatly decrease bio-waste accumulation, but it will also yield products with important medical uses. An ever-increasing amount of attention has been paid to minimizing and controlling garbage as time has progressed. Transforming wastes into value-added products will clear the way for more efficient waste management and contribute significantly to sustainable economic development [12,13].

Therefore, the sustainable synthesis of biomaterials from natural wastes offers a tremendous chance to meet the medical community's needs in the coming years. Studying the use of natural resources and waste recycling will help create highly biocompatible biomaterials, reduce the inflammatory immune response following transplantation, are readily available, affordable, and have the potential to be manufactured commercially to create a green and environmentally friendly planet [14,15].

Prosthetic limb attachment uses osseointegration, or direct skeletal attachment, to connect the device with the patient's bone. This method is an alternative to socket-based prostheses that fit over the residual limb. Osseointegration improves the prosthetic limb's range of motion, proprioception, and functioning. Direct bone contact improves force and sensory transmission, improving prosthetic device control and perception. Osseointegration removes socket-related skin irritation, pain, and other difficulties common to socket-based prostheses. This enhances user comfort and quality of life [16,17].

Tissue engineering has emerged as a promising solution to the challenges mentioned earlier. This has resulted in notable progress in cell and organ transplantation in recent decades. Additionally, it has spurred innovation in areas such as new materials, application models, preparation techniques, and performance evaluation [18].

The performance of the materials has a major role in tissue engineering success. Coatings for bone repair implants are considered a potential strategy in bone tissue engineering. According to Pereira et al. (2020), applying a coating to materials used in implants may increase their mechanical characteristics and boost the physiological reactivity at the interface. Membranes used in bone tissue engineering serve the purpose of creating a separate area for bone regeneration while also acting as a protective barrier against the formation of soft tissues [19,20].

Research on biomaterial uses of graphene-based nanomaterials has shown significant development in recent years. Graphene, a material with exceptional physical, chemical, and biological characteristics, is highly regarded as a revolutionary substance with significant promise in several biomedical fields such as tissue regeneration and medication delivery [20,21].

Due to its distinct molecular structure, graphene may be used to create three-dimensional composites that exhibit excellent electrical conductivity. Furthermore, the substantial specific surface area of graphene may significantly enhance cell adhesion, hence promoting osteogenic activity as well. The large specific surface area of graphene allows for easier functionalization, resulting in enhanced chemical activity and improved hydrophilicity and dispensability [22,23].

Biomimetic graphene-HAP hybrids also show potential as a regeneration medicine for bone repair, anticancer medications, tooth enamel restoration, and spine fusion. In the case of osteosarcoma, for example, a new regenerative medicine tool based on Graphene oxide -HAP was described in research. This study makes available a novel strategy to reach multi-functional applications of biomimetic graphene-HAP hybrid composite [24].

1.2 Literature Review

1.2.1 Literature Review of Material Prepared and Characteristics

Ćurković *et al.* in (**2017**), studied chemically precipitating hydroxyapatite from chicken eggshell bio waste. Eggshell hydroxyapatite is made by converting calcium carbonate to calcium oxide at 1000°C. Calcium oxide is then hydrated to hydroxide. by added phosphoric acid to make hydroxyapatite. The scanning electron microscope with EDS examined the sintered product's round and plate-nanoparticles shaped to submicron grains' surface morphology. Powder X-ray diffraction and scanning electron microscopy "SEM" showed hydroxyapatite as the product's primary phase [25].

In their (**2017**) study, **Emiru** *et al*. examined the impact of reaction parameters, including reaction time, reaction temperature, and the quantity of $KMnO_4$, on the extent of oxidation of graphite powder to graphene oxide using a simplified approach. The graphene oxide and reduced graphene oxide "rGO " samples were analyzed using UV-visible spectroscopy, FT-IR spectroscopy, and XRD. Graphite powder was subjected to treatment with $KMnO_4$ at a temperature of 40 °C for 12 hours. This treatment led to a higher level of oxidation, as shown by the greatest absorption seen at a wavelength of 226 nm in the UV-Vis spectrum [26].

Galindo *et al*. (**2018**) studied a new technique for producing rGO by microwave-irradiating graphene oxide "GO. Microwave-reduced and exfoliated GO was analyzed for water content. Commercial GO samples were irradiated in a microwave oven after exposure to varying air conditions. Different microscopic and spectroscopic methods characterized the materials before and after irradiation. Water trapped in samples is crucial for microwave-reduced and exfoliated GO. These results have been verified by FTIR, Raman, and SEM studies. The reduction and exfoliation of GO were seen macroscopically as a volume change, color change, and conductivity increase, and confirmed by spectroscopy [27].

Sabu *et al.* (**2019**), synthesized HAP complex hierarchical structure from eggshells by using microwave heating as an aid. The morphological characteristics of HAP observed differences significant when processed using microwaves and conventional methods. Microwave processing of HAP results in reduced crystal sizes. The crystal structure of the HAP prepared was measured by XRD, FTIR analysis, and FESEM. The XRD analysis showed good crystallinity of hydroxyapatite can be inferred even though there could be a certain amount of retained amorphous phases. fibrous surfaces of the interwoven network showed, which can be seen from the FESEM micrographs at various magnifications [28].

Tangboriboon *et al.* (**2019**), synthesized HAP nanoparticles from duck eggshells by chemical reaction with phosphoric acid followed by the calcinations at 800, 900, and 1000°C for 2 hours. The average particle size, pore diameter, specific surface, and true density of the sample sintered at 1000°C for 2 hours. The FTIR data of the sample sintered at 1000°C show the intensity peaks of the P-O and C-O stretching vibrations at 1048 and 1092 cm^{-1}, confirming the successful HA preparation. The FESEM image showed some agglomerations with long and particle sizes of less than 50 µm [29].

In **2020**, **Muniyalakshmi** *et al.* used a modified version of Hummer's approach to create graphene oxide "GO" nanosheets. As a starting point, the researchers used graphite powder, H_2SO_4, and $KMnO_4$ solutions. The researchers displayed the creation of GO by the use of XRD, Raman, FTIR, and SEM investigations. The significant peak at 10.44° corresponds to the crystallographic plane (0 0 1), indicating the likely production of graphene oxide (GO). The Raman spectrum exhibits the D and G bands. The FTIR spectra indicate the existence of many functional groups, including O-H, C=O, and C-O, in the GO nanosheets. The scanning electron microscope (SEM) picture clearly shows the flat and layered structure of the graphene oxide (GO) nanosheets in their original form [30].

Andrade *et al.* (**2020**) investigated the synthesis of graphene oxide quantum dots (GOQDs) using chemical exfoliation of carbon fibers, which had evident antibacterial properties. Measurements conducted using transmission electron microscopy revealed that the lateral length varied between a few tens and several hundred nanometres. The UV-vis absorption profile exhibited little alteration upon reduction using sodium borohydride. Consistent outcomes were achieved in the Raman and time-resolved photoluminescence determinations. Interestingly, the samples had observed, although distinct, antimicrobial efficacy *against Staphylococcus epidermidis* cells. The cytotoxicity of GOQDs was much greater compared to that of the chemically reduced equivalents [31].

Umesh *et al.* (**2021**) produced (HAP) from eggshells and Piper betel leaf extract using microwave conversion. XRD, FTIR, SEM, and TEM were used to analyze the synthesized HAP. This research investigated PBL-HAP's biofilm inhibitory activity using the crystal violet test against numerous common diseases and its antibacterial activity using the well diffusion method. The research found that Piper betel leaf extract coated HAP (PBL-HAP) inhibited biofilms of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These data suggest that PBL-HAP might improve dental biomaterial effectiveness. TEM micrograph showed cylindrical PBL-HAP that aggregated and averaged (50-60) nm in size. The SEM image showed that the synthesized HAP (both HAP and PBL-HAP) was agglomerated and varied in form and size [32].

Tewatia *et al.* (**2021**), synthesized of an environmentally benign green reducing agent, ascorbic acid, often referred to as vitamin C, which was used in the reduction process of Graphene Oxide. The synthesis of Graphene Oxide was conducted using the Modified Hummer's technique, whereby the removal of $NaNO_3$ was achieved by increasing the quantity of KMnO incorporated. By changing the quantity of ascorbic acid, the extent of reduction may be regulated while preserving the characteristics of reduced graphene oxide. The surface and structural features were investigated using X-ray diffraction, UV–visible spectroscopy, Fourier tomography infrared (FTIR), and Raman spectroscopy. The FTIR analysis confirmed that a significant number of functional groups, such as hydroxyl (–OH), epoxy (C-O-C), carboxyl (–COOH), and carbonyl (C = O), are eliminated during the reduction process. The UV-visible peak is seen at around 215 cm^{-1} and arises from the G-G* transition originating from C = C double bonds. This peak may undergo temporal shifts in response to the level of oxidation shown by graphite [33].

Goh *et al.* (**2021**) investigated the impact of pH on the characteristics of a wet-chemically synthesized HAP bioceramic made from eggshells with the use of microwave irradiation. The effects of different pH values on the HAP characteristics have been studied. These values range from 8 to 12. Analyses using XRD, FTIR, FESEM, and EDX were performed on the powders that were obtained. More spherical nanoparticles were produced as a consequence of the pH levels. At pH 10, HAP particles that resemble needles were successfully isolated; these particles were 60-80 nm in length and 10-15 nm in breadth [34].

Macedo Castro *et al.* (**2022**) used the Hydrothermal and Microwave Irradiation (MW) techniques to synthesize Hydrogel-Activated Polymers (HAP) from eggshells. Analytical techniques were used to characterize the powders produced, including XRD, FTIR, EDS, and TEM. The preparation of HAP occurred at a pH of 9, resulting in a lower production of carbonated powders compared to those synthesized at a pH of 11. According to the (TEM) examination, the Hydrothermal approach yielded more favorable morphological outcomes. The HAP technique was deemed to be a more appropriate method for the acquisition of hydroxyapatite as a biomaterial [35].

Al-Karim *et al.*, (**2023**) Graphene oxide–hydroxyapatite (GO-HAP) and reduced graphene oxide–hydroxyapatite (RGO-HAP) bio-composites were created to improve the biomaterial's morphology and structure and investigate biomedical applications. The XRD analysis showed that GO-HAP and RGO-HAP nanocomposites exist, with diffraction patterns comparable to HAP. FTIR confirmed functional bands in HAP, GO, RGO, GO-HAP, and RGOHAP. FESEM was used to study the composites' macroscopic structure. The quality of nanocomposites is assessed using Raman and UV spectroscopy. HAP, GO-HAP, and RGO-HAP have Ca/P ratios of 1.56, 1.60, and 1.59, respectively, according to EDS. These readings were close to normal HAP's 1.67 Ca/P ratio. An experimental investigation examined the antibacterial properties of GO-HAP and RGO-HAP *against Bacillus subtilis* and *Staphylococcus aureus*. This research used MTT on Vero cell lines to evaluate GO-HAP and RGO-HAP composite cell viability. GO-HAP/RGO-HAP nanocomposites show promise as biological alternatives [36].

Castro *et al.* (**2023**) synthesized three unique graphene oxides (GOs) with varied oxidation levels and examined these GOs' chemicals also

Abstract

The study highlighted, that a new approach in biomaterials as a therapeutic material in bone engineering and prosthetics, which is a new type of bone differentiation by converting wastes into value-added products will clear the way for more efficient waste management and contribute significantly to sustainable economic development eggshell waste into hydroxyapatite "$Ca_{10}(PO_4)_6(OH)_2$" nanoparticles" HAPn". Part one Calcium oxide was a precursor to synthesize HAPn using the chemical precipitation technique. Chemical treatment and microwave radiation form a novel strategy to create a graphene oxide nanosheet" GNS" that decorates carbon crystal nanostructures from graphite. This approach is proven to be eco-friendly and low-cost.

The structural investigation X-ray diffraction (XRD), test showed that eggshell powder was calcite a stable polycrystalline hexagonal structure calcium carbonate. CaO has a polycrystalline nature with a cubic structure. The XRD analysis results for the hexagonal hydroxyapatite structure also showed that the other main phases, CaO and β-Tricalcium phosphate (β-TCP), appeared as shown for strong peaks. The crystalline size of the prepared materials was calculated using Scherrer’s formula, where the crystalline size values were (44.15, 10,35.4) nm for the eggshell powder, CaO, hydroxyapatite, and β-Tricalcium phosphate. In addition, XRD findings indicated that graphene oxide nanosheet had a hexagonal shape and exhibited a wide peak. The d-spacing of the graphene oxide nanosheet has been measured to be 0.9 nm.

Fourier Transform Infrared Instruments (FTIR), a strong bond between materials prepared materials and their precursors indicated that the bonding bands were compatible. The D and G bands of the graphene oxide nanosheet were seen in the Raman spectra. A peak shift with a

strengthening of the 960 cm^{-1} peak has also been seen in hydroxyapatite Raman spectra. Eggshell had a sharp peak at (280 nm) in UV-vis, whereas CaO and hydroxyapatite had a peak at 320 nm and 207 nm.

Field Emission-Scanning Electron Microscope (FESEM) images have revealed that the eggshell was a nano calcite structure with porous particles of various sizes. Moreover, hydroxyapatite nanoparticles vary in size and shape but are always porous and irregular. Energy Dispersive X-ray spectroscopy (EDS) mapping has shown high C and Ca eggshell nanoparticles. The Ca/P ratio of 1.68 is identical to that of human cortical bone in hydroxyapatite nanoparticles, of 1.67. The FESEM images reveal a porous network with dense layers from a few nanometers to 30nm and visible three-dimensional characteristics.

Using the transmission electron microscopy (TEM) technique, which was developed to determine the size of nanoparticles produced, it was seen that the eggshell nano calcite sample had very aggregated particles with spherical forms and average sizes below 100 nm. While, the hydroxyapatite nanoparticle granules take on a variety of forms before eventually coming together to create a single, bigger one; their particle size is less than 30 nanometers. The TEM images have shown graphene oxide nanosheet monolayers arranged in many survey flakes with a limited number of layers. The nanosheets are characterized by well-identified edges and sharp corners. Graphene oxide nanosheet has aggregative carbon nanoparticles, whose carbon nanoparticles decorated represent promising novel carbon-based structures.

Part two included testing HAP and GNS's biological antibacterial activity two different genus —*Pseudomonas aeruginosa*, a Gram-negative bacteria, and *Staphylococcus aureus*, gram-positive bacteria. incubated with GNS at concentrations of (15, 30, 45, and 60) µg/ml, and HAP at

(50, 100, 150, and 200) μg /ml. The MTT test, a quantitative technique for evaluating *cytotoxicity in vitro*, shows that porous HAPn and GNS-decorated nanomaterials do not have any detrimental effects on the MG-63 cell line. The blue fluorescent signals indicate that the HAPn and GNS have been labeled with a fluorescent dye or probe, allowing their localization and distribution within the MG-63 cells to be visualized under a fluorescence microscope.

part three included the production of the below-knee prosthesis. four laminate composites were used for fabricated below-knee prostheses by a vacuum-forming process. The matrix consisted of polymethyl methacrylate (PMMA) and was reinforced with several layers of fibers (carbon, glass, and perlon) with a weight fraction of 30 %. The best concentration in biological activity was chosen to add to the resin for the other three laminated prosthetic limb materials.

Part three included performing a mechanical test, specifically focusing on tensile and hardness properties. The HAPn/GNS hybrid laminated socket exhibited optimal properties according to tensile strength, Young's modulus, and hardness, with 9 GPa, 115 MPa, and 96 Shore-D values. The theoretical components of this study include simulations of the theoretical parameters related to safety and interface pressure. Continuous monitoring of muscle and bone pressure was conducted inside the prosthesis. Both laminates in the numerical analysis exhibited a maximum equivalent contact pressure of 1.87 kPa. The Von Mises stress observed in all instances remained constant at 7.9 MPa. The maximum interface pressure value obtained in practical use was 219 kPa.

Part four included the osseointegration approach an alternative to traditional socket-based prosthetics, which rely on a socket that fits over the residual limb. In this work, a combination of HAP/GNS-

Polyvinylpyrrolidon (PVP) nanocomposite coatings was constructed for osseointegration on a stainless steel 316L alloy substrate using an electrophoretic deposition (EPD) technique. The composition of the alloy (316L) was verified by energy-dispersive X-ray fluorescence (ED-XRF). The mechanical properties of the alloy (316L) were studied by tensile, impact, and hardness testing where, the results showed Young's modulus was 213 MPa the impact resistance was 5.4J/m^2, and the hardness value was 290 HB. The biological behavior of the coated samples was investigated using the contact angle test. The results indicated that the samples had good wetting characteristics.

FESEM with quantitative and qualitative energy dispersive EDS is used to examine the layer composition and the cross-section related to coating and atomic force microscopy (AFM) to characterize the roughness of the surface morphology. The tape technique was also used to assess the adhesion test results of the EPD layers with the 316L alloy substrate. Additionally, the antibacterial test of nanocomposite coating demonstrated effective inhibition of bacterial growth. Furthermore, in *vitro* experiments, performed over two weeks, showed that the coating layers could form apatite crystals on their surfaces in simulated body fluid (SBF), indicating high osteoconductivity.