



ZINC GRAPHENOXIDE WITH ALGINATE FOR WOUND HEALING

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ABSTRACT:

BACKGROUND:

Oral mucosa wound healing comprises a series of sequential responses that allow the closure of ruptures in this tissue. This process is of critical importance to prevent the invasion of microorganisms into tissues avoiding the establishment of chronic inflammation. Alginate has shown immense potential in oral wound healing because its gel-forming ability maintains the structural resemblance to the extracellular matrices in tissues and can be altered to perform numerous crucial functions. Graphene oxide is a carbonaceous nanomaterial with a large surface area, high thermal conductivity, biocompatibility, and biodegradability. The combination of alginate and graphene oxide can be used as a potential useful material for wound healing in the oral cavity.

MATERIALS AND METHODS:

Six percent of alginate and 500mg of graphene oxide is mixed and stirred for three hours for homogeneous gel formation. The gel is then transferred to petri plates and lyophilised.

RESULTS:

The results of the present study reveals that the GO-alginate gel promoted in vitro endothelial cell migration and proliferation leading to tissue granulation in close proximity with neovascularization. The capability of GO-alginate gel to stimulate the skin wound healing is attributed to graphene oxide's ability to scavenge



reactive oxygen species (ROS) in the wound area. Therefore GO could remove ROS and regulate inflammation to regulate wound healing.

CONCLUSION:

Overall, our results indicate that the GO- alginate gel could serve as a promising candidate as a new wound healing material that is effective and inexpensive. Based on these findings, the GO - alginate gel could be evaluated for clinical applications.

Keywords: Alginate, graphene-oxide, wound healing, Zinc

INTRODUCTION:

Alginate is considered an exceptional biomaterial due to its hydrophilicity, biocompatibility, biodegradability, nontoxicity and low-cost in comparison with other biopolymers. We have recently demonstrated that the incorporation of 1% graphene oxide (GO) into alginate films crosslinked with Ca²⁺ cations provides antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis*, and no cytotoxicity for human keratinocyte HaCaT cells. However, many other reports in literature have shown controversial results about the toxicity of GO demanding further investigation. Calcium alginate exhibits negligible antibacterial and cytotoxic activity.(1) However, zinc alginates have shown strong antibacterial activity against a wide range of microorganisms due to its inhibition of conserved metabolic pathways involved in synthesis of essential biomolecules or antioxidant depletion, and it is well-known that Zn²⁺ may produce toxic effects in human cells . Alternative materials such as graphene oxide (GO), which from the graphene family shows the easiest processing, larger scale production and less expensive cost , has recently exhibited antibacterial capacity and negligible mammalian cytotoxicity in calcium alginate(2).Alginates are commonly used as scaffold materials for cells in regenerative medicine and tissue engineering . This biopolymer possesses a linear chain structure of (1–4)-linked β-d-mannuronic acid (M) residues arranged in block-wise fashion with M and G present in different proportions and sequences, depending on the source of the alginate.(3)

On the other hand, GO is transparent in the visible spectrum and is able to enhance many polymer properties such as water diffusion and mechanical performance in a more efficient way than other carbon nanomaterials. However, the transparency of GO decreases with increasing the presence of the number of GO layers and thus, the darkness of polymer films can increase with increasing GO content .Material wettability is another important surface property to be considered in polymer composite science for many industrial applications.(4)



Graphene and its derivatives have received a great deal of attention in biomedical applications such as drug and gene delivery, imaging, and tissue engineering, due to their high conductivity, good mechanical properties, ability to induce conductivity when combined with biopolymers, and ease of processing(5). The multifunctional properties of graphene-based materials have been reported to enhance the cellular response of different cell lines. Several studies have shown that GO promotes mesenchymal stem cell growth and differentiation toward osteogenic and myogenic lineages.(6)

Zinc is one of the most essential biometals in the human body, as well as Fe, Cu, Mn, and Co. It is found in all body tissues, with 85% of the body's zinc in muscle and bone(1). It is a fundamental microelement involved in cell proliferation and differentiation and a structural constituent of a great number of proteins, including enzymes involved in cellular signaling pathways and transcription factors. Biodegradable metals such as zinc have recently attracted considerable attention in tissue engineering and wound healing. Zinc has been proposed as a new biomaterial for use in bioabsorbable cardiac stents and as a structural material for biodegradable implants. The release of zinc ions from implant during the degradation phase can integrate into normal metabolic activity without producing systemic toxic effects(4).

Both surface bioactivity and antibacterial behavior are two critical factors in determining the success of a biomaterial in an in vivo environment(7).

Thus Zinc graphene oxide combined with alginate has shown potential for wound healing due to their antimicrobial and regenerative properties. The materials contribute to accelerated healing and reduced risk of infection in wounds.

MATERIALS AND METHODS:

The process begins by preparing separate zinc and alginate solutions, ensuring thorough mixing and freedom from impurities. The zinc and alginate solutions are then combined in the desired ratio to create the sample designated for lyophilization. The mixture is transferred into sterile glassware or trays suitable for the lyophilization process using a pipette. The glassware or trays are positioned within the lyophilizer, ensuring proper spacing to facilitate uniform drying. The lyophilization process is initiated, involving the freezing of samples to solidify them, followed by the application of a vacuum to sublimate frozen water, resulting in the formation of the lyophilized product. The lyophilization process is carefully monitored, and necessary adjustments to parameters are made for optimal outcomes. Upon completion of lyophilization, the glassware or trays are extracted from the lyophilizer, and the lyophilized product is securely sealed for appropriate storage.

Cell viability test

The target cells were cultured up to a density of 80%. To do the process of cell dissociation and disperse them, trypsinization and centrifugation of the cell in the complete culture medium was carried out, and then

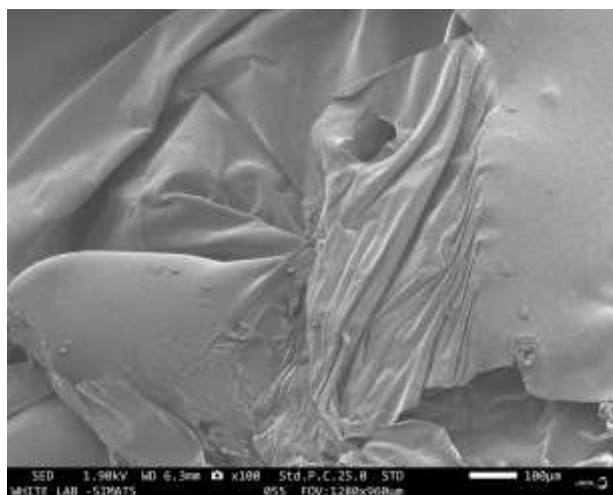


the cells were counted with a Neubauer counting chamber. Then, the cells were seeded into a 96-well plate at a concentration of 15,000 cells per well and dissolved with 100 uL of the culture medium. The cells were kept in an incubator for 24 h in order to attach the cells to the plate surface and grow them.

Then, the cells of each well were treated with the desired agents and incubated for 48 h. The cytotoxicity effect of synthesized nanoparticles was examined at various concentrations of 8, 16, 32 and 64 mg/mL. Then, after 48 h the culture medium was removed and then 20 L of MTT with a concentration of 5 mg/mL was added to each well and incubated for 4 h. Next, after 4 h incubation 150 L of DMSO was added to each well, and absorbance readings of each well were performed. Finally, the absorbance ratio of the number of surviving cells in treated samples was compared with the control group cells.

RESULTS:

FIGURE 1: Scanning electron microscope studies reveals the sheet-like morphology of the gel which is porous and flexible.



The SEM studies have shown that the structure of the gel resembles sheets. This implies that, at a microscopic level, the gel has a flat, layered appearance. Porosity suggests the presence of open spaces or pores within the material, while flexibility indicates its ability to bend or deform without breaking.

CONTACT ANGLE:

FIGURE 2 : Contact angle measures the surface property of the material. As the angle formed is less than 90° the material is hydrophilic having more absorption capacity.

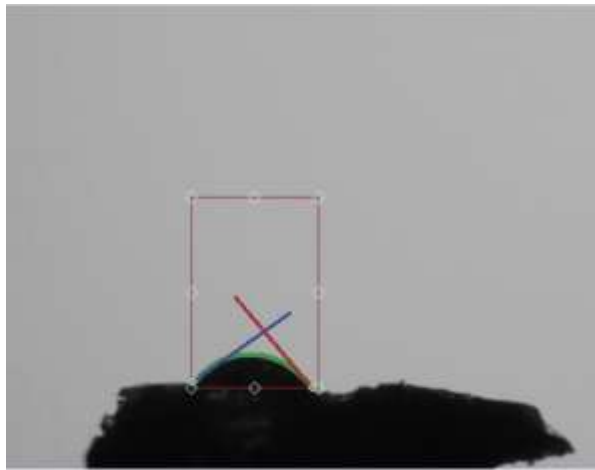
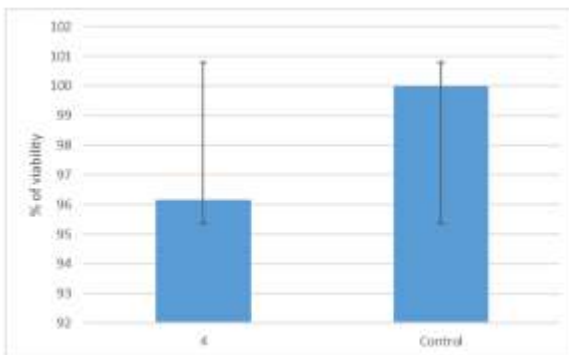


FIGURE 3:

CELL CULTURE:

In the evaluation of cell attachment and toxicity, fibroblast cells were employed. The results indicate a favorable outcome, as 96.5% of the cells were found to be viable. This high viability suggests that the conditions were conducive for the fibroblast cells, promoting their attachment and proliferation. This implies that not only did the cells successfully adhere to the substrate or surface under investigation, but they also exhibited active growth and division, indicating a positive and supportive environment for the fibroblast cells in this particular experimental setting.



DISCUSSION:



Nanofibrous scaffolds' response towards fibroblast cell lines in vitro is crucial to estimate these compositions potential usage.(8) A culture containing fibroblast cell line was prepared to investigate the growth of cells through nanofibers after a period of 3 days. As obvious in Fig. 8a, the fibroblast cell lines tend to be proliferated and spread on the scaffold's surface belonging to ZnO composition. The cells are not only growing on the surface of nanofibers, but they also tend to adhere to the pores of the scaffold(9). These pores possess an essential role in facilitating vascularization formation, besides the transformation of nutrients and oxygen.(5,10) It might be noticed that the spreading of cells exhibits a smaller area than the former one. This trend could be depicted as the difference in biological response between both ZnO and GO. While both are biocompatible, the first one seems to show a higher rougher surface than the other, which could be a critical factor in demonstrating biological behavior for scaffolds(1).

Blending of ZnO/GO could introduce a great interaction between fibroblasts and scaffolds. In other words, cells tend to spread and cover the scaffold surface, while the filopodia follows fibers' curving. These growing cells possess a high tendency to adhere deeply through the nanofibrous scaffold. Furthermore, the latter scaffold's high surface roughness encourages physical adhesions to be conducted via crosslinking between cells and fibers.(3)

The results of the present study reveals that the GO-alginate gel promoted in vitro endothelial cell migration and proliferation leading to tissue granulation in close proximity with neovascularization. The capability of GO-alginate gel to stimulate the skin wound healing is attributed to graphene oxide's ability to scavenge reactive oxygen species (ROS) in the wound area. Therefore GO could remove ROS and regulate inflammation to regulate wound healing.

CONCLUSION:

Combining graphene oxide with alginate to form a gel creates a material with potential healing properties. The synergistic effects of graphene oxide and alginate may contribute to wound healing, tissue regeneration, or other therapeutic application.Both graphene oxide and alginate are generally considered biocompatible, reducing the likelihood of adverse reactions when applied to biological tissues.The healing material could find applications in wound dressings, tissue engineering, or drug delivery, leveraging the properties of graphene oxide and the gel-forming capabilities of alginate for effective therapeutic outcomes.Over all, our results indicate that the GO- alginate gel could serve as a promising candidate as a new wound healing material that is effective and inexpensive. Based on these findings, the GO - alginate gel could be evaluated for clinical applications.

REFERENCES:



1. Mao G, Tian S, Shi Y, Yang J, Li H, Tang H, et al. Preparation and evaluation of a novel alginate-arginine-zinc ion hydrogel film for skin wound healing. *Carbohydr Polym.* 2023 Jul 1;311:120757.
2. Gong CP, Luo Y, Pan YY. Novel synthesized zinc oxide nanoparticles loaded alginate-chitosan biofilm to enhanced wound site activity and anti-septic abilities for the management of complicated abdominal wound dehiscence. *J Photochem Photobiol B.* 2019 Mar;192:124–30.
3. Jagadish C, Pearton SJ. *Zinc Oxide Bulk, Thin Films and Nanostructures: Processing, Properties, and Applications.* Elsevier Science; 2006. 600 p.
4. Díez-Pascual AM. *Antibacterial Activity of Nanomaterials.* MDPI; 2018. 339 p.
5. Cleetus CM, Alvarez Primo F, Fregoso G, Lalitha Raveendran N, Noveron JC, Spencer CT, et al. Alginate Hydrogels with Embedded ZnO Nanoparticles for Wound Healing Therapy. *Int J Nanomedicine.* 2020 Jul 15;15:5097–111.
6. Werner C. *Polymers for Regenerative Medicine.* Advances in Polymer Science; 2006. 256 p.
7. Niculescu AG, Bîrcă AC, Mogoșanu GD, Rădulescu M, Holban AM, Manuc D, et al. Zinc Alginate Hydrogel-Coated Wound Dressings: Fabrication, Characterization, and Evaluation of Anti-Infective and In Vivo Performance. *Gels* [Internet]. 2025 Jun 1;11(6). Available from: <http://dx.doi.org/10.3390/gels11060427>
8. Fischer M, Gebhard F, Hammer T, Zurek C, Meurer G, Marquardt C, et al. Microbial alginate dressings show improved binding capacity for pathophysiological factors in chronic wounds compared to commercial alginate dressings of marine origin. *J Biomater Appl.* 2017 Apr;31(9):1267–76.
9. Wang S, Wang X, Neufurth M, Tolba E, Schepler H, Xiao S, et al. Biomimetic Alginate/Gelatin Cross-Linked Hydrogels Supplemented with Polyphosphate for Wound Healing Applications. *Molecules* [Internet]. 2020 Nov 9;25(21). Available from: <http://dx.doi.org/10.3390/molecules25215210>
10. Segal HC, Hunt BJ, Gilding K. The effects of alginate and non-alginate wound dressings on blood coagulation and platelet activation. *J Biomater Appl.* 1998 Jan;12(3):249–57.