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Comparative Cytocompatibility Evaluation: Two-Dimensional (2D) Vs. Three-Dimensional (3D) Cell Culture Tools

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Abstract— Wound management still represents a significant problem. Furthermore, infection of wounds leads to non-healing conditions. With an increasing need of novel strategies for wound healing as well as treatment of infected wounds, there is an extreme urgency to develop reliable *in vitro* tools for biological evaluation. 3D cell culture has a potential to provide a more physiologically relevant model compared to standard 2D cell culture. For this purpose, the cytocompatibility and antimicrobial activity of different silver-based species occurring in commercially available wound dressings were investigated in this study, comparing the responses of 2D monolayer and 3D cell culture-based testing systems.

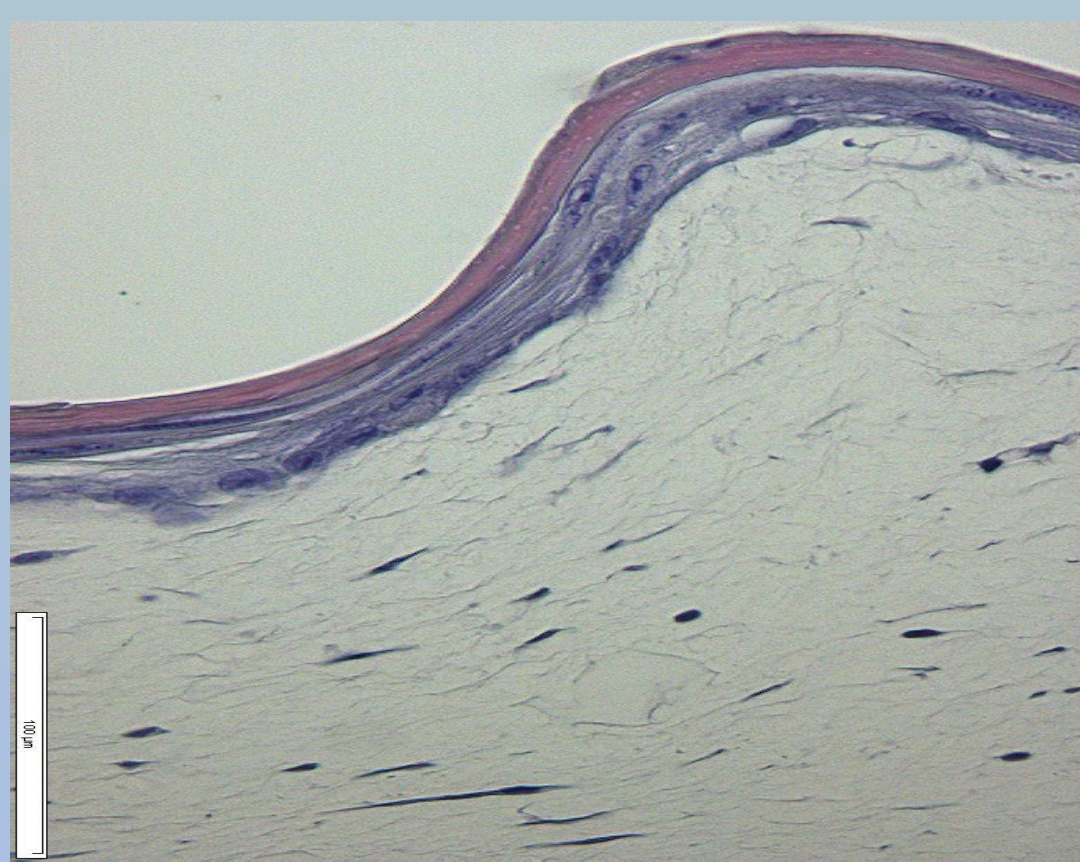
Introduction: Wounds with delayed healing are emerging as serious problems in the clinics causing a considerable morbidity and a high cost of health care. With emerging novel materials for wound management, there is an extreme need to develop reliable *in vitro* tools for biological evaluation [1], [2]. Monolayers of adherent cells have been used for *in vitro* testing for decades. However, they are associated with different limitations, mainly because of their inability to mimic *in vivo* conditions. Three-dimensional (3D) cell culture systems are more physiologically relevant and extremely needed for cytocompatibility testing of biomaterials.

Non-healing wounds are mainly associated with infections of the wounds causing a chronic and non-healing condition. The 3D cell culture system developed in this study was further used to generate a 3D infected wound model, to better mimic *in vivo* infected wound conditions. Considering the emergence of antibiotic resistance, this system will serve as an advanced tool to screen novel antimicrobial biomaterials for wound healing.



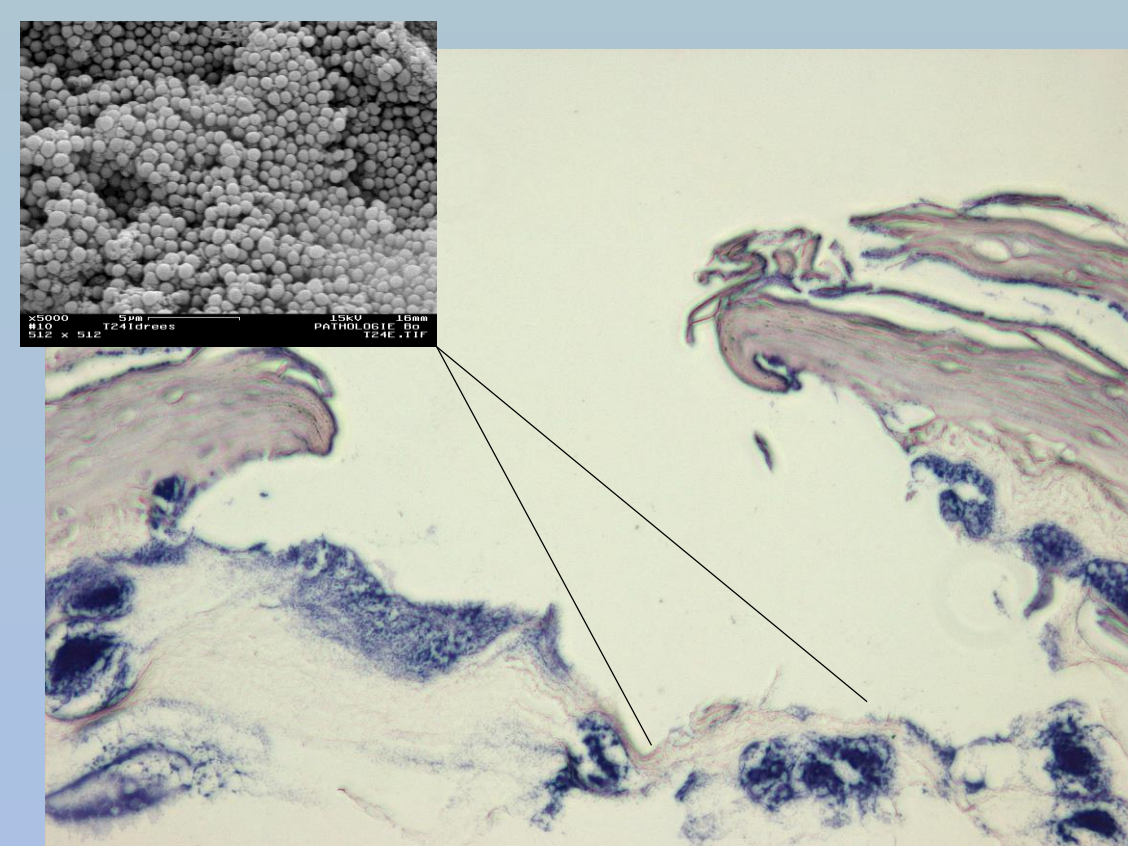
Pat. with an infected wound at the lateral right side of the lower leg. Central part of the highly inflamed pressure ulcer is necrotic. Bacteria found in the wound was MRSA.

In-vitro Human Skin Model

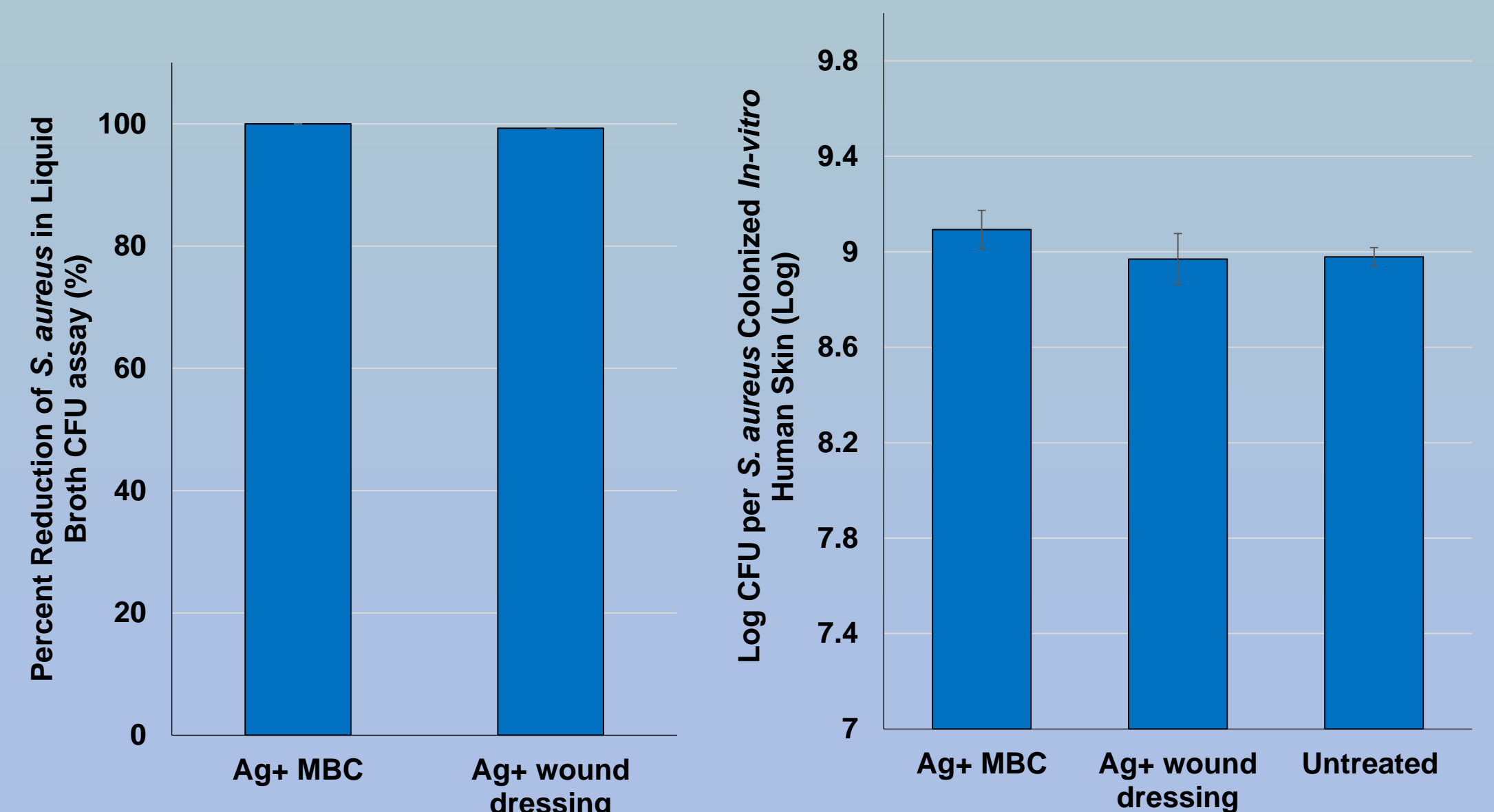


This system was generated by first developing the dermal compartment by embedding human dermal fibroblasts in rat tail tendon collagen type 1 as extracellular matrix (ECM). Once the ECM was remodelled by human fibroblasts, human epidermal keratinocytes were seeded on it and allowed to differentiate by lifting the culture to an air liquid interface (ALI). The fully differentiated 3D human skin model was characterized by histology staining, immunohistochemistry and physicochemistry. The 3D human skin model was further developed as an infected skin model by colonizing *S.aureus* at wound site.

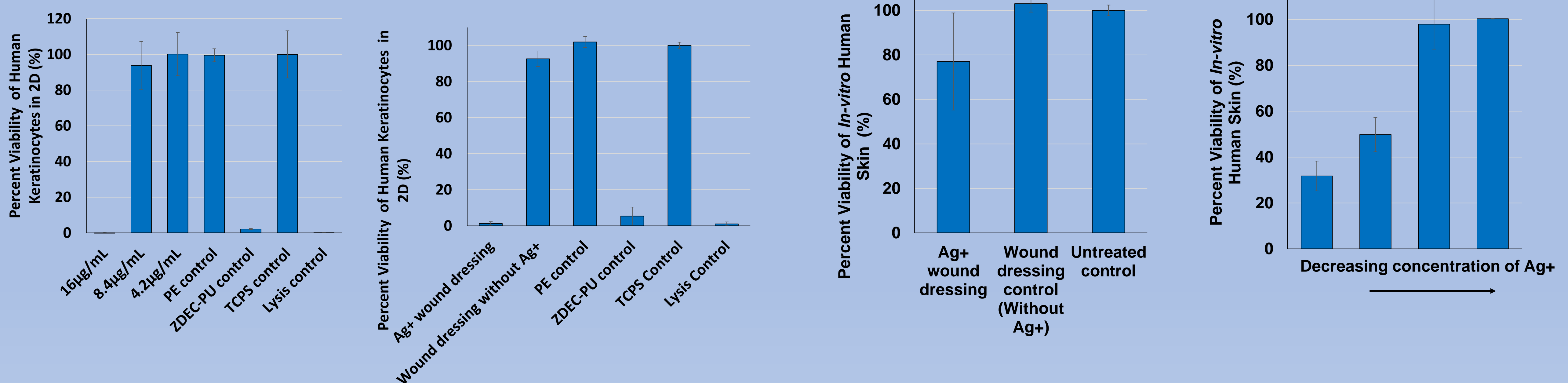
S. aureus Colonized Human Skin Model



Antibacterial Analysis



Cytocompatibility Analysis: Skin Cells (2D Cell Culture) Vs. *In-vitro* Human Skin Model (3D Cell Culture System)



Conclusion: Before testing new materials in clinical trials, predictive and reliable preclinical data are required. Taking this into consideration, responses of a 3D system in comparison to 2D monolayer cells were performed to provide more reliable preclinical outcomes

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