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

Isleidy Ruíza,\*, Katherina Fernándeza, Claudio Aaguayob

a *Laboratory of Biomaterials, Department of Chemical Engineering, Faculty of Engineering, Universidad de Concepción, Chile.*

b *Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy, Universidad de Concepción, Concepción, Chile.*

*Email:* *isruiz@udec.cl*

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 nanomaterial and/or smart material precursors has allowed new formulations based on compounds such as graphene oxide (GO) and its derivatives[2]. This material can be functionalized with other compounds to form structures such as hydrogels due to its biocompatibility, biodegradability, and mechanical, conductive, and thermal 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]. Chitosan (CS) is a natural material widely used in biomedicine due to its biocompatible, biodegradable, anti-inflammatory, low toxicity properties, and ease of functionalization with negatively charged synthetic or natural polymers [3]. Based on these backgrounds, this study aimed to formulate biomaterials based on chitosan-pluronic F127 (CS-PF) and reduced graphene oxide (rGO) for use as patches in wound tissue regeneration and evaluate their cellular viability and antimicrobial action. The materials were synthesized in different mass ratios of rGO (0, 0.5, 1), 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 material) and control wells (without material), respectively. DMEM medium was used as the positive control. The results obtained in the MTT assay showed that the biomaterials are non-toxic, as they 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 antimicrobial capacity was determined using Escherichia coli and Staphylococcus aureus strains. The results of these tests revealed that the biomaterials have an antimicrobial capacity of over 90%. Specifically, SF-rGO-PF15 showed bacterial death of 95.9% against Escherichia coli and 99% against Staphylococcus aureus. Therefore, it can be stated that increasing the CS-PF-rGO ratio improves the antimicrobial capacity of the biomaterials. In conclusion, the results demonstrate that the synthesized materials are biocompatible and possess antimicrobial capacity, 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.