

# FLAMINAL® HYDRO combines non-cytotoxicity with antimicrobial activity



Dave Van den Plas<sup>1</sup>, Kris De Smet<sup>1</sup> and Philippe Sollie<sup>1</sup>

<sup>1</sup>Research Department, Flen Pharma NV, Drie Eikenstraat 661, B-2650 Edegem.

## Introduction

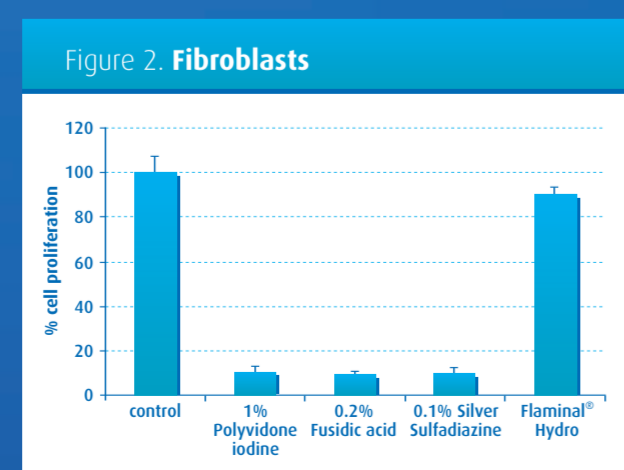
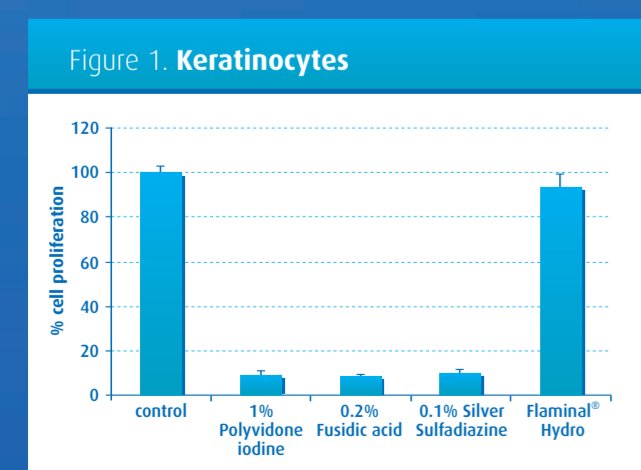
Two important aspects need to be fulfilled by wound products for proper wound healing to occur. Firstly, wound infection needs to be controlled in order to reduce swelling, redness, wound exudate and pain. Secondly, products and dressings need to show, respectively a low degree of cytotoxicity and minimized maceration after application in order not to impair growth of cells and normal healing. However, these two parameters often are conflicting and therefore are difficult to achieve in one product at a time. In this study, the performance of Flaminal® Hydro for the two criteria was assessed.

## Methods

Cytotoxicity was studied by incubating fibroblast (3T3) and keratinocyte cells (HaCat) for 24h with wound products dissolved in growth medium. Next, cell survival was assessed by a MTT assay in which only living cells (metabolic active cells) are able to convert the yellow tetrazolium salt to a blue formazan, which can be measured spectrophotometrically. In a parallel study, the influence on the cell division rate was assessed. After 24h incubation with the products, BrdU was added to the medium and incorporation of this thymidine analogue was measured by standard detection methods. Antibacterial activity was assessed by adding microbial suspensions to Flaminal® Hydro. At different time points, samples were taken and plated on agar plates. After overnight incubation, surviving bacterial and fungal cells were assessed by determining the number of colony forming units (CFU).

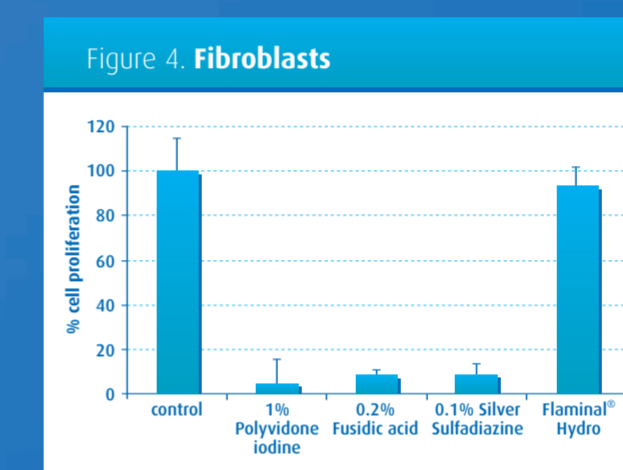
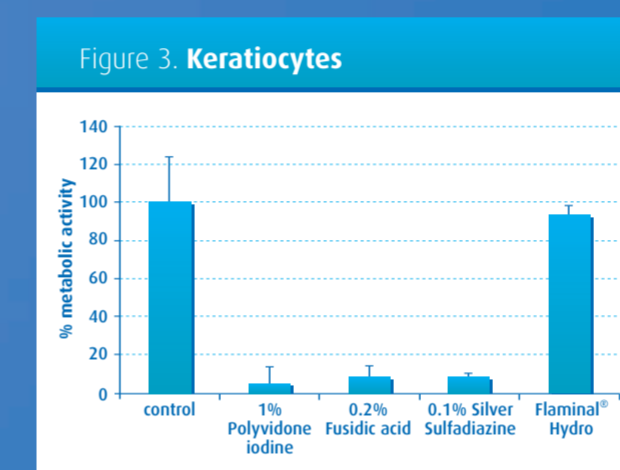
## Cytotoxicity: metabolic activity

Cells were incubated for 24h in a 10% (w/v) Flaminal® Hydro solution, no significant decrease in metabolic activity was detected compared to the control cells. In contrast, 1% (w/v) Polyvidone iodine, 0.1% (w/v) Silver Sulfadiazine and 0.2% (w/v) Fusidic acid showed a high degree of cytotoxicity both towards fibroblasts and keratinocytes.



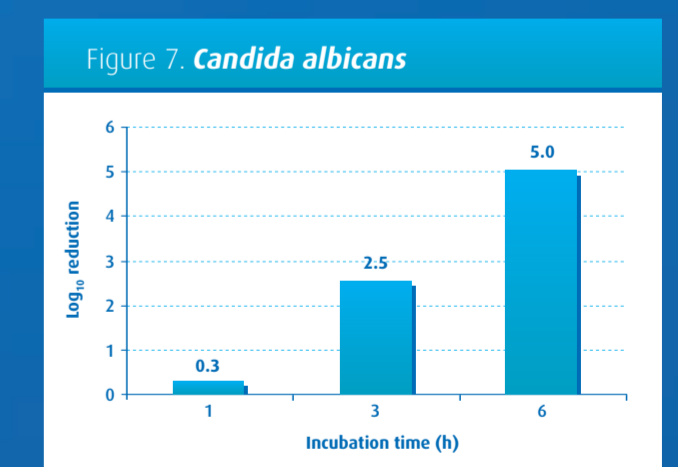
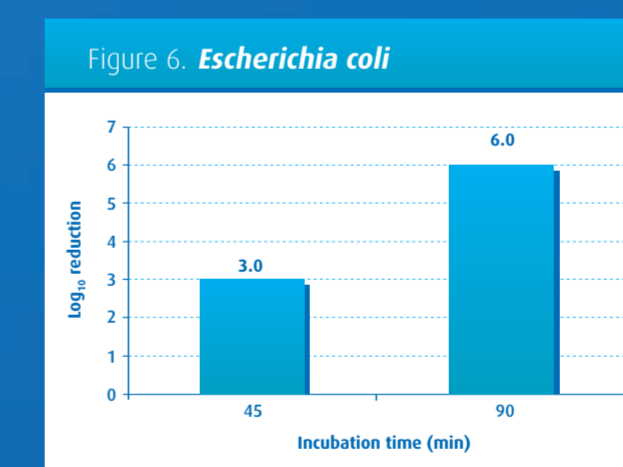
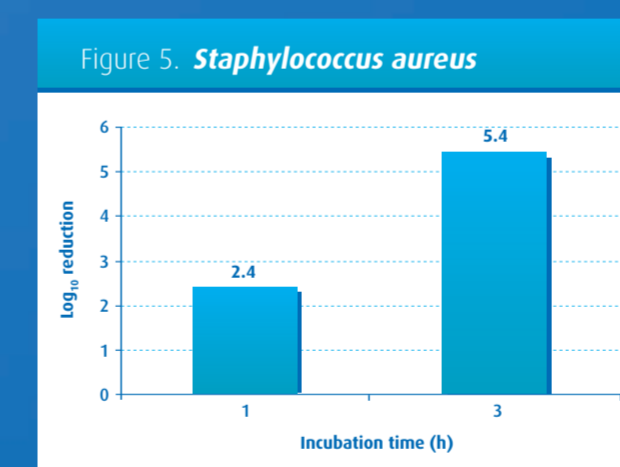
## Influence on cell proliferation

Cells were incubated for 24h in a 10% (w/v) Flaminal® Hydro solution, only minor influence on cell proliferation was seen compared to the control cells. In contrast, 1% (w/v) Polyvidone iodine, 0.1% (w/v) Silver Sulfadiazine and 0.2% (w/v) Fusidic acid clearly impaired cell proliferation.



## Antimicrobial activity

With respect to its antimicrobial effect, Flaminal® Hydro demonstrated a high degree of activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria as well as towards fungi (*Candida albicans*) within 6h of incubation.



## Conclusion

Our data demonstrate that Flaminal® Hydro has no influence on the metabolic activity and proliferation of fibroblasts and keratinocytes in contrast to other antimicrobial products such as Polyvidone iodine, Silver Sulphadiazine and Fusidic acid. In addition, Flaminal® Hydro possesses potent antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as towards fungi. Taken together, the results show that Flaminal® Hydro combines effective control of microbial burden with non-cytotoxicity towards two important cell types in wound healing. Our *in vitro* data might explain the improved overall healing witnessed in a clinical study with patients suffering from ulcers (de la Brassine *et al.*, JADV [in press]).