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Influence of preoperative skin sealing with cyanoacrylate on microbial contamination of surgical wounds following trauma surgery: a prospective, blinded, controlled observational study

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SUMMARY

Objective: Intraoperative bacterial contamination is a risk factor for surgical site infections (SSIs). This prospective, randomized, blinded, controlled trial (Reg. No. BB08/12) investigated the effect of a cyanoacrylate-based skin sealant (IntegüSeal) on intraoperative wound contamination during trauma surgery.

Methods: A total of 128 patients undergoing trauma surgery were assigned randomly to an intervention (n = 62) or a control group (n = 66). Surgical sites were investigated at three locations: maximum incision depth (base), wound margin prior to wound closure (margin), and the surgical sutures (suture). Colony-forming units (CFU) were counted after 48 h of incubation.

Results: Overall, significantly lower CFU counts were obtained for samples from the intervention group at all three sample sites compared to the control group. The difference, however, was only significant for the suture site (p = 0.040).

Conclusions: Preoperative sealing reduced microbial contamination on sutures during surgery, while the overall wound contamination remained unchanged. Hence, prevention of the clinically more relevant deep SSIs may not be expected. However, this study was not designed to detect differences in the rate of SSI. The role of the reduction in suture contamination with regard to the prevention of SSI remains to be evaluated.

1. Introduction

Most surgical site infections (SSIs) may arise endogenously, with the skin and nasal flora providing a source of infection.[1–3] In a study of 40 healthy participants, the median numbers of bacteria yielded from skin swabs following preoperative skin antisepsis with 70% v/v propan-2-ol applied for 1 min to skin with a low concentration of sebaceous glands, or for 10 min to skin with a high concentration of sebaceous glands, were 1.3 log and 3.4 log, respectively.[4] Hence, even after meticulous skin antisepsis, the resident skin flora will prevail at the incision site; this can then be transferred intraoperatively into the surgical site. In addition, standard skin antisepsis will have no effect on bacteria in the lower skin layers such as the border between the epidermis and dermis, or areas with high numbers of bacteria inhabiting the skin appendages, such as hair follicles[5] and sebaceous glands.

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Since preoperative skin antiseptics are not able to completely eradicate the resident skin flora and do not reach the deep skin layer, they do not prevent re-colonization of bacteria inhabiting the deeper skin areas and glands. Therefore, the patient and most of the skin around the incision site are covered with sterile drapes after the preoperative application of an antiseptic. However, this form of covering does not completely prevent the incision site from subsequent contamination with bacteria residing in the vicinity, as the gap between the drape and the surgical site may still allow bacterial contamination.

One preventive strategy has been the use of incision drapes placed directly onto the incision site, tightly sealing the surrounding skin area from the surgical site. Because of the formation of moisture and accumulation of sweat beneath the incision drapes, bodily fluids with a high concentration of bacteria may be spread into the surgical site, particularly after removal of the drape for the final skin closure. Indeed, the subsequent introduction of non-antimicrobial incision drapes has been demonstrated to be associated with an increase in the rate of SSIs.[6] Therefore, antimicrobial incision drapes impregnated with either povidone–iodine or chlorhexidine were developed to kill off emerging skin organisms and to decrease SSI rates. However, the results of meta-analyses have yielded inconclusive findings.[7]

A modification of the above strategy is the application of skin sealants by direct application of a liquid cyanoacrylate-based adhesive (IntegueSeal; Kimberly Clark Health Care, Atlanta, GA, USA) with the intention of blocking skin pores during the entire surgical procedure. The sealant polymerizes and hardens within 4 min to form a coating that adheres completely to the skin. By doing this, bacterial release will be blocked and endogenous contamination of the surgical site will be prevented.[8–11]

The clinical evidence for the prophylactic use of cyanoacrylate-based sealants to prevent SSI is currently controversial. However, the barrier effect of the sealant has so far been studied mostly by measuring bacterial numbers at one location of the surgical site, or by comparing SSI rates in intervention and control groups. To our knowledge, the exact anatomical location at which the sealant may support the prevention of bacterial contamination has not been ascertained microbiologically. Therefore, the aim of this study was to measure the number of bacteria at the base of the wound, along the wound margin, and on the wound sutures in patients undergoing surgery with and without the use of a cyanoacrylate-based adhesive sealant.

2. Materials and methods

2.1. Study design

The study was designed as a prospective, blinded, controlled, randomized clinical trial.

A total of 128 patients were studied (Figure 1): group A ($n = 66$; control group, no sealant) encompassed 38 male and 28 female patients (mean age: 50.7 ± 18.8 years; range: 18–85 years) and group B ($n = 62$; intervention group, IntegueSeal®) encompassed 29 male and 33 female patients (mean age: 53.6 ± 20.4 years; rang: 18–89 years). In group A, 63 suture samples and in group B, 56 suture samples were included.

2.2. Patient management

Patients were included if they were scheduled for spinal surgery (skin with a high density of sebaceous glands) or surgery to the lower extremities (skin with a low density of sebaceous glands). Patients were excluded if they had infected wounds, AIDS, a hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, or if they were known drug users.

The study was designed as a blinded controlled observational study and was approved by the ethics committee of Ernst-Moritz-Arndt University Greifswald (Reg. No. BB 08/12). After assessing eligibility, patients were randomized to one of the two study arms by opening a sealed envelope, which contained the randomization code. Randomization was performed by use of a pre-set computer-generated allocation table.

All patients received perioperative antibiotic prophylaxis using a single shot of 1.5 g cefuroxime intravenous, administered 10–30 min prior to skin incision. All patients provided informed consent to participate in the study.

2.3. Surgical conditions and postoperative surveillance

Surgery was always carried out in the same surgical unit with a laminar airflow ventilation system (ceiling area 3.20×2.40 m) and disinfection of the floor and contact surfaces close to the patient between each operation. The single-use drapes and protective clothing worn by the surgical team were high performance quality (3 M GmbH, Neuss, Germany). Skin antisepsis was performed using a propan-2-ol (70% v/v)-based product (Antiseptica GmbH, Pulheim, Germany); the exposure time was 1 min on skin that had a low density of sebaceous glands (surgery of the lower extremities) and 3 min on skin with a high density of sebaceous glands (spinal surgery).[4] All operative procedures were performed by the same surgeon who has more than 20 years of experience.

Following skin antisepsis, patients allocated to group A (controls) were covered with sterile surgical drapes prior to incision. For group B (intervention) patients, a cyanoacrylate-based sealant (IntegueSeal) was applied after skin antisepsis and before application of a sterile surgical drape. The sealant was applied to the incision site as per the manufacturer's recommendations, using an integrated applicator with a width of 4 cm. The sealant was allowed to dry for 4 min, thereby transforming into a flexible film. In group A, the incision was not made until 4 min after antisepsis. Therefore, the duration of skin antisepsis and the allowed application time was identical in the two groups. The mean operation time for spinal surgery in both groups was 59 ± 28 min, and for surgery of the lower extremities was 74 ± 40 min.

After the procedure, patients were followed-up for up to 3 months to record the development or absence of SSI. Since the frequency of SSI was not the primary study measure, the assessment of SSI (A1–A3) followed a modification of the US Centers for Disease Control and Prevention (CDC) definitions[12] in terms of duration of the surveillance, yet was always observed by the attending surgeon.

2.4. Microbiological sampling

Three intraoperative swabs were taken from each surgical site in both groups by the same trained investigator, the surgeon performing the operation. After reaching the maximum incision depth, a swab was taken from the base of the wound. A second swab was then obtained from the internal upper dermal/epidermal margin of the wound, directly before wound closure using subcutaneous and intra-cutaneous sutures. After closure of the skin incision, a final swab was obtained across the entire length of the closed surgical site.

2.5. Microbiological investigation

After sampling, each swab, which had a polystyrene shaft and pure viscose tip (BBL CultureSwab; Becton Dickinson, Heidelberg,

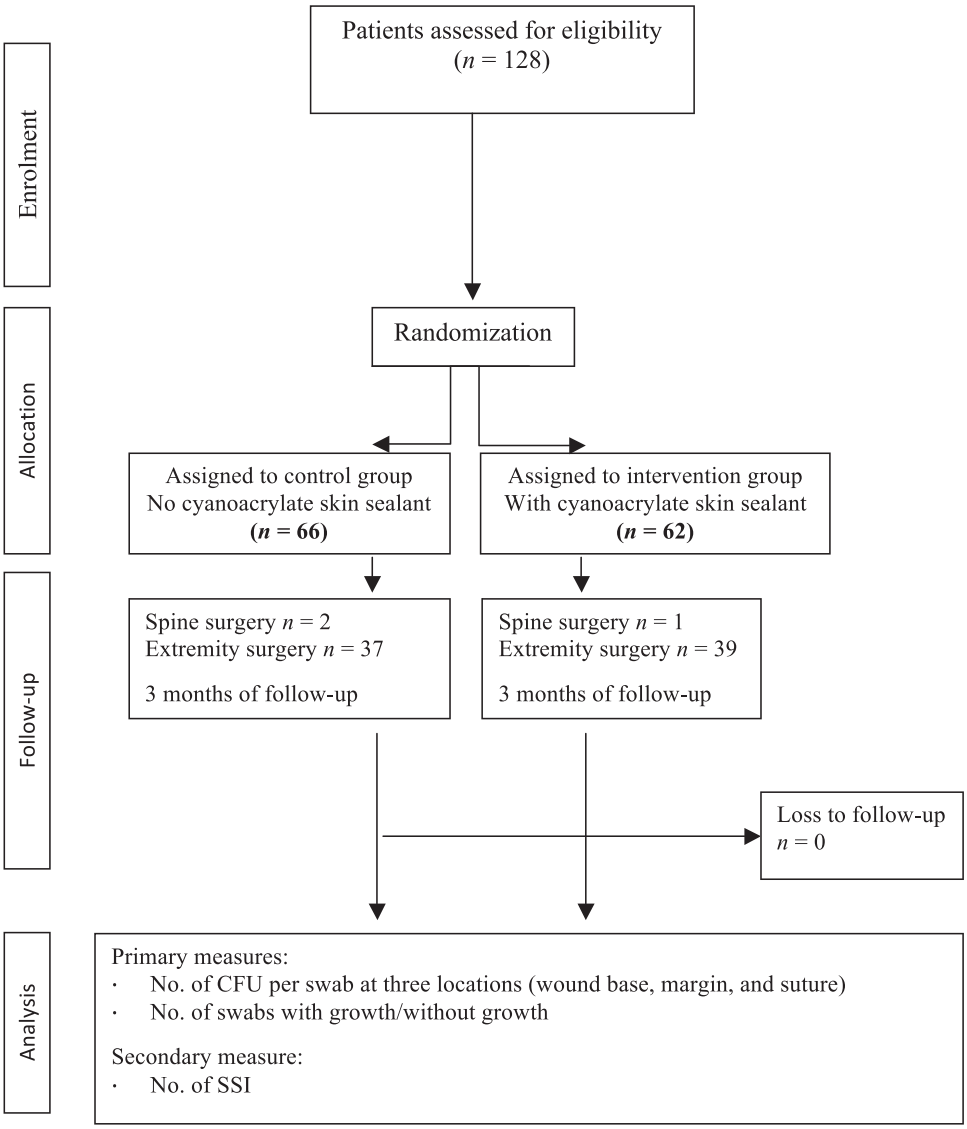

Figure 1. Enrolment, randomization, and follow-up.

Germany), was placed directly into a sterile tube containing Amies transport medium.[13] Thereafter, swabs were immediately cooled and brought to the laboratory. Each swab was vortexed for 30 s in 5 ml sterile sodium chloride solution (0.9%) and then sterile filtered. The filters were applied to Columbia agar (5% sheep blood; Oxoid, Wedel, Germany) and incubated for 48 h at 36 °C, and then the colonies were counted and differentiated (Vitek Systems; bioMérieux Deutschland, Nürtingen, Germany). The extracted swabs were placed in 5 ml thioglycolate nutrient broth and incubated for 7 days at 36 °C. The nutrient broth culture was further cultured on solid medium aerobically and anaerobically if there was visible clouding. Further aerobic and anaerobic subcultures were taken to provide evidence of strictly anaerobic bacteria, and anaerobically growing pathogens were differentiated biochemically. All tests were carried out in accordance with validated standard methods.[13]

2.6. Statistical analysis

Continuous numbers of colony forming units (CFU) at the three wound sites in both groups were compared using a one-tailed *t*-test ($\alpha = 0.1$, respectively 0.5). The categorical variable 'growth' or 'no growth' for the number of samples showing bacterial growth or no growth in both groups were analysed by cross-tabulation and application of the Fisher's exact test (hyper-geometric distribution). It was calculated that a total number of 165 independent microbiological samples per study arm was necessary to achieve 80% power to detect an absolute 10% difference between the intervention and the control group in the proportion of qualitative reduction in representative skin flora, at a significance level of 0.05 using a two-sided *Z*-test with continuity correction. Assuming that on average any patient had three independent representative microbiological samples from different anatomical locations, the number of patients per treatment group was approximately $n = 55$. Allowing for a 10% dropout rate, this study aimed to enrol a minimum of 60 patients per study arm.

3. Results

Independent of the sampling site, 69% of all samples (134/193 samples) obtained from the control group (group A) and 80% of all samples (141/176 samples) obtained from the intervention group (group B) yielded no bacterial growth (Table 1). The overall difference between the two groups was statistically significant ($p = 0.023$; Fisher's exact test).

Table 1
Proportion (%) of samples with no bacterial growth

Group	Wound base *n*/*n* total (%)	Wound margin *n*/*n* total (%)	Closed wound, suture *n*/*n* total (%)	All samples *n*/*n* total (%)
A Without sealant	49/65 (75.4)	44/65 (67.7)	41/63 (65.1)	134/193 (69.4)
B With sealant	50/60 (83.3)	48/60 (80.0)	43/56 (76.8)	141/176 (80.1)

Lower bacterial contaminations were retrieved from all three sample sites in the intervention group (Figure 2). This difference was only significant for the suture site ($p = 0.040$), and did not reach statistical significance for the wound base ($p = 0.089$) or the wound margin ($p = 0.057$).

The bacterial species identified in both groups are typical representatives of the physiological skin flora and can also be found in the patient's environment. No marked differences in species pattern distribution in the two groups were found (data not shown). The following Gram-positive species were identified in both study groups: *Staphylococcus aureus*, anaerobic and aerobic spore-forming bacilli, *Micrococcus spp*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Streptococcus spp*, and alpha-haemolytic streptococci. Samples obtained from group B patients showed a slightly broader diversity with additional yield of *Corynebacterium spp*, *Kocuria rosea*, *Kocuria varians*, *Staphylococcus lugdunensis*, *Staphylococcus auricularis*, *Staphylococcus simulans*, *Staphylococcus cohnii*, *Staphylococcus warneri*, *Staphylococcus capitis*, and *Leuconostoc spp*. The only Gram-negative species identified was *Morganella morganii*, in one sample obtained from a patient in group B.

No SSI occurred in either group during up to 3 months of follow-up.

4. Discussion

Skin antisepsis is one of the important measures within the multi-barrier strategy for the prevention of SSI.[8,14] However, despite the development of highly effective alcohol-based skin antiseptics, the resident skin flora cannot be fully eliminated.[4] The risk of developing SSI depends on the bacterial count in the wound at the end of the operation.[15,16] Since an infection at a primary sterile site will develop only after microbial contamination, multiple studies have investigated bacterial contamination of a wound site as a surrogate for SSI.

A new and potentially promising approach to solve the problem of endogenous bacterial contamination is the use of skin-sealing technology to immobilize the surviving skin flora following preoperative skin antisepsis. The feasibility of such microbial sealants was first evaluated by Wilson et al. in a skin incision model where the sealant was able to demonstrate reducing the recovery of *Acinetobacter baumannii* by $10^{1.5}$-fold.[17] Using a similar model, recovery of methicillin-resistant *Staphylococcus aureus* (MRSA) was reduced by 99.9%, recovery of *S. epidermidis* by 99.5%, and recovery of *Escherichia coli* by 96.6%, respectively.[9] Furthermore, this study also compared the efficacy of the sealant against an antimicrobial incision drape. After skin antisepsis with 10% povidone–iodine, either a microbial sealant or an antimicrobial drape was applied to the skin of pigs. Wounds were sampled for bacterial contamination immediately after incision and before wound closure. The sealant resulted in a six-fold reduction in wound contamination compared with the antimicrobial drape before wound closure.[9]

These experimental findings were later supported by case-controlled studies in humans published by Dohmen et al.[8,10] Towfigh and colleagues[18] demonstrated significantly reduced bacterial contamination of wounds in elective inguinal hernia repair by use of antimicrobial sealant in a prospective, randomized, multi-centre clinical trial. However, because of the sample size, the authors were not able to demonstrate a significant reduction in SSI as well. An early review of the literature in 2010 found conflicting evidence in support of the use of cyanoacrylate skin sealants and supported its omission as part of preoperative skin preparation; further work to support preventive aspects of the sealants was recommended.[19,20] Accordingly, several clinical studies have been conducted, again with mixed results. Waldow et al.[21] conducted a large randomized controlled trial (RCT) in almost 1000 patients and reported no effect on the prevention of SSI within 30 days after cardiac surgery. The observed frequency of SSI in the intervention group was 2.3% and in the control group was 3.2%, with no statistically significant difference. Dromzee et al.[22] also conducted an RCT and found no effect of the cyanoacrylate skin sealant in spinal surgery. This RCT, however, included only 56 patients, and it

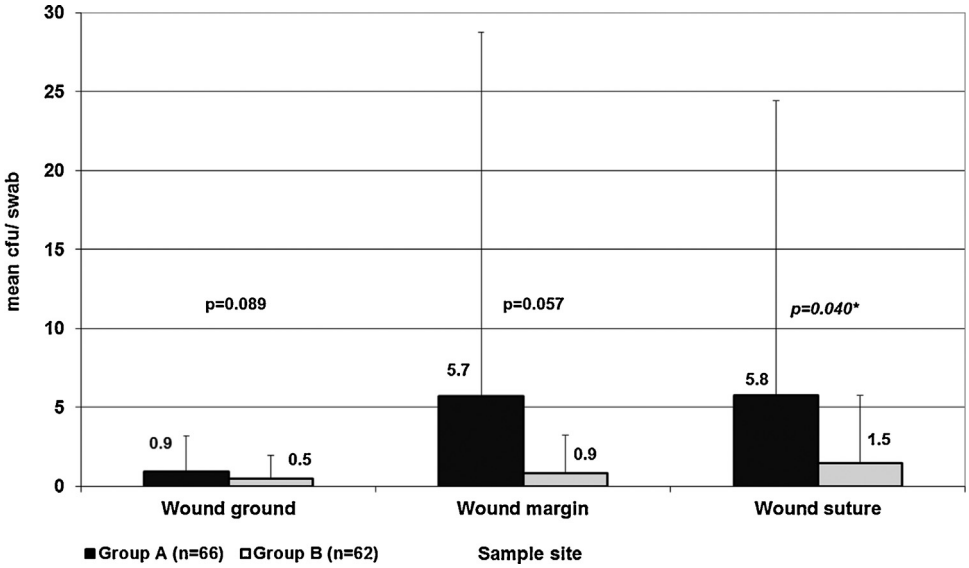

Figure 2. Intraoperative bacterial counts (mean ± standard deviation CFU/swab) at the wound base, margin, and the closed wound, in the groups without (group A) and with (group B) cyanoacrylate skin sealant.

was highlighted by the authors themselves that the study was underpowered to detect a difference if there had been one. Furthermore, in the control group, incision drapes were used, and in the intervention group an incision drape was placed over the cyanoacrylate sealant, making it difficult to assess the performance of the sealant alone.

In contrast to these negative results of RCTs, other authors have found statistically significant reductions in SSI by using cyanoacrylate skin sealants as part of preoperative skin preparation. A large multi-centre RCT studying 300 elective coronary artery bypass graft (CABG) procedures observed 6.2% SSI in the skin sealant group versus 9.5% in the control group. Furthermore, an 83.3% relative risk reduction for SSI was observed in the subgroup of obese patients.[15] A small, but well-designed RCT investigating the effect of skin sealant on the prevention of SSI was conducted in 47 patients undergoing CABG surgery, serving as both an intervention and control group. The SSI rate at the leg harvest sites was 2.1% in the intervention group versus 25.5% in the control group ($p = 0.001$).[23] In a retrospective, non-randomized study including a total of 580 CABG patients,[11] the SSI rate in the intervention group was 2.3% versus 6.8% in the control group ($p = 0.011$). Later, Dohmen et al. confirmed these findings in a prospective study among 910 cardiac surgery patients, reporting 1.1% SSI in the intervention group and 4.8% in a prospective control group ($p < 0.025$).[24]

Investigating studies with comparable surgical procedures, at present three out of five RCTs have demonstrated a significant clinical benefit in terms of SSI prevention. One of the explanations for this disparity may be that the cyanoacrylate skin sealant is only one measure among a bundle of preventive measures used, and hence RCTs performed by different study groups in varying settings should have controlled for all other, equally or more important factors. This, however, has not always been the case. Analysing, for example, the protocols for preoperative skin antisepsis of such studies, a broad variation can be seen. Two studies did not state the modalities used for skin antisepsis at all.[6,23] One RCT used povidone–iodine alone;[15] another RCT used only an alcohol-based skin antiseptic.[21] Even the same study group used different antiseptics, povidone–iodine[10] or povidone–iodine + 70% alcohol.[11] Therefore, future studies should take into account the influence of the skin antiseptic used and its composition.

The intention of this study was not to explore the ability of the sealant to prevent SSI but to identify the potential reduction in the contaminating flora in the wound sites during the operation in order to draw conclusions regarding potential infection control measures to be taken against surviving or newly appearing contaminating flora in the localized wound area. Therefore, the results of this study do not allow any conclusions to be drawn on the possible effects on SSI.

As a result, only a weak overall reduction in wound contamination in the intervention group could be demonstrated, supporting studies showing a low infection prevention outcome. Further, the only significant action conferred by the sealant was the reduction in surface contamination in the suture area. Regarding the importance of defined contaminations, this flora results in clinically less important healing difficulties (superficial low grade wound infections, A1, compared with deep contaminations leading to A2 and A3 infections). However, these more superficial contaminations may lead to relevant pathogens spreading in the hospital environment (mostly during wound dressing changes or related manoeuvres in the ward). This supports the relevance to infection control of the sealant in the prevention of transmission and superficial wound healing deterioration (A1), but lacks further clinical evaluation. Finally, deeper wound areas (wound base and margin) did not benefit from the sealant; therefore the results do not support the potential for direct infection prevention in patients undergoing surgery.

In conclusion, the application of a cyanoacrylate skin sealant may have some beneficial impact on the prevention of superficial SSIs (A1) and on pathogen transmission in hospitals, but clinically more important infections may not be prevented. However, this study was not designed to detect differences in the rate of SSI. The role of the reduction in suture contamination in the prevention of SSI remains to be evaluated.

Conflict of interest: This study was financed through the routine research grant of the Institute for Hygiene and Environmental Medicine, University Medicine Greifswald, Germany. The authors have no competing interests to report.

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