ORIGINAL RESEARCH

In vitro evaluation of Tungsten oxide decorated Hydroxyapatite membrane towards guided tissue regeneration with enhanced antibacterial efficacy

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Abstract

Background: Tungsten oxide (WO_3) has recently garnered attention for advanced healthcare applications due to its promising biological properties. Hydrogels, as water-insoluble three-dimensional polymer networks, are capable of retaining large volumes of water and are particularly suitable for use as membranes in dentistry, especially for replacing periodontally diseased tissue as a flap or graft.

Objective: This study aims to investigate the antibacterial and regenerative potential of hydroxyapatite (HAp) membranes incorporated with WO_3 nanoparticles, with a focus on their application in guided tissue regeneration in regenerative dentistry.

Materials and Methods: Hydrogel membranes were prepared using hydroxyapatite (HAp), a biocompatible and bioactive calcium phosphate material widely used in hard tissue restoration. WO_3 nanoparticles were incorporated into these HAp membranes. The antibacterial efficacy of the resulting composite was evaluated against oral pathogens. The potential of the membranes for tissue regeneration was assessed through their structural and functional properties.

Results: The integration of WO_3 nanoparticles with HAp membranes demonstrated enhanced antibacterial activity against common oral pathogens. The composite material showed favorable characteristics for use in guided tissue regeneration, including biocompatibility and potential for restoring diseased tissues.

Conclusion: WO_3-decorated HAp membranes present a promising approach for developing model therapeutics in regenerative dentistry. Their dual functionality—enhanced antibacterial efficacy and tissue regenerative capacity—suggests potential applications not only in dental therapy but also in broader medical fields.

Keywords: Tungsten, Hydroxyapatite, Antibacterial, Tissue regeneration, Oral pathogens.

Introduction

Tungsten oxide has multiple crystalline phases, numerous stoichiometric forms, the highest melting point and is an effective electrochromic material. It exceeds the hardness of steel in its purest form, because of its properties it is utilized as coating nanowires, nanofibres etc[1]. In dentistry, Tungsten is used in coating dental burs, surgical tools and punches.[2]

Tungsten oxide (WO_3) is a chemical compound comprising of metal tungsten and oxygen and garnered considerable attention particularly in lieu of it being a nanomaterial[3]. It has special quantum confinement effects and behaves in an entirely different manner when compared to the bulk metal oxide. It is a much researched transition metal oxide. Strong colloidal elements in the range of 10-1000nm are considered as nanoparticles. Tungsten oxide is hydrated in its natural form. Hydrogels are water-insoluble, three-dimensional networks of polymer chains capable of holding large amounts of water[3,4].

Hydrogel (HG) membranes can be used in dentistry to replace the periodontally diseased tissue as flap or graft[5]. Due to their soft and elastic three-dimensional (3D) networks made of hydrophilic homopolymers, copolymers, or macromers, hydrogels have demonstrated advantages as scaffolds[6]. In addition to being simple or hybrid, HG exhibits chemical, mechanical, and biological activities like the incorporation of cells in their structures, the retention of high-water content

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to improve the transportation of cell nutrients and waste, and elastic and flexible qualities to mimic the native extracellular matrix[7]. Thus on incorporation of nanoparticles, we can find the antibacterial activity of it against oral pathogens to utilize it in treatment[8].

Hydroxyapatite (HAp) is considered the most appropriate calcium phosphate for the preparation of biomaterials for hard tissue restoration, owing to its elevated stability, admirable biocompatibility and bioactivity[9]. The purpose of HAp, either independently or in combination with diverse materials, widened from coatings, to cements, to scaffolds for regenerative medication[10]. Consequently, on integration of WO_3 with HAp, we can analyze the antibacterial activity against oral pathogens and also can regenerate and restore the diseased tissues, by developing it as model therapeutics mainly in regenerative dentistry[11].

Angiogenesis, chemotaxis, proliferation, biomineralization, and the expression of particular tissue biomarkers are a few of the cellular processes that HG might alter, accelerating the regeneration process[12]. Other than that, several of them contain anti-inflammatory and antibacterial properties. HG has become a substitute for the regeneration of tissues in dental organs. and they offer a promising option for therapeutic use due to their qualities[13].

Previous studies have documented the use of Collagen based hydrogel, hyaluronic acid hydrogel, gelatin hydrogel, Chitosan hydrogel, alginate hydrogel, agarose hydrogel, extracellular matrix hydrogel etc., to determine dental organ tissue regeneration and pulpal regeneration. The current study aims to create a hydrogel membrane incorporating tungsten oxide (WO^3) and hydroxyapatite to harness the beneficial properties and evaluate its characteristics by Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDS) and its efficacy as a material with antibacterial properties by evaluating its antibacterial activity.

Materials and methods

Hydroxyapatite was prepared through dropwise addition of $(NH_4)_2HPO_4$ (50 mL, 0.65 M) to Ca $(NO_3)_2$ (50 mL, 1.08 M) (Figure 1), at pH 10. After the addition, the solution was kept under magnetic stirring for 5 h during which the pH was maintained at about 10 with ammonia solution (NH_3). The solution was then centrifuged at 10000 rpm for 10 min. The precipitate was washed 3 times with distilled water and dried at 37 ºC.

Briefly, 200 mL of a sodium tungstate solution ($Na_2WO_4.2H_2O$, 1.649 g, 0.005 mol measured to a quantity of 1mM and 3 g of sodium alginate dissolved in 100 ml of distilled water. It was stirred in the magnetic stirrer for 30 minutes, to which 1g of HAp was added and again stirred for 1 hr. After completion the mixture was poured in a petri plate and frozen for 12 hours (Figure 2). Calcium chloride solution is added

Fig. 1: 50ml of alginate measured.

Fig. 2: Prepared hydrogel in a petriplate.

Fig. 3: The SEM results depicting the porous morphology of hydrogel with embedded tungsten particles.

Fig. 4: The EDS results showing the presence of W, Na, Ca, P, and Chlorine in the fabricated hydrogel membrane.

Fig. 5: This shows the antibacterial results illustrating the prepared hydrogel tested against E.coli, S.aureus, E.faecalis and C.albicans.

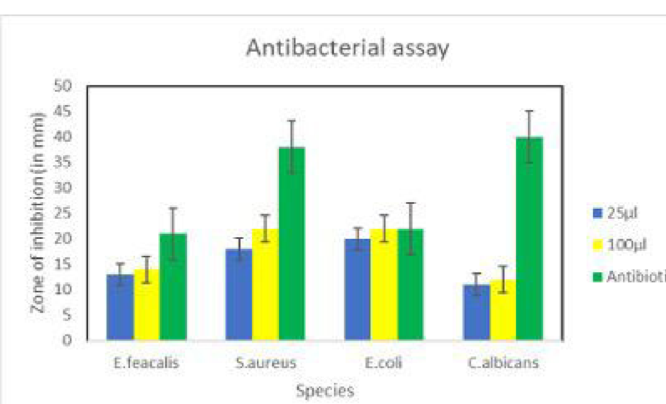
as the crosslinker. After 3 hours of setting time a hydrogel membrane is formed. SEM-EDS and Antibacterial activity was tested.

The antibacterial activity of the hydrogel membranes was tested on common oral pathogens (Staphylococcus aureus, Enterococcus faecalis, Candida albicans and Escherichia coli) using the agar well diffusion method. Membranes with a diameter of 10 mm were placed on the inoculated agar plates and incubated at 37 degrees Celsius for a period of 24 hours, after which they were evaluated for zone of inhibition (measured in mm).

Scanning Electron Microscope (SEM) analysis was performed to examine the surface morphology and microstructural properties of the hydrogel membranes and the corresponding particles. The micrographs were evaluated to determine the morphology of the particles, pore structure, and the degree of uniformity of WO_3 incorporation into the surface of the membrane. It is also used to validate the effective coating of HAp with WO_3 nanoparticles and analyze to interface the properties related to bacterial resistance with the surface features.

Result

The antibacterial assay results show the sensitivity of test compound on four microbial species: E. faecalis, S. aureus, E. coli, and C. albicans. A distinct dose-dependent inhibition is noticed with E. faecalis and S. aureus since increasing the volume from 25 μL to 100 μL leads to larger zones of inhibition. Moreover, S. aureus gave a notable response suggesting high sensitivity to the test compound though still less than the antibiotic control. On the other hand, E. coli has been shown to have a uniform inhibition zone at both doses which is comparable to the control antibiotic which gives the impression that the test compound is almost effective as standard antibiotics in this species. Notably, C. albicans showed no inhibition at both doses while the antibiotic had a significantly larger inhibition zone which shows the poor activity of the compound towards fungi. In general, the test compound displayed selective antibacterial activity especially on S. aureus and E. coli, however, lost effectiveness on C. albicans showing a lack of antifungal capability.



Graph 1: Tungsten nanoparticle loaded hydrogel has increased zone of inhibition against E.coli and S.aureus (22mm) whereas the zone of inhibition for E.feacalis is 14mm and C.albicans is 9mm in 100μl of preparation.

The energy-dispersive X-ray spectroscopy (EDS) spectrum reveals the elemental composition of the analyzed sample. The major elements identified are carbon (C) and oxygen (O), comprising 40.4 wt% and 38.5 wt% respectively, suggesting that the material has a significant organic or oxidized matrix. Minor elements present include calcium (Ca) at 8.6 wt%, chlorine (Cl) at 6.6 wt%, and aluminum (Al) at 2.5 wt%, which may indicate the presence of mineral phases or additives. Tungsten (W) appears in several peaks across the spectrum with a total contribution of 2.3 wt%, possibly from a coating or inclusion, while sodium (Na) is detected at 1.1 wt%. SEM confirmed the porous and flexible morphology embedded with tungsten nanoparticles.

Discussion

Based on the previous research tungsten is used in prosthetic agents such as crown, dentures, implant etc. Here we have prepared tungsten loaded hydrogel membrane for tissue regeneration as hydrogel preparation reduces the toxicity of W which makes it a potent antibacterial agent to use as flaps and grafts in periodontally diseased tissues[14,15]. In a study by Thanakkasarane et al WO_3 was found to exhibit maximum antibacterial activity with regard to E.coli at 10 mg concentration, compared to S.aureus.[16]

The hydrogels with calcium alginate have been used in various drug delivery methods as it has an ability to retain and release the drugs[17]. The dextran and aniline trimer-based hydrogel is used in delivering the drug as it does a precise drug delivery and it is cytocompatible based on the study reported by Jin Qu et al in 2019[18]. AgNP-loaded hydrogels showed broad spectrum antimicrobial activity and antioxidant activity[19]. WO_3 nanocomposites in combination with poly (hydroxyethylacrylate) and poly(acrylamide) gel has been used to counter bacterial keratitis in contact lens care solutions (Ejeromedoghene et al, 2021). Optimal photochromic a-WO_3 with sustained controlled release of H_2O_2 has found desirable application against Cancer.

Tungsten oxide acts through various mechanisms, one which is by creation of a photogenerated hole. Reactive oxygen species in the form of superoxide anion and hydroxyl ion is created which is implicated in the water disinfection property of the tungsten oxide against Escherichia coli[20]. In the study by Wang et al, solar spectrum effects irradiation on the photochromic WO_3 and produces sustained tunable release of radicals. This sustained oxidative stress results in enhanced tumor inhibition[21,22, 23].

The limitations of the study are inclusion of more criteria, further anti-inflammatory and cytotoxicity properties were not tested. Hence more studies have to be done to elucidate the nature of the hydrogel completely. The future scope of the study is that provided its structural characteristics and antibacterial activity, further evaluating the cytotoxicity, anti-inflammatory property, antioxidant property of the tungsten loaded hydrogel, it can be taken for further In vivo and clinical trials.

Conclusion

The morphologically porous and flexible tungsten nanoparticle loaded hydrogel has potent antibacterial properties against E.coli, S.aureus, E.faecalis and C.albicans and can be potentially used in tissue regeneration for periodontal flap applications.

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