In Vitro Evaluation of the Cytotoxicity and Antimicrobial Efficacy of Silver-Containing Nanofiber Wound Dressings

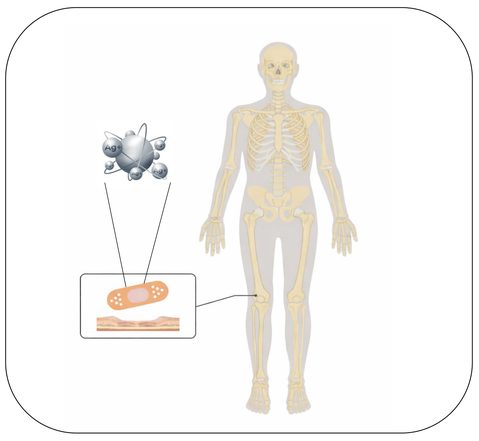
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Background

Chronic wounds are characterized by persistent inflammation and impose a substantial clinical and economic burden. Advanced **wound dressings** represent an established therapeutic strategy. Silver coatings are widely used to prevent and control **wound infections**.



Research Question

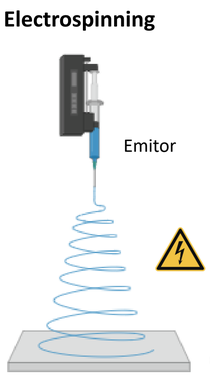
Although silver promotes tissue **regeneration** due to its antimicrobial properties, it may also have adverse effects. This in vitro study evaluates the **biocompatibility** of silver-containing biomaterials, aiming to further define a therapeutic window that balances **antimicrobial efficacy** and **cytotoxicity**.

Experimental Setup

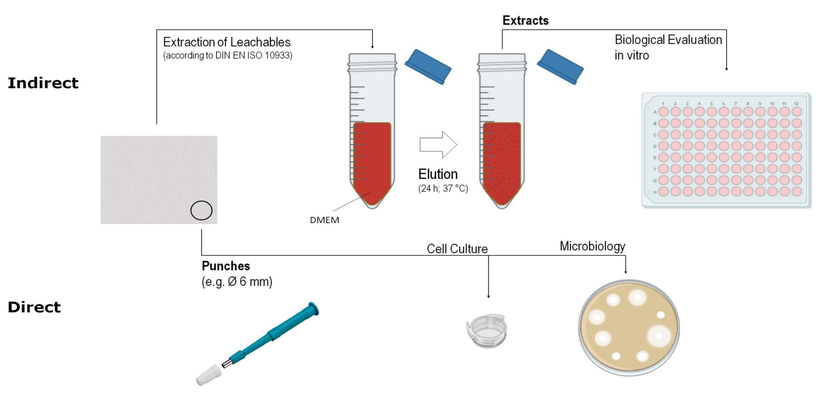


Commercial silver-containing wound dressings

Simplified material platform



PPDO nonwovens with incorporated silver nanoparticles



Results

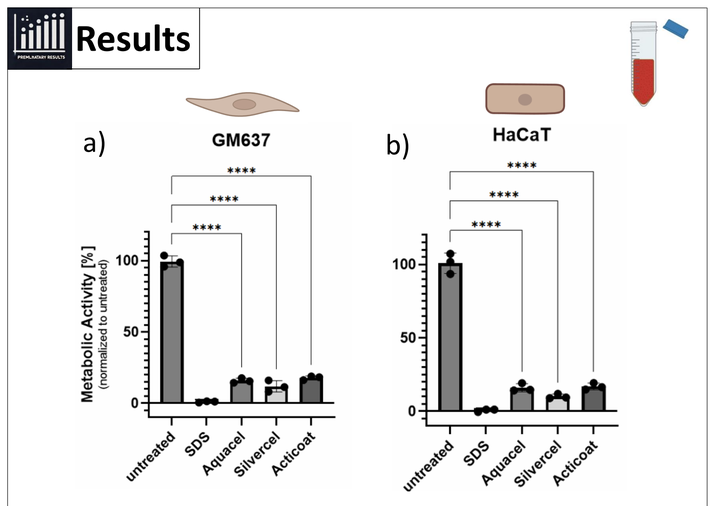


Figure 1: Assessment of the metabolic activity of **a)** GM637 (human fibroblasts) and **b)** HaCaT (human keratinocytes) after treatment with commercial wound dressing extracts using the XTT assay; *Mean of n=3 independent experiments. One-way ANOVA with Šidák correction. ns = not significant; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001*

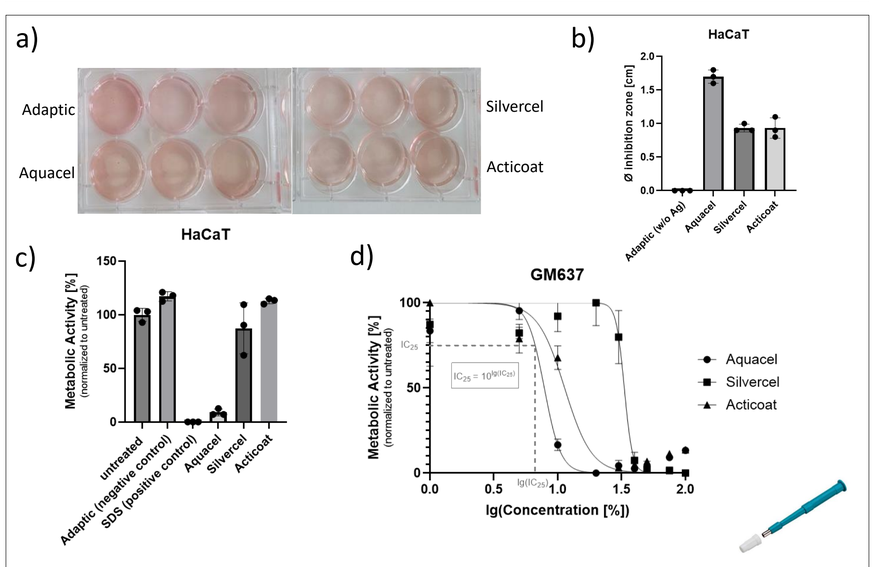


Figure 2: Assessment of **a)** the impact of wound dressing punches (ø 10 mm) on overlaid HaCaT in neutral red stained agar (6-well) with **b)** measured inhibition zones and **c)** the measurement of the cell viability (XTT) after direct treatment of wound dressing punches (ø 5 mm) fixed in CellCrown™ inserts (96-well) and **d)** the metabolic activity (XTT) after treatment with serial dilutions of wound dressing extracts in order to calculate inhibitory concentrations ('IC values')

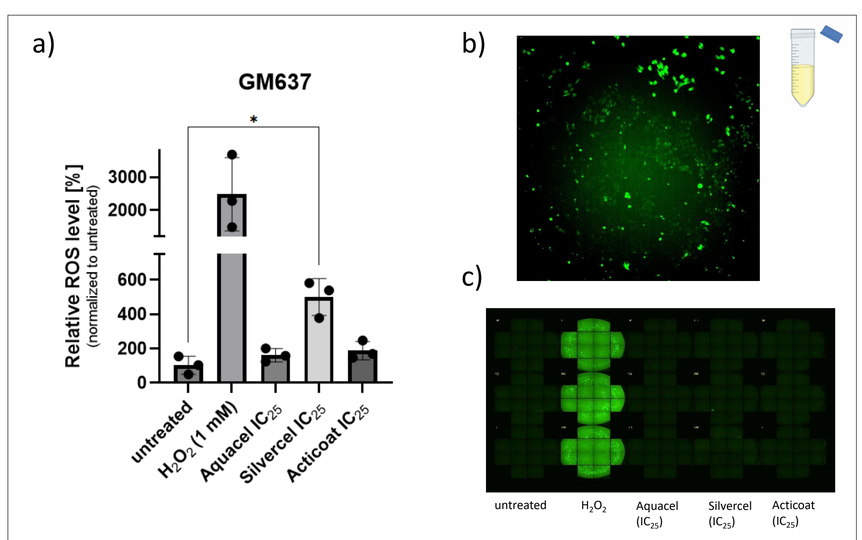


Figure 3: Assessment of **a)** the intracellular oxidative stress response of GM637 using a fluorescent probe (H_2DCFDA) after treatment with commercial wound dressing extracts at the calculated IC_{25} value with **b)** the microscopic appearance (FITC) of the positive control treated with H_2O_2 (1 mM) and **c)** the overview of all measured sites of each well in MetaXpress® (ImageXpress Confocal HT.ai High-Content Imaging System; Molecular Devices); *Mean of n=3 independent experiments. Brown-Forsythe and Welch ANOVA tests; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001*

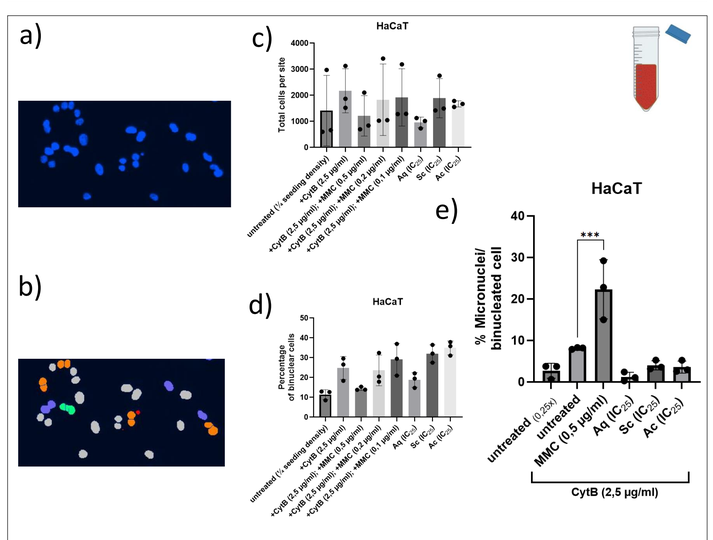


Figure 4: Microscopic appearance of **a)** DAPI-stained HaCaT with **b)** the evaluation mask in order to detect binucleated cells (orange) with micronuclei (pink) as an indicator for mutagenicity analyzed in MetaXpress®: **c)** total cells per site, **d)** percentage of binuclear cells and **e)** micronuclei per binucleated cell after treatment with commercial wound dressing extracts at the calculated IC_{25} value; *Mean of n=3 independent experiments.*

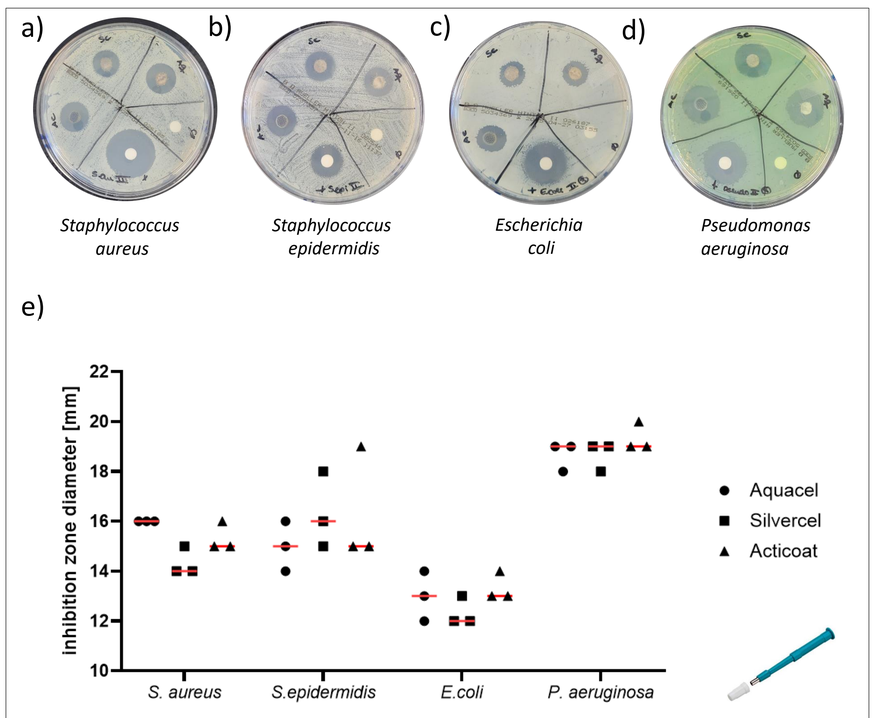


Figure 5: Assessment of the antimicrobial activity of wound dressing punches (ø 6mm) against wound-associated pathogens: **a)** *Staphylococcus aureus*, **b)** *Staphylococcus epidermidis*, **c)** *Escherichia coli* & **d)** *Pseudomonas aeruginosa* and an overview of the measured inhibition zones with a median value (red line); *Mean of n=3 independent experiments. Two-way ANOVA with Tukey's multiple comparisons test. ns = not significant; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001*

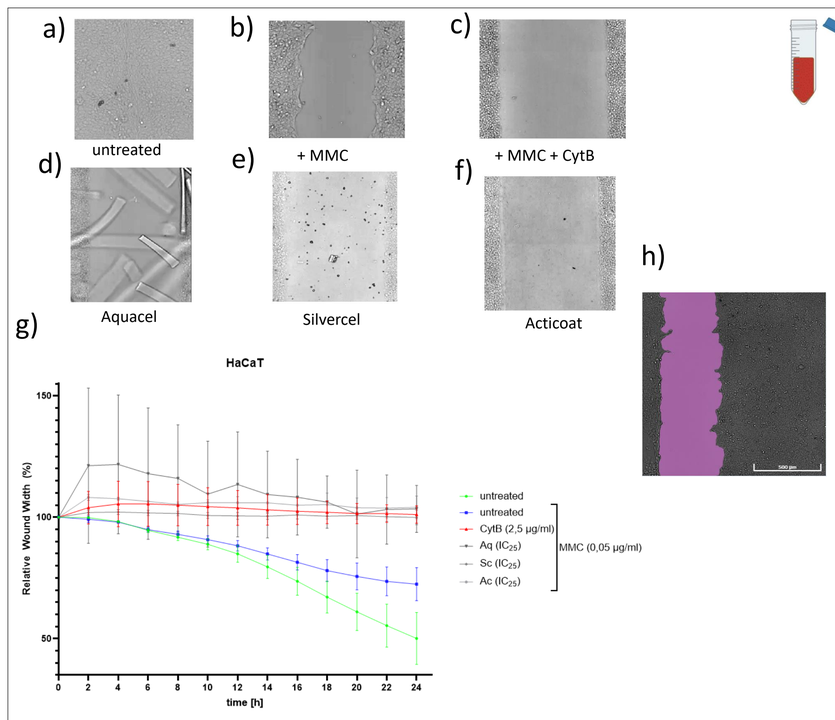


Figure 6: Assessment of the migration (scratch assay) of HaCaT within 24 h after removal of ibidi 2 well chamber inserts (24 well) and microscopic appearance (transmission light in MetaXpress®): **a)** untreated, **b)** + MMC (proliferation inhibitor), **c)** + MMC + CytB (migration inhibitor), **d)** Aquacel extract (IC_{25}), **e)** Silvercel extract (IC_{25}), **f)** Acticoat extract (IC_{25}) and **g)** overview of the wound closure (%) normalized to the initial timepoint (t= 0 h) **h)** while the total scratch area was determined by using the IN Carta® Image Analysis Software

Conclusion & Outlook

▶ measurement of the **metabolic activity**:

▷ wound dressing extracts exhibit a significant decrease (*Figure 1*)

▷ wound dressing punches exert a partially decrease (*Figure 2*)

▶ diluted wound dressing extracts (IC_{25}) do not significantly increase the intracellular **reactive oxygen species** (**ROS**) level (*Figure 3*) and did not induce the formation of micronuclei as an indicator for **genotoxicity** (*Figure 4*)

▶ wound dressing punches possess an **antimicrobial activity** against gram-negative as well as gram-positive wound-associated bacterial strains (*Figure 5*)

▶ diluted wound dressing extracts (IC_{25}) appear to inhibit **cell migration** (*Figure 6*)

Future work will explore zinc as a potential alternative and include comparison with PPDO nonwovens.

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