Summary

Chronic wounds pose a socioeconomic problem and burden to patients worldwide. There are several causes of wound chronicity. One of the major issues of the non-healing state of a wound is its infection. Bacteria provoke a proinflammatory response, which disrupts the proper sequence of wound healing phases. Also, inflammation raises the production and activity of proteases. These enzymes digest the extracellular matrix, resulting in deceleration or hindrance of granulation tissue maturation. In addition to the inflammation-induced proteases, some bacterial species produce proteases that contribute to tissue degradation and increase bacterial virulence.

Antiseptics are used to lower the bacterial burden in chronic wounds. Antiseptics act non-specifically, which may be their asset as well as drawback. They act as a double-edged sword. Antiseptics act on bacterial cells. However, host cells are caught in the crossfire as antiseptics may damage eukaryotic cells as well. The damage of eukaryotic cells complicates wound closure. Silver is probably the most used antiseptic to treat chronic wounds. Silver is also suspected of inducing nonspecific damage to eukaryotic as well as prokaryotic cells.

This doctoral thesis is aimed at investigating the multitude of effects that silver may exhibit. We chose *in vitro* settings mimicking specific aspects of chronic wounds. Four commercially available wound healing dressings impregnated with silver were compared side-by-side. The antimicrobial properties of the dressings were compared in planktonic bacteria in liquid culture as well as seeded on a Petri dish. The silver content in the dressings or dressing extracts was evaluated with ICP-OES. We observed that the antimicrobial activity did not directly correspond to silver content.

Similarly, the dressing extracts differed in their cytotoxic effect. The least cytotoxic and also one of the least antimicrobial efficient was Silvercel. We applied the silver dressings onto porcine dermis to evaluate the relevance of our in vitro cytotoxicity observations. We detected silver with ICP-OES and with a histological method – silver autometallography. Silver penetrated into the skin and caused DNA damage and the upregulation of stress-response genes. We further confirmed that silver from the dressing extracts induced oxidative stress and DNA breaks using cultured skin cells. We showed that silver may decrease proinflammatory response of neutrophils (in contrast to monocytes) while concomitantly increasing their cell death.

We investigated the distribution of silver in chronic human wounds treated with a silver dressing and a dressing with octenidine in different parts. Silver was observed in macrophages, as well as associated with ECM and capillaries. The wounds healed faster under the dressing

with octenidine, where inflammation receded faster, and collagen matured more prominently. Wound slough decreased in the parts treated with the dressing with hyaluronan and octenidine more prominently. The gene expression of metallothioneins was increased in the parts of the wounds treated with the silver dressing.

The antiprotease activity was observed using the salt of silver – silver lactate. Silver lactate inhibited proteases produced by human neutrophils, fibroblasts, and keratinocytes, as well as recombinant MMP-2 and trypsin. Also, silver lactate inhibited bacterial proteases produced by species isolated from chronic wounds. The inhibitory effect was dose-dependent. However, we did not observe any specificity towards any of the tested proteases; the proteases were inhibited within a similar concentration range. As a next step, we observed inhibition of endogenous proteases in porcine skin by silver lactate. Again, we showed that silver may penetrate the dermis.

Silver is widely used in wound dressings due to its well-documented antimicrobial properties. The inhibition of excessive protease activity in chronic wounds is considered beneficial. Cytotoxicity towards stromal or immune cells is negative. We showed that silver penetrated into the dermis, which we compared in laboratory settings to the exposition of chronic wounds to silver dressings. Silver penetration through extracellular matrix tissue was subsequently confirmed in chronic human wounds.

We revealed some of the silver (side) effects, but there may be indeed more (effect on inflammatory mediators, growth factors etc.) that may play a role in chronic wound healing. However, the respective side effects are hard to observe or dissect from other events (i.e. ongoing inflammation) due to the complex nature of the chronic wound and its underlying causes.

Our results complement published clinical observations, which showed silver in the blood and organs of patients treated with silver dressings. One should consider applying a silver-impregnated dressing for a minimum period or choosing another antiseptic with a better cytotoxicity profile (i.e. octenidine dihydrochloride or polyhexanide).