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In vitro model based on human keratinocyte to evaluate relaxin activity on wound healing exploiting time-lapse video microscopy

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Summary

Wound repair is a well-ordered but complex process involving many cellular activities including inflammation, growth factor and cytokine production, and thus cell migration and proliferation. *In vitro* wound healing assays have been used and are well accepted, for the discovery and validation of biomolecules that may influence cell migration and improve repair (Liu et al. 2009). The aim of our research was to exploit time-lapse video-microscopy (Okolab, Italy) to observe and analyse *scratched monolayers of* keratinocyte cell line (HaCat), with specific interest in characterizing different relaxin preparations.

Key words

Relaxin, wound healing, time-lapse.

Relaxin (RLX), is a molecule with the potential to speed wound closure and enhance the formation of new epidermis (Samuel et al., 2007). The experimental set-up used here proved a powerful method to directly observe and characterize several important biological processes such as motility and proliferation (Schiraldi et al., 2012), concurrently evaluating *in vitro* wound repair . Images are acquired at specific intervals and successive analysis is performed through tailored softwares providing a robust and accurate investigation of the biological phenomenon. Relaxin preparations improved wound healing *in vivo* (Stewart et al., 2009) enhancing both the repair time and smoothing of the surgical scars on pigs. However, to assess a dose-response analysis numerous trials are needed. For this reason a reliable comparative evaluation *in vitro* prior to animal testing has to be ethically considered. In these experiments the commercial porcine extractive relaxin (p-RLX) and the recombinant human one (r-h-RLX) could be compared with newly obtained extractive porcine preparation from IBSA (p-RLX IBSA). Our results show that relaxins treatment stimulates keratinocyte migration and proliferation. The different relaxins (10^{-6}M) shortened by 50% the wound closure time compared to untreated cells (figure1). In particular, as showed by representative micrographs and reported in figure 1 B, at 20h the percentage of wound repair is 80% for porcine IBSA and human recombinant RLX indicating their strong similarity. However, while r-h-RLX reached complete closure at 45h, the p-RLX IBSA needed approximately 70h. The p-RLX differred from the others since it induces 60% of repair in the first 20h and determines a complete reparation after about 60 hours.

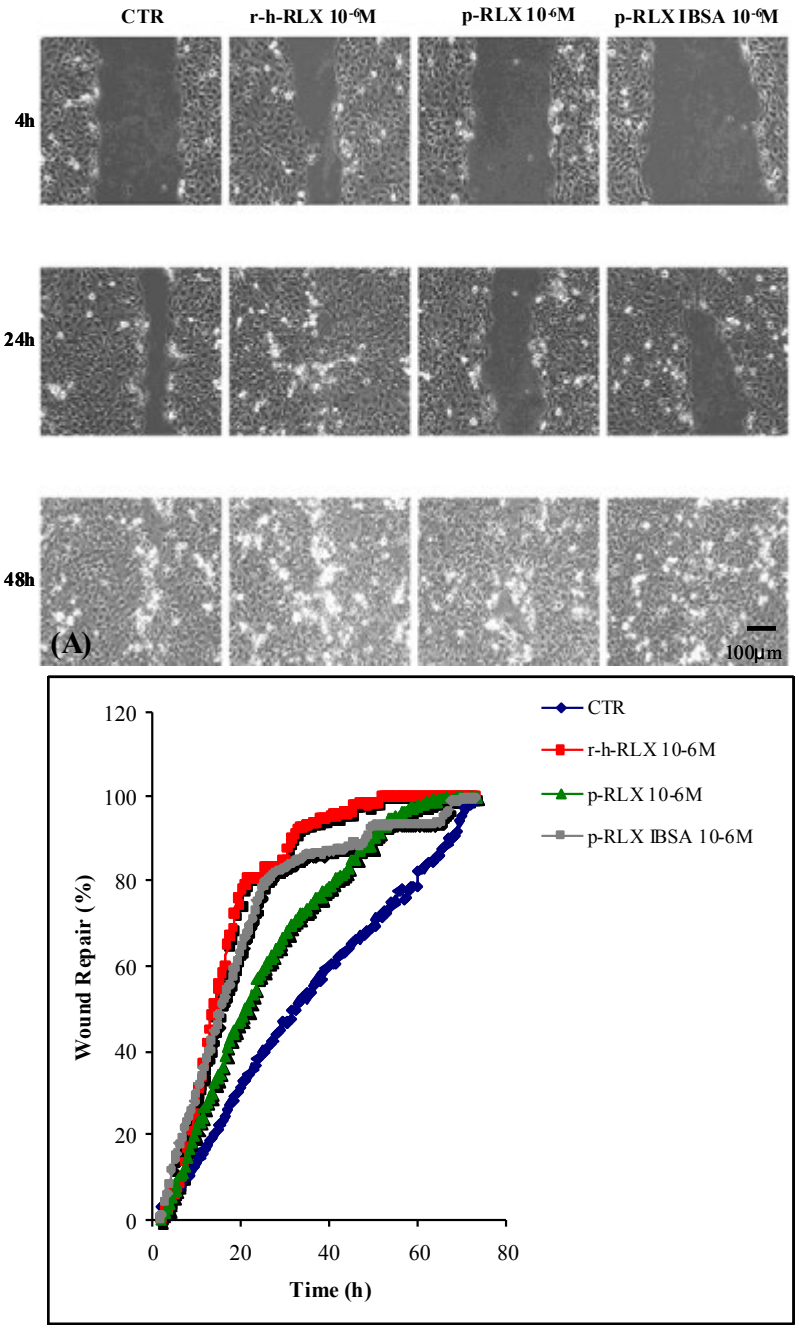

Figure 1. Representative time-lapse images relative to scratched HaCat alone and treated with relaxins (10^{-6}M) at different time (4, 24, 48 hours) (**A**) percent of healing (%) (**B**) relative to control and to cells treated with relaxins (10^{-6}M).

The average repair rates in the first 24h were 250 $\mu m^2/h$ for p-RLX, 500 $\mu m^2/h$ for r-h-RLX and 380 $\mu m^2/h$ for p-RLX IBSA, all significatively higher compared to that of the control (150 μ^2/h).

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