



POLITECNICO DI TORINO  
Repository ISTITUZIONALE

Antimicrobial and Cytotoxicity Evaluations of Hydrogen peroxide - Towards Clinical Application of Antimicrobial Biomaterials for Wound Dressings

*Original*

Antimicrobial and Cytotoxicity Evaluations of Hydrogen peroxide - Towards Clinical Application of Antimicrobial Biomaterials for Wound Dressings / Idrees, Ayesha; Pacharra, S.; Marlinghaus, L.; Chiono, V.; Ciardelli, G.; Vasquez, J. M.; Greiser, U.; Wang, W.; Viebahn, R.; Gatermann, S. G.; Salber, J.. - (2017). ((Intervento presentato al convegno Annual Meeting of the German Society for Biomaterials 2017 – Würzburg, November 09 – 11 tenutosi a Würzburg, Germany nel November 09 – 11, 2017.

*Availability:*

This version is available at: 11583/2691360 since: 2019-01-11T19:02:29Z

*Publisher:*

Biomedical Engineering / Biomedizinische Technik

*Published*

DOI:

*Terms of use:*

openAccess

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

(Article begins on next page)

# Antimicrobial and Cytotoxicity Evaluations of Hydrogen peroxide-Towards Clinical Application of Antimicrobial Biomaterials for Wound Dressings

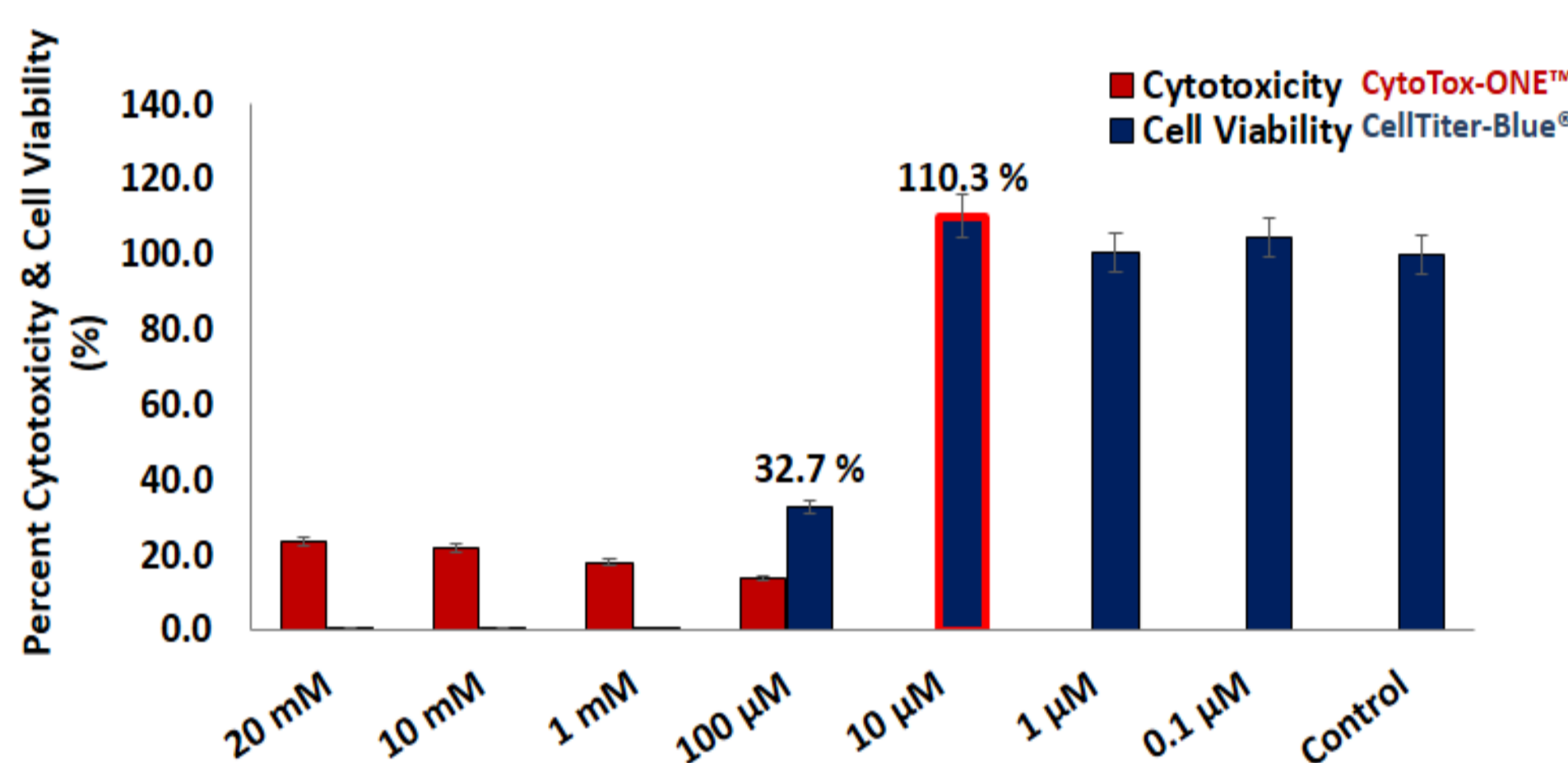
A Idrees<sup>1,2,3</sup>, S Pacharra<sup>3</sup>, L Marlinghaus<sup>4</sup>, V Chiono<sup>1</sup>, G Ciardelli<sup>1</sup>, JM Vazquez<sup>1,5</sup>, U Greiser<sup>5</sup>, W Wang<sup>5</sup>, R Viebahn<sup>2</sup>, SG Gatermann<sup>4</sup>, J Salber<sup>2,3</sup>

<sup>1</sup>Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Corso Duca Degli Abruzzi 24, 10129 Turin, Italy.  
<sup>2</sup>Clinic of Surgery, Knappschaftskrankenhaus Bochum, Hospital of the RUHR-University, In der Schornau 23-25, 44892 Bochum, Germany.  
<sup>3</sup>Clinical Research Center, RUHR University Bochum, Universitätsstraße 150, 44801 Bochum  
<sup>4</sup>Department of Medical Microbiology, Institute for Hygiene and Microbiology, Ruhr-University Bochum, Universitätsstraße 150, 44801, Germany.  
<sup>5</sup>Vornia Biomaterials Ltd, Dublin.

**Introduction:** Chronic wound infections and emerging drug resistance are serious problems in the present world causing a considerable morbidity and a high healthcare costs. For this purpose, investigation on novel antimicrobial strategies is of great interest. Use of honey from ancient times is reputed for its wound-healing and antibacterial properties. It has been reported that the major antibacterial factor in honey is the release of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) produced by glucose oxidase. This approach can be exploited to prepare novel polymer-based antimicrobial biomaterials for wound healing purposes. In this study, the inhibitory effect of H<sub>2</sub>O<sub>2</sub> on the growth of numerous bacteria of clinical significance was investigated. To determine the “safe” antimicrobial concentration of H<sub>2</sub>O<sub>2</sub>, cytocompatibility analysis was also performed for H<sub>2</sub>O<sub>2</sub> induced cellular cytotoxicity.

**Methods:** The effect of externally added H<sub>2</sub>O<sub>2</sub> was performed by exposing L929 fibroblasts to various H<sub>2</sub>O<sub>2</sub> concentrations. At different time points after exposure with H<sub>2</sub>O<sub>2</sub>, cell viability was assessed by measuring cell metabolic activity, cell membrane integrity and cell morphology. Antimicrobial efficacy was evaluated against a wide range of gram-positive and gram-negative bacteria that are involved in chronic wounds namely *S. aureus*, *S. epidermidis*, *S. lugdunensis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii*. Antimicrobial tests were performed using broth microdilution method for the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). MIC is the lowest concentration of antimicrobial agent that inhibits bacterial growth, while the MBC is the lowest level of antimicrobial agent that kills the bacteria (by reducing the viability of the initial bacterial inoculum by ≥99.9%).

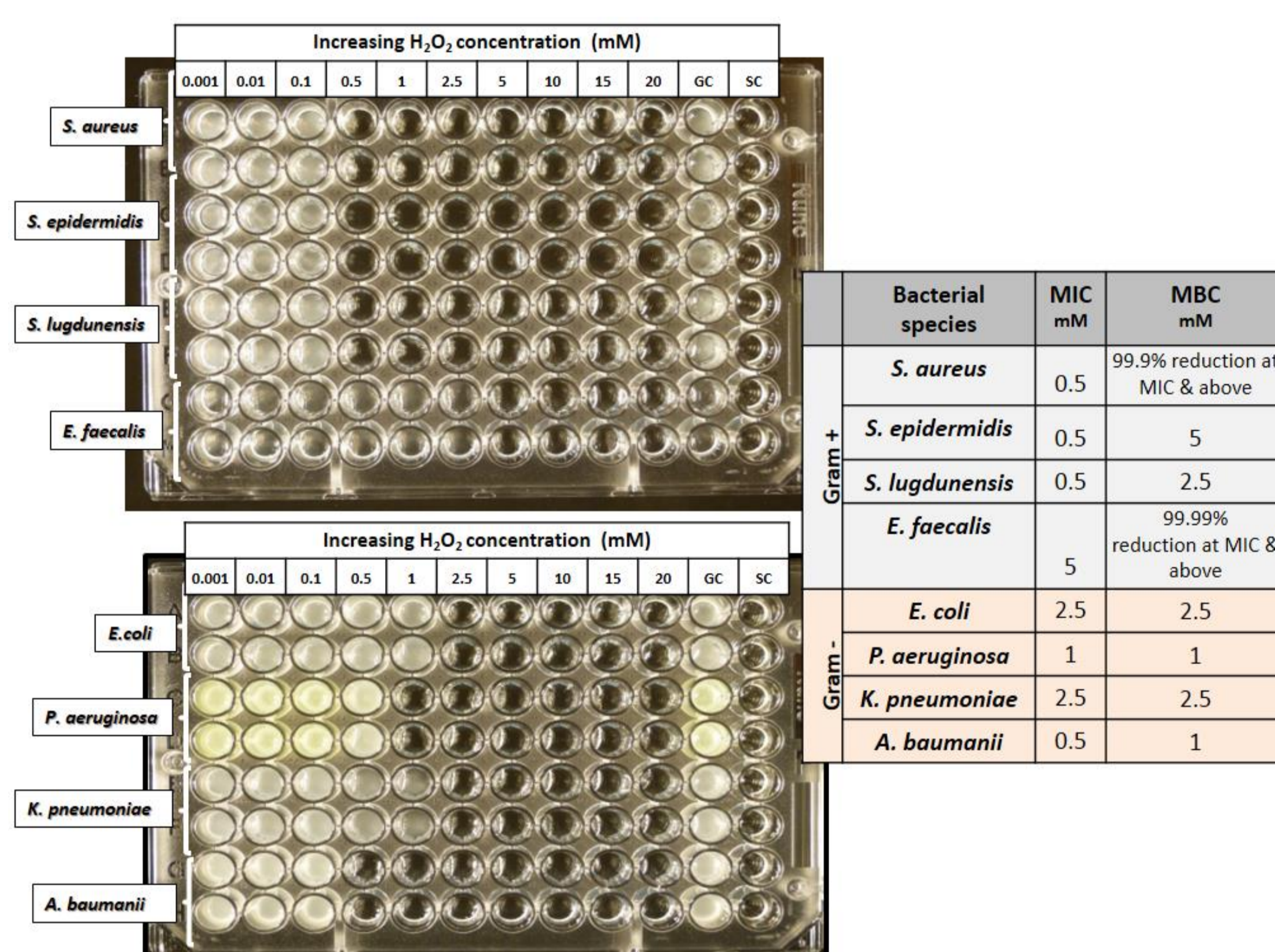
**Figure 1: Evaluation of cytotoxic effects of H<sub>2</sub>O<sub>2</sub> on L929 fibroblasts**



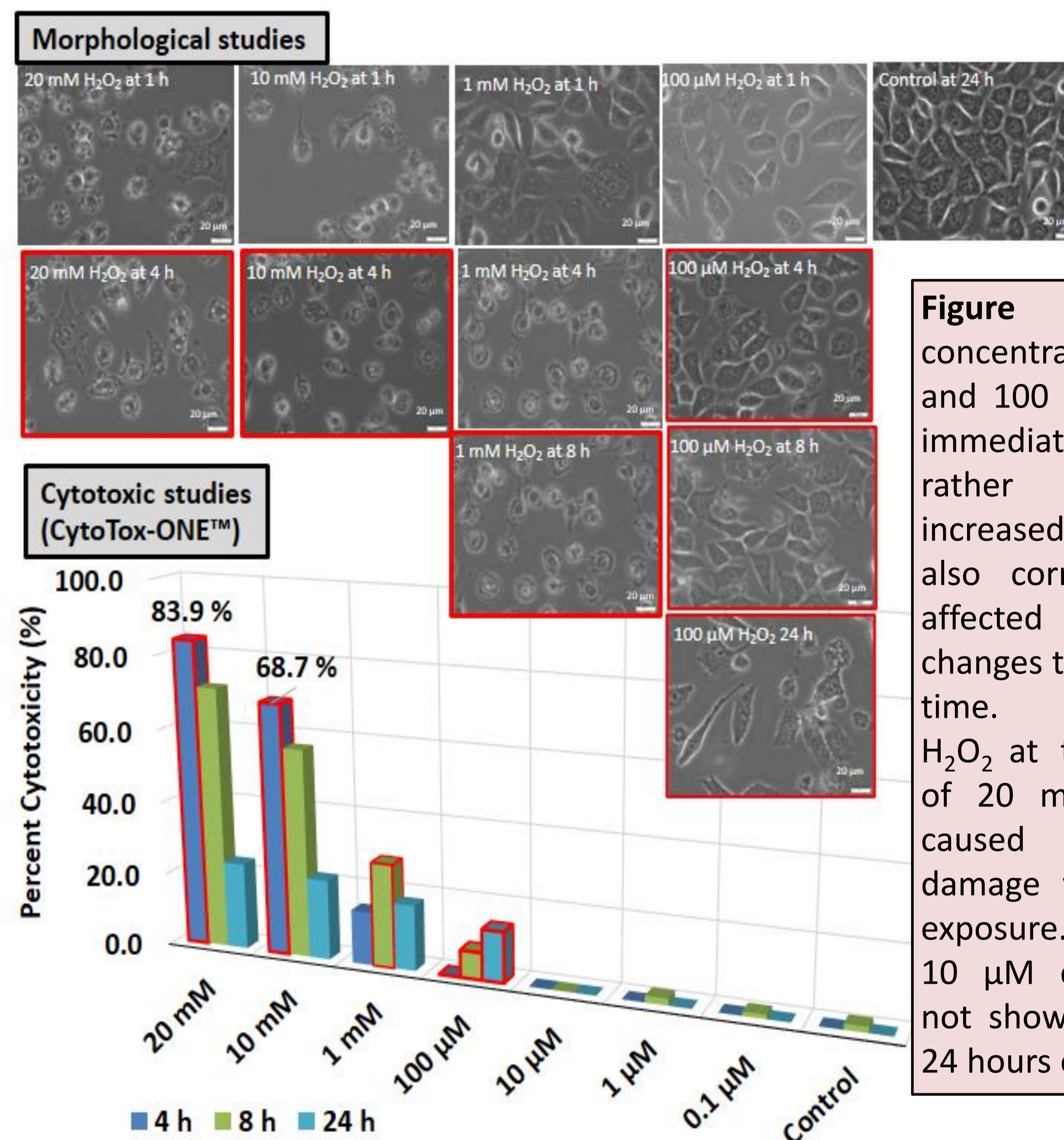
**Figure 1: Cell viability data shows that:**

- H<sub>2</sub>O<sub>2</sub> did not favor cell viability at 20 mM, 10 mM, and 1 mM concentrations in our experimental set-up (L929 cell culture).
- H<sub>2</sub>O<sub>2</sub> concentration of 10 μM did not alter cell viability with respect to control conditions (cells only) and further that H<sub>2</sub>O<sub>2</sub> might be stimulating cell growth at the concentration of 10 μM.<sup>1,2</sup>
- Cytotoxic effect started decreasing below 100 μM H<sub>2</sub>O<sub>2</sub> concentration and was no more relevant at further lower concentrations (10, 1, 0.1 μM). H<sub>2</sub>O<sub>2</sub> concentration of 10 μM did not affect cell behavior.

**Figure 3: Antimicrobial activity of H<sub>2</sub>O<sub>2</sub> against different clinically significant bacterial species**

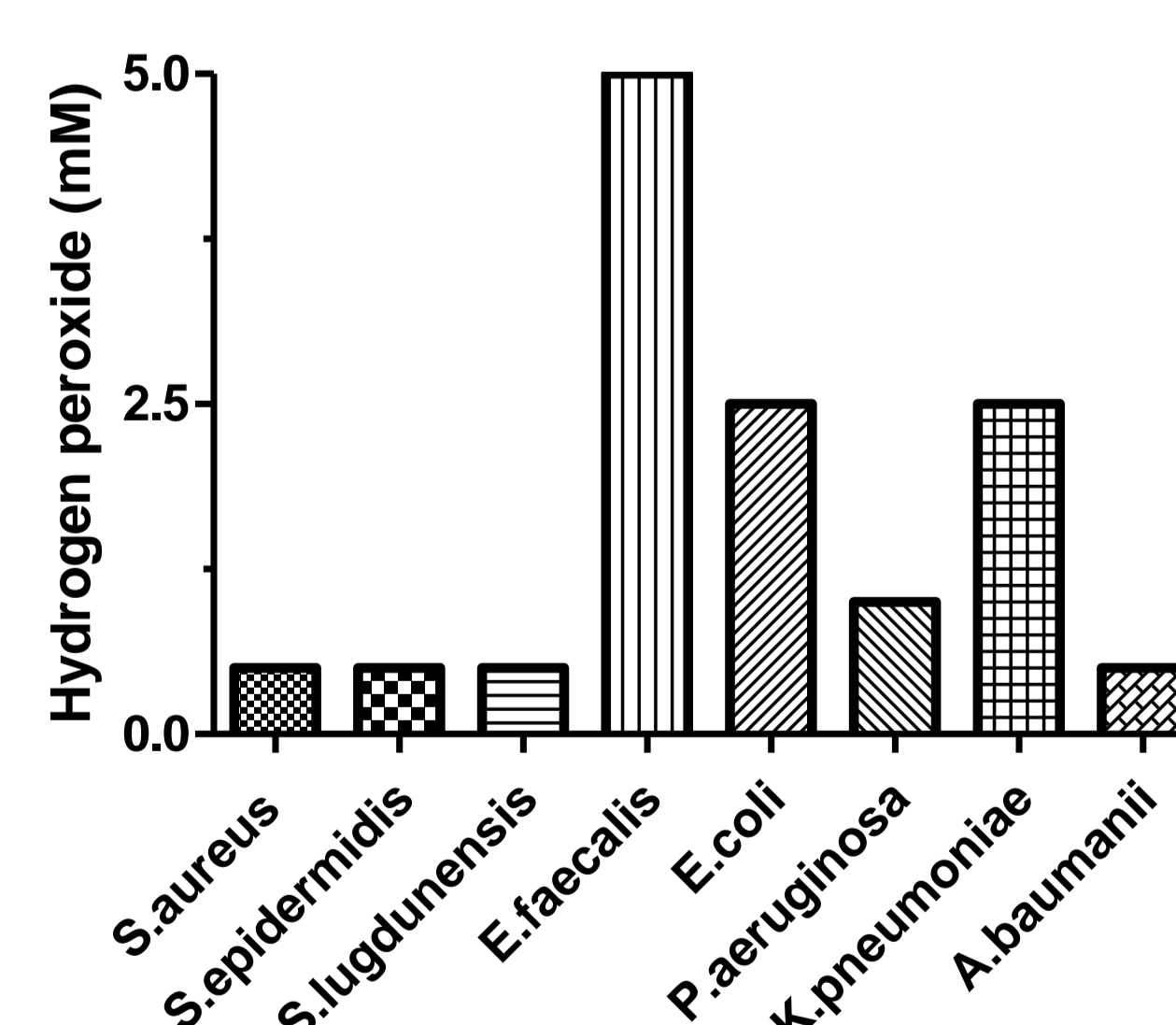


**Figure 2: Evaluation of cytotoxic effects of H<sub>2</sub>O<sub>2</sub> on L929 fibroblasts over time**

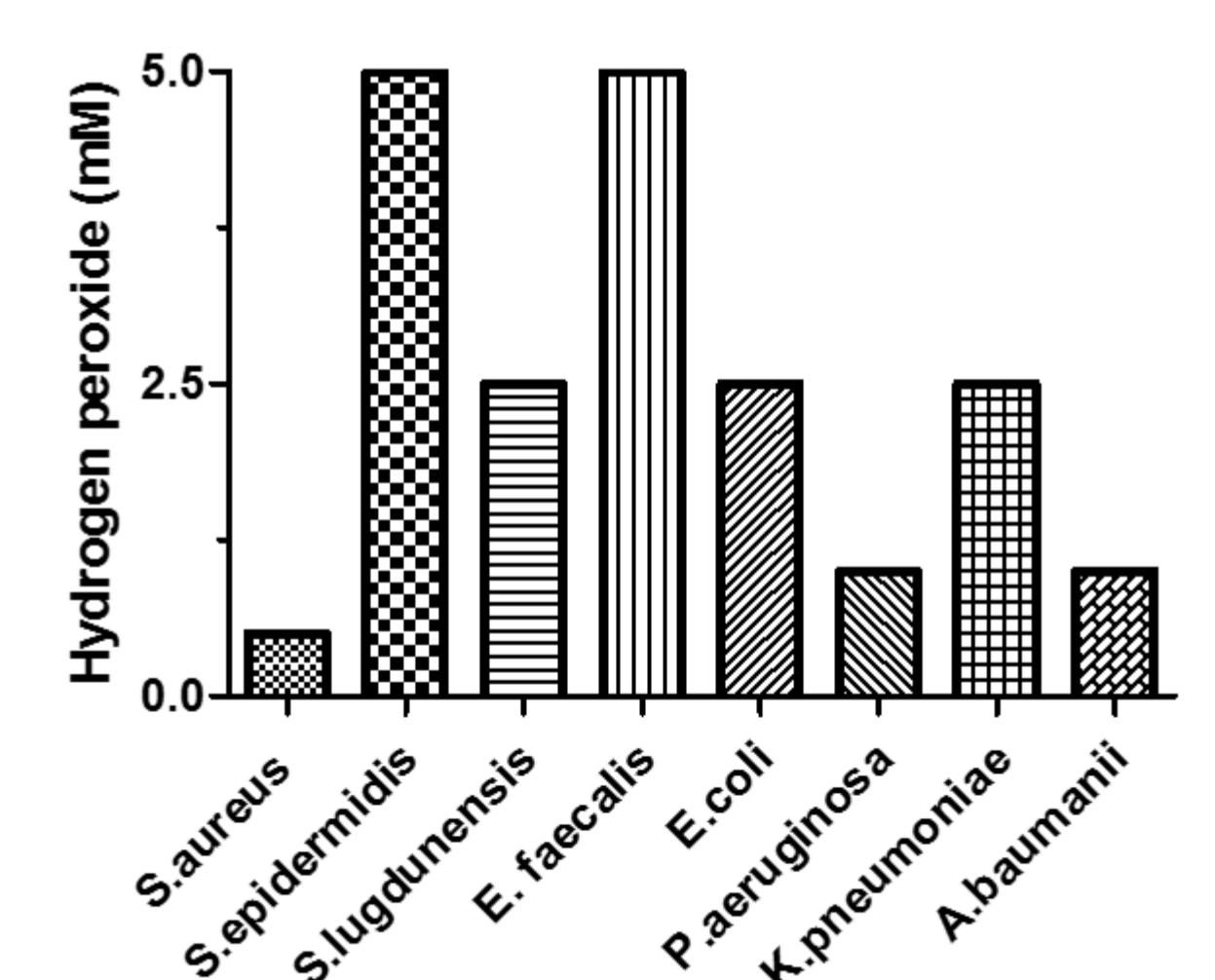


**Figure 2:** H<sub>2</sub>O<sub>2</sub> concentrations of 1 mM and 100 μM did not show immediate cytotoxic effect, rather cytotoxic effect increased with time. This also correlated with the affected morphological changes that appeared over time. H<sub>2</sub>O<sub>2</sub> at the concentration of 20 mM and 10 mM, caused cell membrane damage within 4 hour of exposure. 10 μM concentration did not show cytotoxicity until 24 hours of exposure.

**Figure 4: MIC of H<sub>2</sub>O<sub>2</sub> against different bacterial species**



**Figure 5: MBC of H<sub>2</sub>O<sub>2</sub> against different bacterial species**



**Results & Conclusion:** Results (Figure 3, 4 and 5) showed different MIC and MBC values of H<sub>2</sub>O<sub>2</sub> for different bacterial species indicating their differences in susceptibility to treatment. There was no clear preference between Gram positive and Gram negative bacteria. Among the tested bacteria, *S. aureus* showed 99.9% bacterial reduction at the concentration of 0.5 mM, while *E. faecalis* showed the highest MIC value of 5 mM. H<sub>2</sub>O<sub>2</sub> was found most effective against *S. aureus* (one of the most pathogenic bacteria) and less effective against *E. faecalis*. *Acinetobacter baumannii* being one of the most pathogenic bacteria involved in serious skin wound infections also showed the same MIC value (0.5 mM) as *S. aureus*. Cytotoxicity results (figure 1 and 2) showed two distinct patterns in our experimental set-up: the highest concentrations rapidly induced cell death characterized by morphological evidence and plasma membrane damage as compared to the concentrations of 1 mM and 100 μM where the cytotoxic effect only gradually increased with time. Results showed that 10 μM concentration did not show cytotoxicity. This data also indicated the concentration dependent distinct pathways of H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity. Also, we speculated that the cytotoxic effects would differ depending on “at once” H<sub>2</sub>O<sub>2</sub> exposure or exposure to “gradually” produced H<sub>2</sub>O<sub>2</sub> by glucose oxidase and glucose entrapped into a matrix. Different H<sub>2</sub>O<sub>2</sub> administration influences the ability of the cells to eliminate and detoxify H<sub>2</sub>O<sub>2</sub> and needs further investigation.