Wound healing (WH) is a complex process involving several stages as hemostasis, inflammation, re-epithelialization, and remodeling, often the impaired wound healing may be associated with microbial infection which translated in hindered resolution of the wound [1]. In this field water-soluble carvacrol prodrgs (WSCPs) have been synthesized as carvacrol (CAR) hydrophilic formulations, with improved water solubility as well as antimicrobial activity against a specific panel of Gram-positive bacteria and a lack of toxicity towards Human immortalized keratinocytes (HaCaT cells) [2,3]. Unfortunately, WSCPs/CAR formulations tend to hydrolysis in simulated fluids and human plasma. To further deepen these data, WSCPs/HA formulations - based on WSCPs and hyaluronic acid (HA) - were prepared.

**Preparation of WSCP1-2/HA formulations**

HA-based formulations were prepared by physical interactions between HA (350 kDa) and WSCP1-2 (Figure 1). WSCP1-2/HA formulations were obtained following a hydration treatment involving a mixture of dried HA and the investigated WSCPs (Figure 2). In the drug-loaded samples, the prodrg concentration was kept constant at 2% w/w, while the HA concentration examined was 3% w/w, which yielded the HA3 formulations. The ability of WSCPs/HA formulations to modulate the wound repair was evaluated in vitro model of WH, using HaCaT cells at 6, 18, and 24 h. Moreover, the effects of conditioned medium (CM) from M1/M2-like human leukemia monocyctoid cell line (THP-1 cells) cultured in presence of WSCPs/HA, were tested on the in vitro WH model.

**In vitro Biological assays**

**EFFECTS OF HA, WSCP-CAR/HYALURONIC ACID FORMULATIONS ON THE RE-EPITHELIALIZATION OF HACAT CELL LINE SCRATCHED MONOLAYERS**

The treatment of HaCaT cells with WSCP1/HA for 18 h increased expression of both MMP-2 and MMP-9 with higher levels of MMP-9 in presence of WSCP2/HA. While TGFβ expression was higher in cells incubated with WSCP1/HA for 6 h, and with WSCP2/HA for 18 h. After 24 h, both formulations induced a similar trend of expression decline of MMP-2, MMP-9, and TGFβ (Figure 6). To clarify if WSCP1 formulations can modulate the expression of TNFα, as pro-inflammatory cytokines and IL-10, as an anti-inflammatory cytokine, M1 and M2 polarized cells were incubated with the previously selected concentration of 10 μg/mL of WSCP1/HA. Results indicate that WSCP5/HA treatment of polarized M1 cells leads to a down-regulation of IL-10 and TNFα gene expression, compared with M1 untreated cells (Figure 7). Since CM from polarized M1 - or M2-like THP-1 cells in the presence of 10 μg/mL WSCP1/HA or WSCP2/HA can mirror a microenvironment comparable to the inflammatory or proliferative phase of WH, it was added to scratched HaCaT monolayer and the effects on the re-epithelialization were evaluated. As reported in Figure 8, after 18 h of the mechanical wound 54.6% of cell-free was observed in the untreated cells, while in presence of CM from M1 and M2 cells incubated with WSCP1/HA cell-free area of 40.2% and 78.9%, respectively, was observed. In the presence of CM from M1 and M2 cells incubated with WSCP2/HA, the percentages of wound cell-free area were reduced to 25% and 18.7%, respectively.

**Conclusions**

Polymer-based wound dressing material loaded with bioactive compounds can result in enhanced therapeutic outcomes when used in the treatment of wounds. In this work, the important roles of HA-based formulations loaded with carvacrol prodrgs for skin WH were elaborated. WSCPs/HA formulations arise the wound closure rate by increasing the cell migration at the wound borders and modulating anti-inflammatory mediators. Further studies will be conducted in vivo to better evaluate the involvement of WSCPs/HA formulations in the complex WH process.

**References**