**Título:** **Cell viability and antimicrobial capacity of chitosan-pluronic F127 and reduced graphene oxide hydrogels as wound healing dressings**

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Hydrogels are one of the most widely used biomaterials in biomedical studies for tissue regeneration, as they are polymeric networks with high fluid absorption capacity[1]. In this sense, synthesizing new smart material precursors has allowed new formulations based on compounds such as reduced graphene oxide (rGO) and its derivatives[2]. This material can be functionalized with other compounds to form structures such as hydrogels given its biocompatibility, biodegradability, and mechanical, conductive, thermal, and antibacterial properties [3].

Conductive hydrogels have attracted interest in the scientific community due to the role of electric fields in the natural wound healing process, which is directly related to cellular migration and tissue reorganization [1].. Based on these backgrounds, this study aimed to formulate biomaterials based on chitosan-pluronic F127 (CS-PF) and rGO evaluating their cellular viability and antimicrobial action, to be used as patches, for wound tissue regeneration

The materials were synthesized in different mass concentrations of rGO (0, 0.5, 1 % w,w) while keeping the mass of CS-PF constant (1). The terminology used for the materials is as follows: CS-PF15 (1:0), CS-rGO0.5-PF15 (1:0.5), and SF-rGO-PF15 (1:1). The cytotoxicity assay (in vitro) was performed using human dermal fibroblasts (HDF) and a cell density of 104 cells/mL. Absorbance was determined using a microplate reader at a wavelength of 540 nm. Cellular viability (%), relative to control cells, was calculated as Atest/(Acontrol) × 100%, where Atest and Acontrol are the absorbance values of wells (with the material) and control wells (without material), respectively. as the positive control. DMEM medium was used The antimicrobial capacity was determined using *Escherichia coli* and *Staphylococcus aureus* strains.

The biomaterials developed were non-toxic; all exhibited cell viability values above 80%. Additionally, it was observed that increasing the CS-PF/rGO ratio favored cell viability, reaching a value of 120% in the case of SF-rGO-PF15. The biomaterials had an antimicrobial capacity of over 90%. Specifically, SF-rGO-PF15 showed bacterial death of 95.9% against E. coli and 99% against *S. aureus*. Therefore, it can be stated that increasing the CS-PF-rGO ratio improves the antimicrobial capacity of the biomaterials. the materials were biocompatible, making them potential candidates for wound-healing dressings.

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References:

[1] M.F.P. Graça, B.L. Melo, R. Lima-Sousa, P. Ferreira, A.F. Moreira, I.J. Correia, Reduced graphene oxide-enriched chitosan hydrogel/cellulose acetate-based nanofibers application in mild hyperthermia and skin regeneration, Int J Biol Macromol 229 (2023) 224–235. https://doi.org/10.1016/J.IJBIOMAC.2022.12.291.

[2] Y. Wang, S. Liu, W. Yu, Functionalized Graphene Oxide-Reinforced Chitosan Hydrogel as Biomimetic Dressing for Wound Healing, Macromol Biosci 21 (2021). https://doi.org/10.1002/mabi.202000432.

[3] N.O. Gegel, A.B. Shipovskaya, Z.Y. Khaptsev, R. V. Radionov, A.A. Belyaeva, V.N. Kharlamov, Thermosensitive Chitosan-Containing Hydrogels: Their Formation, Properties, Antibacterial Activity, and Veterinary Usage, Gels 8 (2022). https://doi.org/10.3390/gels8020093.