Project PN-III-P2-2.1 – PED-2021-2193

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Acronym: ARGOS Stage 2023

Abstract

The second stage of the ARGOS project aimed to obtain and characterize multifunctional dressings based on collagen (Col) incorporating Au, Ag and magnetic nanoparticles (NPs) functionalized with natural compounds (gallic acid-AG, tannic acid-AT, ferulic acid-AF and quercetin-Q), as potential alternatives for the treatment of malignant skin tumors. In order to achieve this objective, the following activities were carried out: i) the synthesis and physico-chemical characterization of Au NPs, MNPs and Ag functionalized with natural compounds. Using different synthesis and characterization methods, Au-PEG-PEI-AG, Au-PEG-PEI-AF, Au-AT, Au-Q, MNP-AT, MNP-AF and MNP-AG nanoparticles were obtained, with a spherical morphology and sizes between 14-681 nm. The antimicrobial activity evaluated against reference microbial strains, by qualitative and quantitative methods, allowed the selection of NPs with a broad spectrum, respectively MNP-AT, AuNP-AT, AgNP-AF and those with a limited spectrum on Gram-positive bacteria (MNP-DEX -AG, MNP-DEX-AF, AuNP-Q, AgNP-AT, AgNP-AG and AgNP-Q). Most of the NPs selectively inhibited the microbial adhesion of *S. aureus, P. aeruginosa* and *C. albicans* strains. Antioxidant activity determined by DPPH and CUPRAC methods decreased significantly for Au-PEG-PEI-GA and Au-PEG-PEI-FA NPs and increased for AuNP-TA, AuNP-Q, MNP, AgNP-AF and AgNP -Q compared to pure antioxidants, AG and AF. The NPs were tested for cytotoxicity against normal HaCaT and non-melanoma Cal-27 cancerous cells, by MTT and LDH assays, the obtained results suggesting that MNP-AG, MNP-AF, MNP-AT, AuNP-AT, AuNP-Q and AgNPs-AT show selective cytotoxic effect on Cal-27 tumor cells; ii) optimization of the method of obtaining functional dressings based on collagen and functionalized NPs. By corroborating the results obtained in the first activity, MNP-DEX-AG, AgNP-AT2 and AuNP-AT were selected for loading in the collagen matrix; iii) obtaining functional dressings based on collagen – AuNPs, MNPs or AgNPs containing compounds derived from plants were achieved using type I Col, obtained and characterized in stage 1 of ARGOS project, obtaining the composites Col-AuNP-AT, Col- AgNP-AT and Col-MNP-AG; iv) the physico-chemical characterization of the multifunctional dressings carried out by FT-IR, EDX and SEM, revealed a structure with high porosity, with a pore diameter of 50 - 200 μm, in which NPs form aggregates of different sizes; v) the evaluation of the bioactive compounds release from the dressings performed by spectrophotometric methods demonstrated that collagen systems loaded with NPs carrying bioactive compounds represent viable systems that can be used in the controlled and prolonged release of active principles; vi) the evaluation by qualitative and quantitative methods of the viability and growth of prokaryotic and eukaryotic microorganisms on the newly synthesized hydrogels was carried out on the samples previously sterilized with the help of gamma radiation (Co-60). Col-AgNP-AT showed significant microbicidal effect against the clinical strains of *P.*

mirabilis, K. pneumoniae and *C. albicans*, and Col-AuNP-AT against those of *S. aureus, P. mirabilis*, *K. pneumoniae* and *C. albicans;* vii) the analysis of the capacity of microbial adhesion and biofilm formation revealed that the anti-biofilm activity correlates with the concentration of exogenous NO released by NO donors, the most effective anti-biofilm material being Col-AgNP-AT; viii) the *in vitro* evaluation of multifunctional dressings based on Col and bioactive molecules involved the analysis of the apoptotic profile of HaCat and Cal-27 cells. If in the case of normal diploid HaCaT cells the expression of pro/anti-apoptotic markers remains almost unchanged, the apoptosis of Cal-27 tumor cells is strongly influenced in the presence of the tested materials, with the stimulation of the expression of pro-apoptotic factors, in correlation with the inhibition of the anti-apoptotic ones, especially by Col-AuNP-AT and Col-AgNP-AT materials; ix) the evaluation of the antioxidant activity highlighted the classification of the tested materials in the order Col-AgNP-AT > Col-MNP-AG> Col-AuNP-AT; x) the evaluation of the inflammatory response of human diploid cells HaCat and Cal-27 cultured in the presence of functional dressings revealed that the extracellular and intracellular cytokine profile varies according to the cell fraction, the tested material and the cell line, the expression variations being more numerous and of greater amplitude in the case of lysates compared to the supernatant, respectively in the case of normal cells compared to tumor ones. In the case of normal HaCat cells, the stimulation of the production of an anti-inflammatory response and stimulator of epithelial regeneration, has been potentiated by the inhibition of the expression of the pro-inflammatory cytokines TNF-α and IL-1β by all tested materials. By corroborating the extra- and intracellular cytokines profiles, the most promising material, with anti-inflammatory and pro-tissue regeneration activity, is Col-AgNP-AT, followed by Col-MPN-AG and Col. In the case of Cal-27 tumor cells, the marked inhibition of intracellular IL-10 production, by all tested materials, could indicate a pro-inflammatory effect of these materials, which, in the context of local application in case of a malignant wound or skin tumor, may suggest the triggering/stimulation of antitumor immunity; xi) the critical evaluation of the biological performances of nanostructured collagen-based dressings was carried out in all stages of the characterization of the functionality of collagen matrices containing NPs functionalized with phenolic compounds. Therefore, the variants recommended to be scaled up from laboratory studies (TLR4) to *in vivo* studies and micropilot production will be subsequently selected according to the reproducibility of their functionality. In conclusion, for this stage, all the foreseen documentation, experimentation and dissemination activities were carried out all deliverables have been achieved.