

Basic evaluation of an antimicrobial gel for peri-implantitis treatment

Authors: Dr Georg Bach & Christian Müller, Germany

Introduction

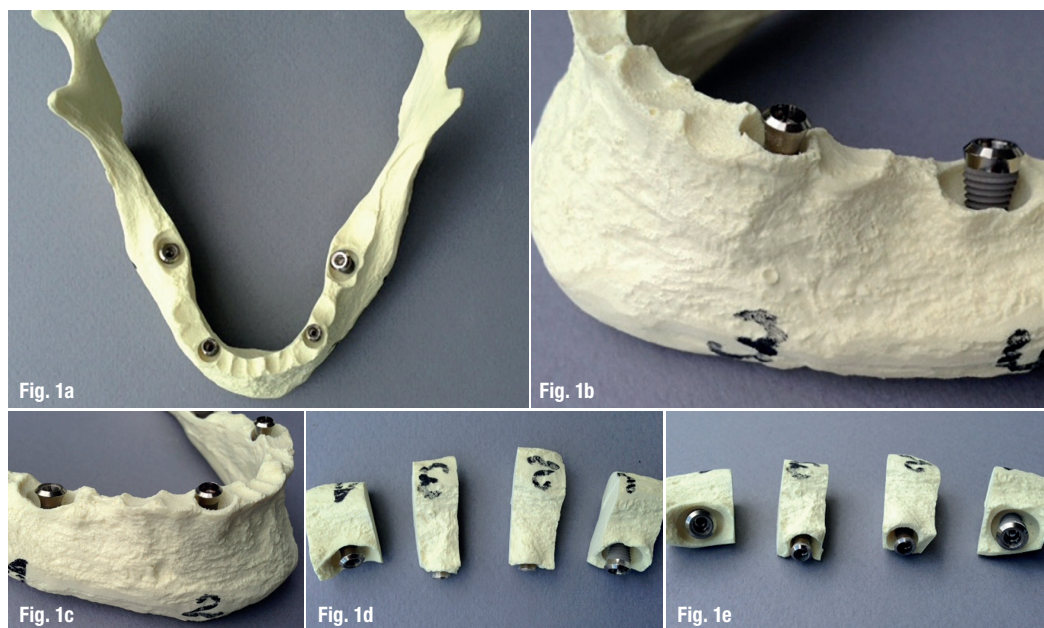
Early complications, which have been regarded as the major dread in the initial phase of oral implantology, have become a rare phenomenon for a fairly long time. Reasons for this positive development can be found in significant improvements of the implant surfaces, improved insertion techniques as well as in new ways to improve the prospective implant site.

Nevertheless, with the enormously increased number of inserted implants, a significant increase of late complications has meanwhile been recorded.^{1,4,12} These complications typically manifest themselves many years after installation of the superstructure by means of peri-implant bone loss around artificial tooth pillars.^{17,20,21,25} Often

associated with an insufficient or declining oral hygiene of the patient, these peri-implant lesions lead to the loss of the artificial tooth pillar and the corresponding suprastructure in case they are not treated.^{5,11,13,14} Many authors regard the development of peri-implantitis therapies as one of the current key challenges of implantology.^{15,18–20,26}

Cleaning and disinfection of the exposed implant areas represents an undeniable requirement. For the latter step the term "decontamination" has been generally established.^{3,16} For decontamination, various methods are indicated for their suitability.^{3,6,8,16,21–24} The aim of this study was to evaluate the suitability of using an antimicrobial gel for peri-implantitis treatment in an *in-vitro* experiment.

Figs. 1a–e: Peri-implant defect—
Simulated model: Crater-shaped defects were prepared in plastic jaws typically used for insertion exercises. Brand-new implants were placed in the middle of these defects in a way that at least three threads were exposed (**a–c**). The jaws were divided into smaller units (**d & e**) and autoclaved before conducting phase II examinations (bacterial cultivation—Perisolv application—Microbiological diagnostics etc.) in order to allow better fit into the furnace as well as in vials containing culture medium.



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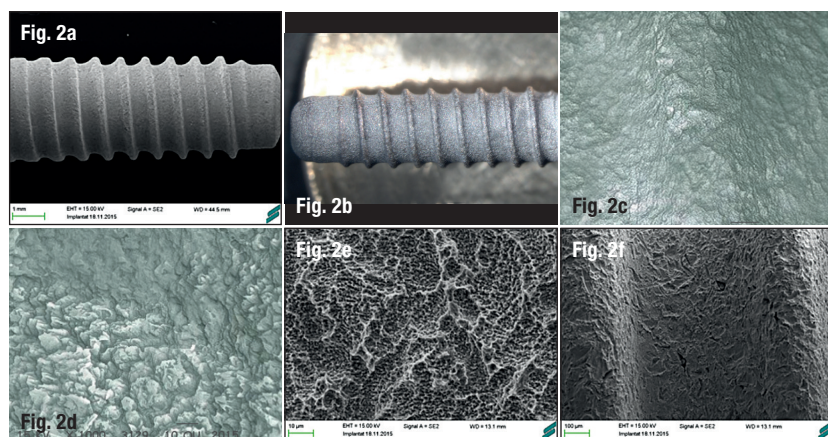
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Figs. 2a–f: SEM analysis: Brand-new, sterile implants were inoculated and incubated with a microbial suspension. **Figure 2a** shows a scanning electron micrograph of this starting material. **Figure 2b** shows the bacterial turf on an implant thus processed. After Perisolv application, many areas showed a detached bacterial coating, the implant surface is virtually free from bacterial turf (**c & d**). These “exposed spots” feature an unchanged implant structure (**e & f**), therefore Perisolv application does not alter the implant surface per se.

Material and Methods

Two test phases were performed:

a) Phase I: Decontamination procedure of brand-new sterile implants, which have been inoculated with bacteria and subsequently coated with antimicrobial gel.

b) Phase II: Decontamination procedure of brand-new sterile implants placed in a plastic jaw with simulated bone defects after subsequent inoculation with bacteria and final exposure to antimicrobial gel.

Phase I: Decontamination procedure to implants inoculated with bacteria

To evaluate general suitability of the decontamination process, brand-new ITI implants (Institut Straumann AG, Basel, Switzerland) were microbiologically processed and analysed at the Institute for Medical Diagnostics Bioscientia (Freiburg, Germany).

Implant contamination—microbial procedure:

The implants were exposed and inoculated with a bacterial suspension (overnight cultures of *MRSA* ATCC 33591):

By means of sterile forceps, the implants were placed in 10 ml peptone yeast extract broth each. The tubes were incubated for 48 h at 36 °C and 5–10 % CO₂. After 48 h of incubation, the liquid was removed by means of vacuum filtration and the implant was transferred back to the initial container with sterile forceps for immediate further process-

ing. Exclusively, implants with a medium bacterial growth were used for further examinations, implants with low or very low bacterial growth were excluded. Two test series were conducted with four implants each.

Decontamination procedure with contaminated whole implant bodies:

After completion of the microbiological work, three out of four implants were confronted with antimicrobial gel for two min in the sense of a decontamination procedure and immediately transferred to the Institute for microbiological analysis. One implant served as positive control, without conduction of the decontamination procedure.

- Antimicrobial Gel: An antimicrobial gel known for its application in periodontology was used (PERISOLV, REGEDENT AG, Zurich, Switzerland). It is typically used for adjuvant cleaning and decontamination of the outer tooth root area and the surrounding tissue.¹⁰ Furthermore, in the literature the gel is described to feature a softening effect towards degenerative tissue before debridement of periodontal pockets.⁹ According to the manufacturer, the gel does not affect healthy tissue⁹ and, however, features an antimicrobial effect.^{2,7}
- Gel composition: The gel contains amino acids (glutamic acid, leucine and lysine), carboxymethyl cellulose, titanium dioxide as well as ultra pure water and features a pH value below 10. The transparent liquid represents a 0.95 % sodium hypochlorite solution and is admixed immediately before the application. After mixing hypochlorite and amino acids, so-called Chloramines (NCA), a short-lived active substance class, are formed. These substances are part of the body's own immune system.⁹
- Gel Preparation: The set (gel and liquid) is stored in the refrigerator. One hour prior to planned application, the set is removed from the refrigerator to allow the contents of the kit to warm up to room temperature. Both components (gel and liquid) are arranged in separate syringes and are connected by means of screwing (Luer-lock connection). Both components were thoroughly mixed by moving the stamps back and forth 10–15 times. The activated and operational gel was finally left in the transparent syringe. A non-invasive/blunt application tip is attached and the implants are coated with the gel.

Table 1: Results of Phase I.

Bacterial growth on implant		Implant 1	Implant 2	Implant 3	Implant 4 control
A:	MRSA	–	–	–	+++
B:	MRSA	–	+	–	+++

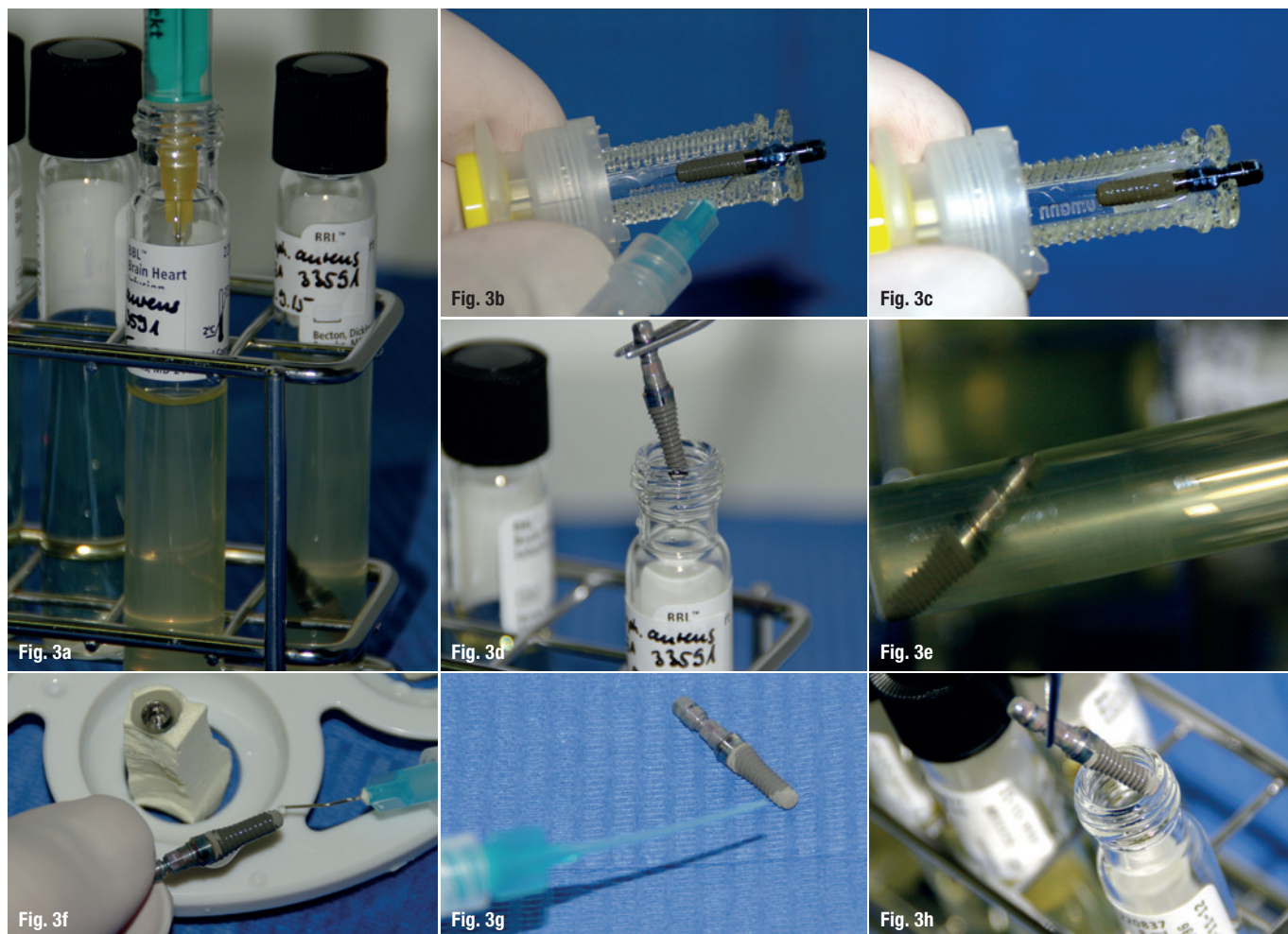


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Figs. 3a–h: Phase I: Brand-new, sterile implants were used for the study. Implants supposed for SEM evaluation were initially kept in their original containers. The MRSA bacterial suspension was drawn in a sterile, disposable syringe (**a**) and applied directly on the respective implant in its original container (**b & c**).

Subsequently, the shipment for immediate SEM analysis was carried out. Implants supposed for microbiological testing were removed from their containers and placed directly into the MRSA bacterial suspension (**d & e**). After a one-minute inoculation period, the implants were removed and coated with Perisolv gel (**f & g**). After the exposure time specified by the manufacturer, the implants were introduced into the tube containing the nutrient medium and sent to the microbiological examination (**h**).

Implant preparation for microbial investigations

Immediately after application of the gel, the implants were introduced into tubes with a sterile nutrient solution and sent to the Institute for microbiological analysis. The samples were processed in the Microbiological Institute by means of conventional (plate) cultivation.

Scanning electron microscopic studies of the implants

Some of the implants were investigated by scanning electron microscopy (Institut Straumann AG).

Results of Phase I—Decontamination procedure with contaminated whole implant bodies (Tab. 1)

Scanning electron microscopic studies

In some areas, where Perisolv had been applied, the "bacterial turf" on the implants was interrupted or rather dissolved/removed. Underlying areas, freed from bacterial turf, displayed an intact, unaltered implant structure. For implants only confronted with Perisolv without previous inoculation, no gel-induced change of the implant surface were observed.

In summary, SEM analysis after treatment with the gel revealed no change of implant surface as and a partial dissolution of the inoculated bacterial layer.

Microbiology

Phase I investigations revealed bacterial inactivation in the highest degree, remaining MRSA bacteria were detected in one test item of series B1 only.

Summary of Phase I—Decontamination procedure with contaminated whole implant bodies

The investigated gel is capable to induce a pronounced destruction of pathological bacteria present on implant surfaces without altering this implant surface structure.

Phase II: Testing the effect of the antimicrobial gel on contaminated implants placed in a plastic jaw with a simulated peri-implant tissue defect

After the first test phase to evaluate the principle suitability of the gel application, a second test phase was conducted.

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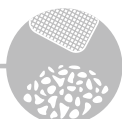
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Table 2: Results of Phase II.

	Bacterial DNA in simulated bone defect	Unit 1	Unit 2	Unit 3	Unit 4 Control
A:	MRSA	++	+	++	+++
B:	MRSA	–	++	+	+++

Preparation of simulated peri-implant defects

Implants (Institut Straumann AG) were placed in a plastic jaw, which was prepared with standardised crater-shaped (peri-implant) defects prior to implant placement. The implants were placed in the centre of these defects by means of allowing the upper three threads not to be sunk into the plastic. Thus, a defect situation simulating a typical manifested peri-implantitis was generated. For better further processing, the jaws were sawed into small implant/plastic jaw units. These implant/plastic jaw units were steam sterilised (autoclaved).

Implant contamination

Afterwards, the exposed implant surfaces were contaminated with a bacterial suspension. The circumferential defects were completely filled with the bacterial suspension as well. Two test series were conducted with four implant/plastic jaw units each.

Microbiological procedure:

The bacterial suspension (*MRSA ATCC 33591*–ATCP strain) was prepared and suspended in BHI broth. The bacterial count of this “stock suspension” represented approx. 10^8 – 10^9 bacteria/mL. To inoculate the implant/plastic jaw units, each 100 µl of the cultured MRSA stock suspension were pipetted into one simulated bone defect. This corresponds to approx. 10^7 – 10^8 bacteria/100 µl respectively.

Decontamination procedure with simulated peri-implant defects

Perisolv gel was administered into three of four simulated bone defects (details s. Chapter “Phase I”). The gel was allowed to operate for two minutes. One

implant/plastic jaw unit served as a positive control, where no decontamination was performed.

Implant preparation for microbial investigations

The units were subsequently placed into 10 mL of BHI broth (Brain Heart Infusion Glucose), each by means of a sterile forceps. The implant/plastic jaw units were placed in a culture oven. To establish a humid environment, a small Erlenmeyer flask filled with sterile distilled water was added into the pot. The units were incubated under aerobic conditions at 36 °C.

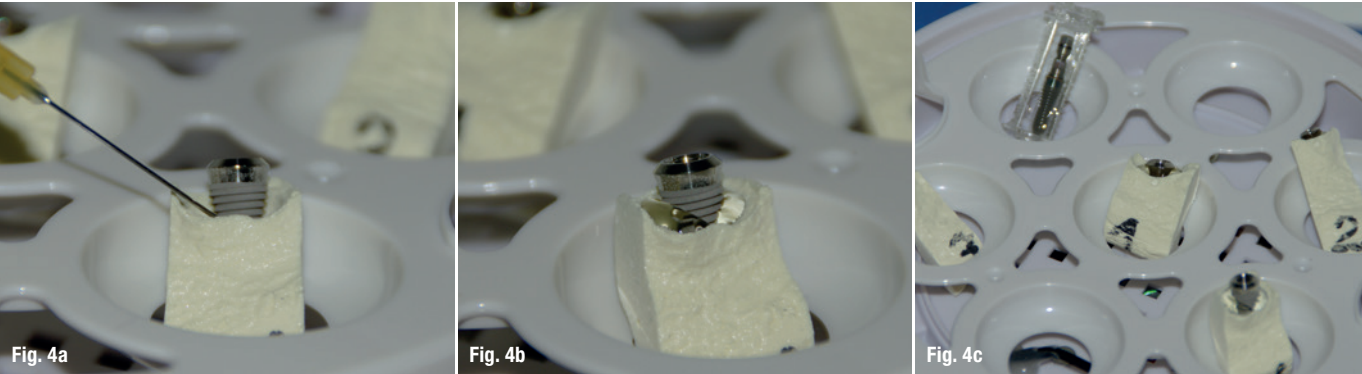
After two days of incubation, the simulated bone defect of unit 1 was dry, whereas bone defects of units 2–6 were still slightly humid. The remaining liquid from these units was removed by means of a pipet.

The implant/plastic jaw units were introduced in tubes with a sterile nutrient solution and forwarded to the Institute Bioscentia for microbiological analysis. The samples were processed by means of conventional (plate) cultivation.

Results of Phase II (table 2)

Remaining MRSA bacteria were detected in five of six decontaminated implant/plastic jaw units as well as in the control unit. This finding can be categorised as “significant” in three out of five units and as “distinct” in the other two out of five units. In addition, a bacillus species was detected in one unit. This can be regarded as an environmental contaminant.

Figs. 4a–i: Phase II: Brand-new, sterile implants were placed in simulated bone defects in a plastic jaw. These implant/plastic jaw units were autoclaved. Afterwards, a MRSA solution was introduced into the simulated peri-implantitis defects (a–c). Afterwards, the units were incubated in a special oven and a proof for the presence of “massive” MRSA bacteria was performed. At this time, the Perisolv gel was applied (d–f). After the exposure time specified by the manufacturer, the samples were placed directly into a BHI broth (g & h) and the samples were passed for further microbiological examination (i).





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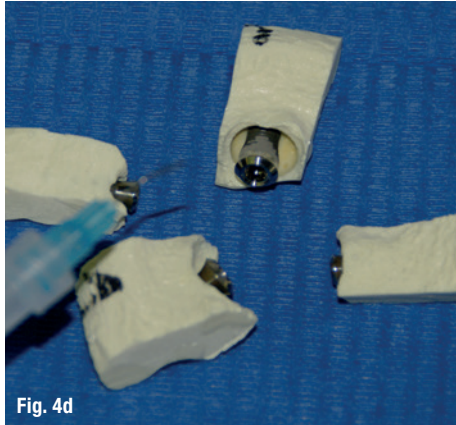


Fig. 4d

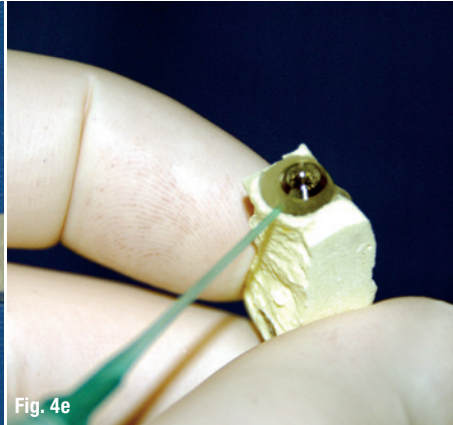


Fig. 4e

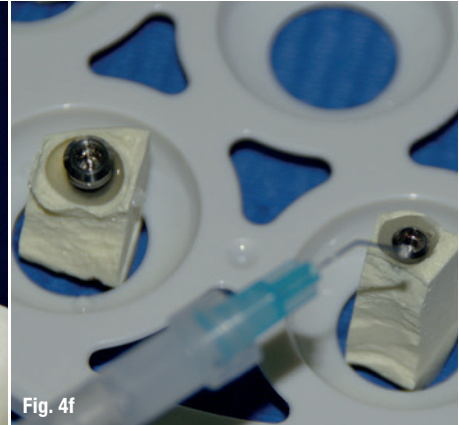


Fig. 4f



Fig. 4g



Fig. 4h

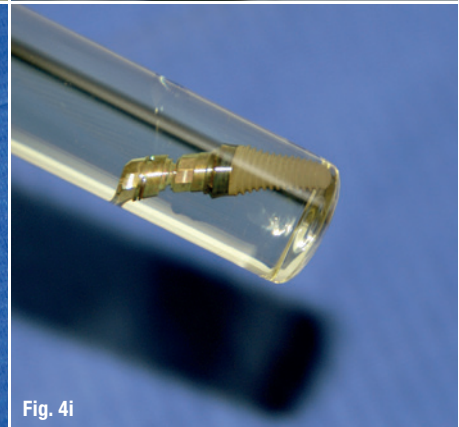


Fig. 4i

Breeding trial after decontamination

It was possible to recultivate bacteria sporadically after decontamination and simple drying.

Preliminary Summary

Compared to other decontamination procedures, the application of the antimicrobial gel Perisolv achieved satisfactory decontamination results from a microbiological point of view in both *in-vitro* study phases. In all samples, a significant reduction of the bacterial count was observed. However, a bacterial elimination only was achieved in the first study phase, but not in the second phase.

SEM images of the implants that have undergone the procedure described above, pointed out that the antimicrobial gel did not induce any changes to the implant surface and that it has certain potency for dissolving the (inoculated) bacterial turf.

As a limitation to the evaluated results, it should be clearly stated that the presented investigation was performed in an *in-vitro* environment with a "non-human milieu" and without a real inflammatory component. Thus, our results about the basic applicability of the presented method can be regarded as a first approach, but in no case a clear statement about the definitive decontamination efficacy of the tested methods can be done.

Acknowledgement

In particular, we want to thank Dr Brodner (Institute Bioscientia, Freiburg, Germany) and Institut Straumann AG (Basel, Switzerland) for their valuable support in the microbiological testing phase and in the preparation of scanning electron images. We want to thank Straumann Germany GmbH for providing the plastic jaws and the implants. Without their elaborate and valuable work, this study would not have been possible.

Contact

Dr Georg Bach

Rathausgasse 36
79098 Freiburg im Breisgau
Germany
doc.bach@t-online.de
www.herrmann-bach.de

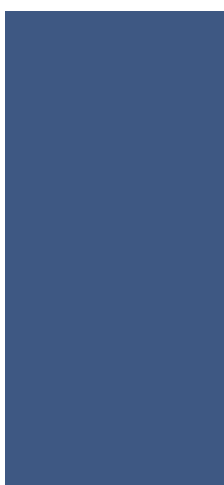


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