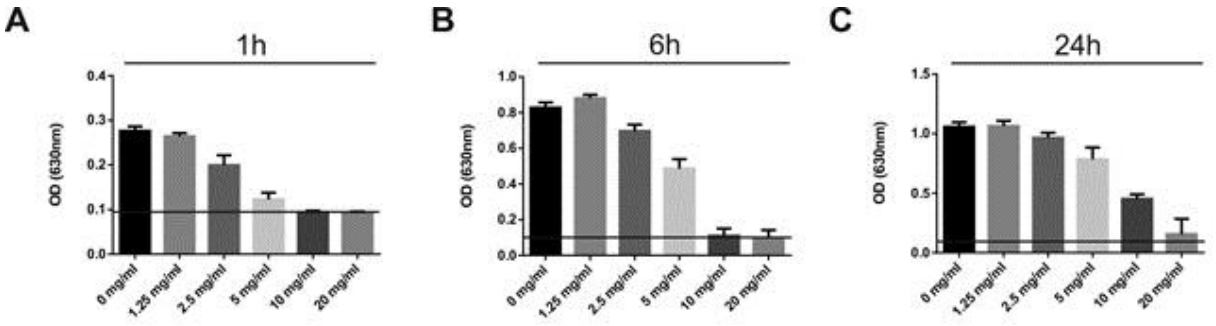
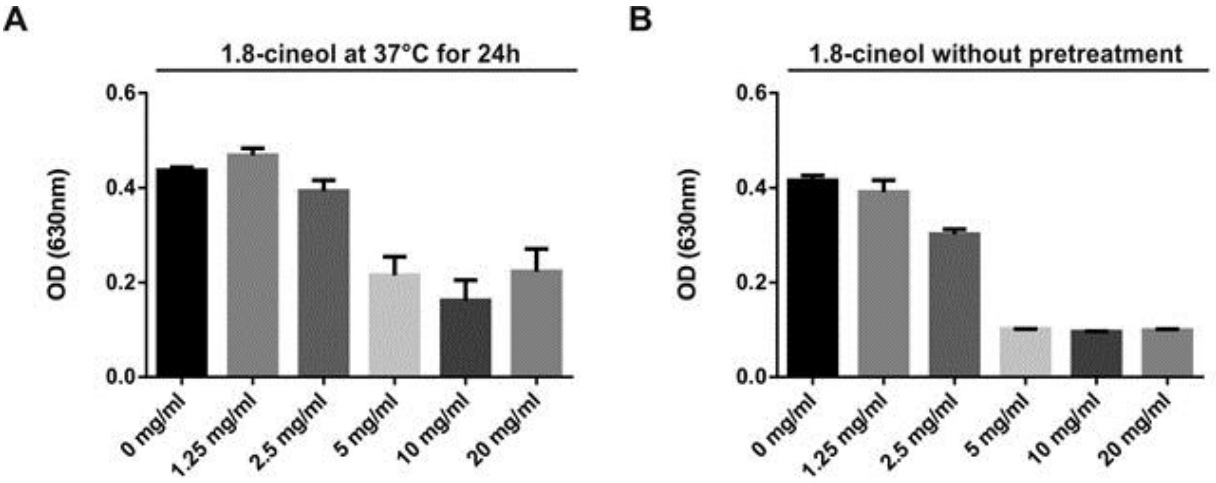
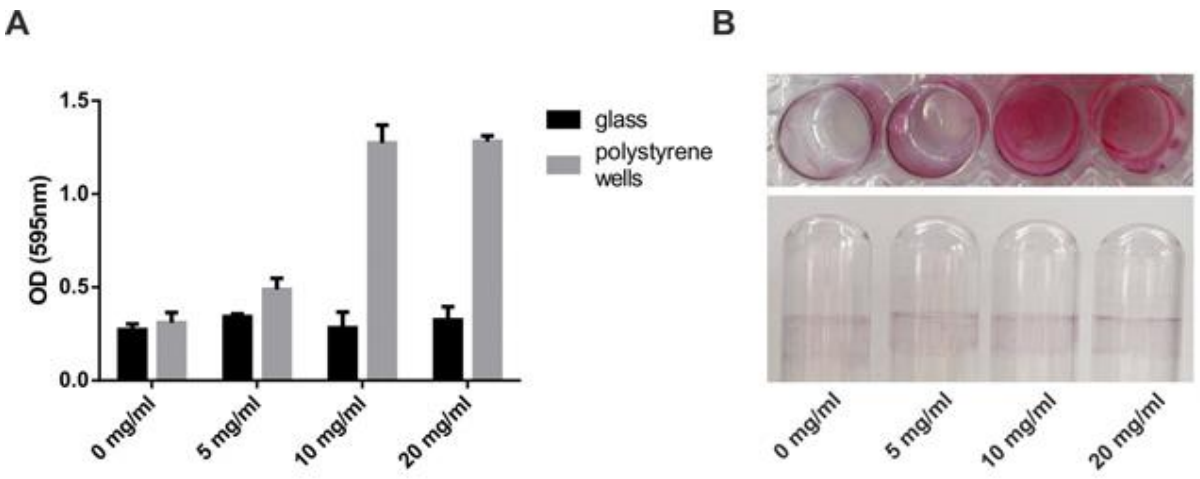
Supplementary information



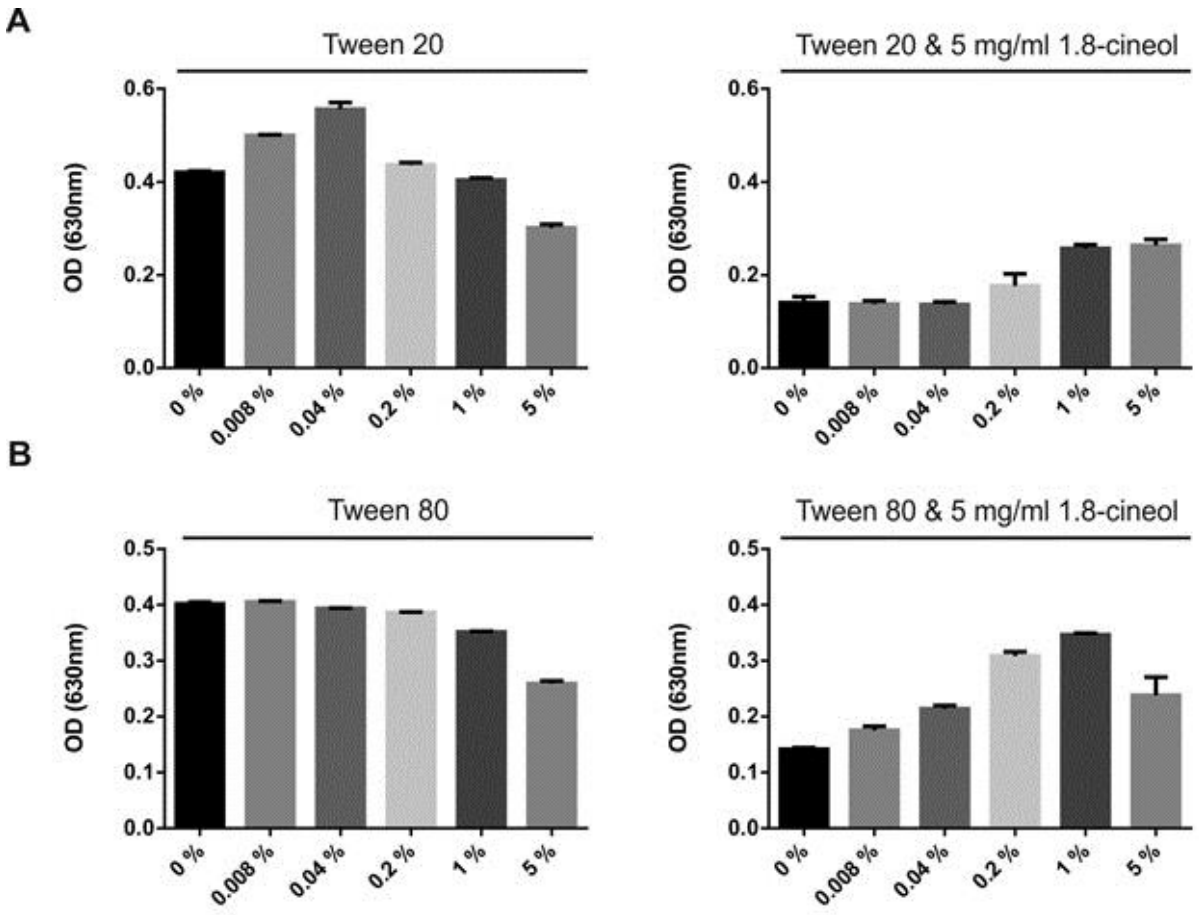
Supplementary figure S1. *Determination of the optimal time to verify the MIC of 1.8-cineol on the Staphylococcus aureus strain.* Investigation of the MIC of 1.8-cineol after 1 h **A**, 6 h **B** and 24 h **C** of bacterial culture in polystyrene 24-well plates. After 1 h a slight growth can be detected at 5 mg/ml but the OD is not clearly distinguishable from an inhibited growth. An incubation over 6 h led to a distinct difference in inhibition of growth between 10 mg/ml and 5 mg/ml 1.8-cineol and hence a MIC of 10 mg/ml 1.8-cineol can be ascertained. A longer incubation of 24 h resulted in a reduction of the inhibitory effect of 10 mg/ml 1.8-cineol and is therefore not suitable to enhance the sensitivity of the macrodilution assay.



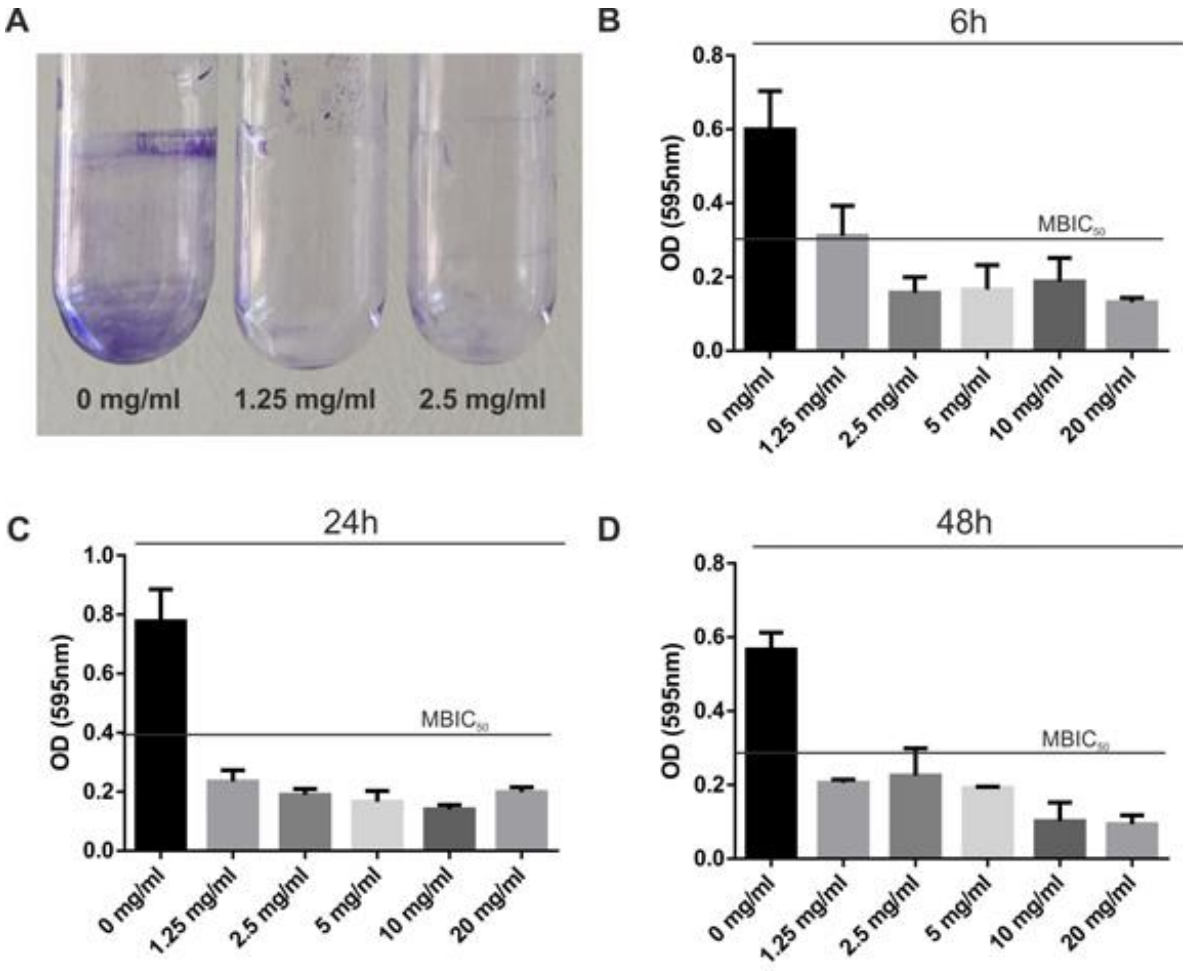
Supplementary figure S2. *Biological instability of 1.8-cineol after thermal treatment at 37°C for 24 h in comparison to untreated fully active 1.8-cineol.* **A** Determination of the MIC of pre-treated 1.8-cineol after 6 h of incubation. Only a slight inhibitory action could be detected at 1.8-cineol concentrations above 5mg/ml. **B** Concentration series to determine the MIC of un-treated 1.8-cineol after 6 h of incubation. The MIC was found to be 5 mg/ml as this is the lowest 1.8-cineol concentration that showed complete inhibition of bacterial growth.



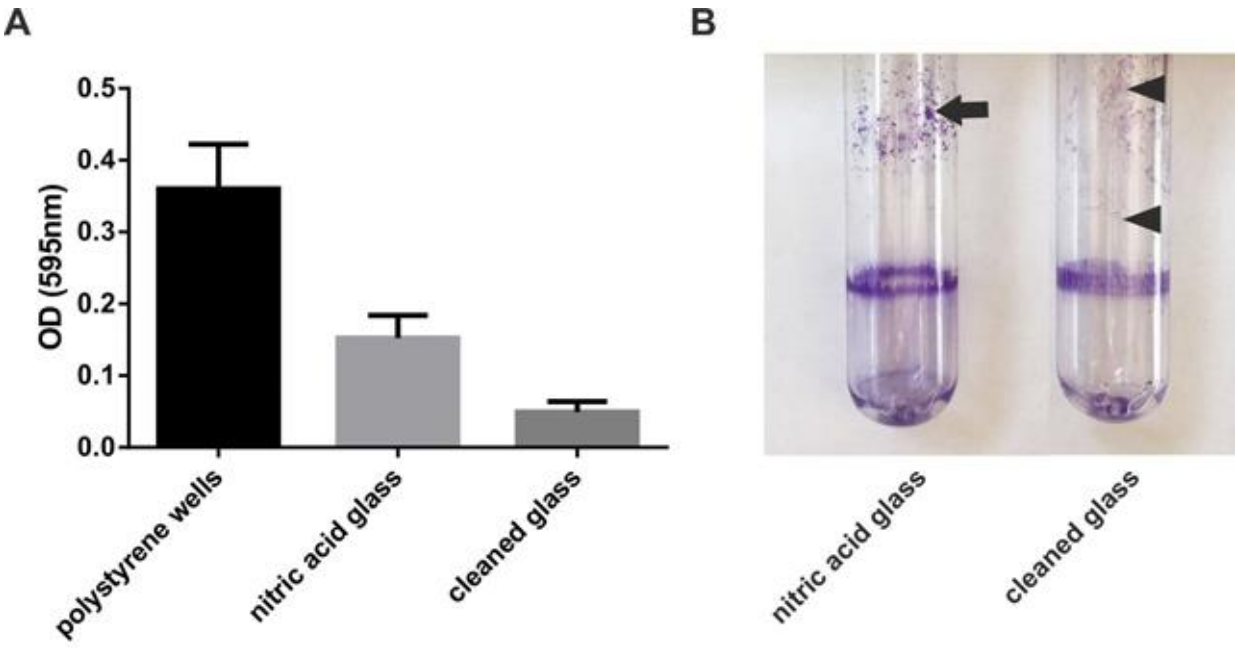
Supplementary figure S3. *The adsorbance capability of glass and* polystyrene *surfaces at different 1.8-cineol concentration.* **A** Amount of recovered Sudan red from glass (black columns) and polystyrene surface (grey columns) incubated with 0 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml 1.8-cineol for 6 h. The adsorbance of 1.8-cineol on the polystyrene surface was observed even at a 1.8-cineol concentration of 5 mg/ml and a saturation of the adsorbtion occurred at 10 mg/ml 1.8-cineol. No adsorbance of 1.8-cineol on glass could be observed. **B** Sudan red staining after 6 h of incubation in polystyrene and glass vessels at different 1.8-cineol concentrations. The highest 1.8-cineol absorption was observed in the polystyrene wells, at 10 and 20 mg/ml. In return, glass vessels showed only unspecific adsorbtion of Sudan red to the gas surface, indicating no absorption of 1.8-cineol.



Supplementary figure S4. *The bacterial growth in MHB supplement with different concentrations of the surfactants Tween 20 or 80 with or without supplementation of 5 mg/ml 1.8-cineol.* **A** Bacterial growth was investigated after incubation in 0 – 5 % Tween 20 with or without 5 mg/ml 1.8-cineol. Tween 20 without 1.8-cineol showed an increase in bacterial growth until 0.04 % and an inhibition till 5 %. With supplementation of 1.8-cineol an increased growth was observed when 0.2 – 5 % Tween 20 was added into the media. **B** Bacterial growth was investigated at 0 – 5 % Tween 80 in the presence or absence of 5 mg/ml 1.8-cineol. Without 1.8-cineol the bacterial growth is continuously reduced between concentrations of 0.04 – 5 % Tween 80. Increased bacterial growth was observed already at a concentration of 0.008 – 1 % Tween 80, whereas at 5 % the growth slightly decreased.



Supplementary figure S5. *Time series for the determination of the optimal incubation time to detect the MBIC$_{50}$ of 1,8-cineol.* **A** The picture shows the localization of a biofilm at a concentration of 0 mg/ml after 24 h of incubation. The biofilm is localized both at the bottom of the glass vessel and at the level of the meniscus of the nutrient medium. **B** After 6h, differently strong biofilm formations were detected, however the MBIC$_{50}$ was found at 1.25 mg/ml. **C** After 24 hours a MBIC$_{50}$ of less than 1.25 mg/ml of 1,8-cineol cloud be detected. **D** Further maturation of the biofilm over the period of 48 h showed no further increase in sensitivity and also resulted in a MBIC$_{50}$ of less than 1.25 mg/ml.



Supplementary figure S6. *The effect of different surface topography on biofilm formation of S. aureus.* **A** Cristal violet quantification of biofilms formed for 24 h in polystyrene 24-well culture plates, reused glass vessels treated with nitric acid as well as fabric new cleaned glass vessels. The most efficient biofilm formation was observed on the polystyrene surfaces. Biofilm grown in glass vessels only cleaned with detergent showed rather weak development. In contrast, glass vessels cleaned and subsequently treated with nitric acid showed enhanced biofilm growth compared to the unetched glass vessels. **B** Cristal violet staining after biofilm formation for 24 h. Glass vessels treated with nitric acid showed an enhancement in biofilm formation compared to cleaned vessels. The crystal violet stained deposits above the filling level of the nitric acid (arrow) which could not be removed mechanically with the aid of detergents. Even though the staining intensity of the deposits differs in dependence on the time of reuse even fabric new glass showed slightly stained deposits (arrowhead).