INTRODUCTION: Chronic wounds with impaired status are often infected with bacteria in form of biofilm (1). The prevalence of bacterial biofilm in chronic wound was increased 10 times compared to acute wounds (2). Bacteria in form of biofilm can resist many standard procedures of wound care, which also includes antimicrobials. Debridement is useful support for removal of microbial load, however after the procedure, biofilm can quickly reproduce itself. Therefore tight combination of antimicrobial treatment and debridement is preferred (3). Biofilm bacteria are more resilient against external influences and are more resistant to antimicrobials. Some works have shown up to 100-1000 fold increase of effective concentration of antimicrobials against biofilm compared to effective concentration against planktonic suspended bacteria (4), (5).

AIMS: The antibiofilm efficacy of iodine antiseptics has not been well studied to date (6). However, available information from literature suggests that iodine in different forms could have positive effect on eradication of microbial biofilm (7), (8). In some cases it performed better against biofilm than other antimicrobials (9). The purpose of this work was to evaluate the effect of certain iodine and non-iodine antimicrobials against in vitro biofilm produced by wound present bacteria and to select antimicrobials with highest antibiofilm potential.

Development of substances for effective biofilm elimination could lead to improvement of wound care.

METHODS: Strains were cultivated and identified from 191 swabs from 19 patients hospitalized in University Hospital Hradec Králové. Out of 21 selected strains two strains of Pseudomonas aeruginosa and Staphylococcus aureus, were selected according to screening the biofilm formation in TSB using crystal violet staining (Fig.1/10). Due to wound environment imitation, the biofilm formation on the polystyrene surface at 35°C was also measured in the presence of different concentration of blood plasma. Biofilm was checked by scanning electron microscopy (SEM). MBEC (minimal biofilm eradication concentration) and MIC (minimal inhibitory concentration) were determined using the MBEC Innovotech assay and microtitre plates cultivation. MIC and MBEC were determined for antisepsics (K13, K13 in complex with 1.5 MDa sodium hyaluronate, polyvinylpyrrolidone iodeine, chlorhexidine digluconate, octenidine), and for antibiotics (gentamicine, cefazidine, ciprofloxacin, amikacine and chloramphenicol).

MIC was defined as the lowest concentration of iodine antiseptics against biofilm which shows no visible growth of bacteria was present after 24h treatment with antimicrobial. MBEC was defined as the lowest concentration with no growth for 24 hours incubation of biofilm in fresh media after 24 h antimicrobial treatment. The initial inoculums contained 106 suspended cells or 24 hours old rinsed biofilm. Ratios of MBEC to MIC were then calculated.

RESULTS: The most frequently isolated strains from the complicated wounds were Staphylococcus sp., Corynebacterium sp., P. aeruginosa, Enterococcus faecalis, Serratia marcescens and E.coli (Fig. 2). Out of the 21 screened strains one strain of P. aeruginosa and one S. aureus with highest biofilm formation were selected. The content of 20% plasma in P. aeruginosa media and 40% plasma in St. aureus media caused the most significant biofilm formation. These conditions were used for subsequent analysis. SEM images depicted in vitro biofilm as multilayered structure and cells were engulfed by glyocalyx (Fig. 3A-E).

It has been shown that biofilms of P. aeruginosa and St. aureus were very resilient against all selected antibiotics (ratio of MBEC/MIC was up to 512), except gentamicine (Table 1). Biofilm was also resilient against non-iodine antiseptics (MBEC/MIC varied from 4 to 64) and only slightly resistant against iodine antiseptics (MBEC/MIC was about 3) Table 1. Effect of all the forms of iodine antiseptics against biofilm was comparable. SEM showed that after the iodine treatment eliminated biofilm elimination was effective; however remnants of death disfigured cells still adhered on the surface with remaining glyocalyx around.

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