Original Article

Biofilm on patient-ready orthopaedic screws acquired through the loaner system

Biofilme em parafusos ortopédicos prontos para uso adquiridos por meio de sistema de consiganação/comodato

Biopelícula en tornillos ortopédicos listos para uso adquiridos por medio del sistema de consignación/comodato

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Abstract

Objective: Assess the surface integrity and microbiological conditions of patient-ready screws in orthopaedic trays that had been multiply reprocessed.

Methods: After full reprocessing, clinical trays used for small fragment surgery provided through a loaner system to a Brazilian hospital were randomly selected during four months. The most (numbers 14, 16 and 18 – Group 1) and least (numbers 10 and 38 – Group 2) frequently implanted screws, therefore, the ones estimated to be the most and least exposed to biological, chemical and physical agents, were randomly removed and subjected to visual inspection (n=126), followed by bacterial culture (n=6 screws/tray, 9 trays), protein test (n=6 screws/tray, 9 trays) and Scanning Electron Microscopy (SEM) (n=2 screws/tray, 9 trays). Positive cultures were subjected to automated bacterial identification and antimicrobial susceptibility tests.

Results: Grooves were detected on 8.7% screws, predominantly in Group 2 (8/11). Residual protein was detected on 96,3%, and there was no statistically significant difference in the amount of protein between the groups (P=0.07). Bacterial growth was identified in 3/54 screws. Surface damage and soil were visualized on all screws subjected to SEM. Extensive biofilms were detected on eight screws, three from Group 1 and five from Group 2.

Conclusion: Recovery of bacteria, biofilm accumulation and surface damage were detected on patient-ready screws. Screws frequently remain in surgical trays for multiple reprocessing; thus they are repeatedly exposed to contamination and damage. These findings point to the need to discuss and review the way these single-use implants are currently made available for surgeries.

Resumo

Objetivo: Avaliar a integridade da superfície e as condições microbiológicas de parafusos prontos para uso em bandejas ortopédicas após múltiplos processamentos.

Métodos: Após o processamento completo, as bandejas utilizadas em cirurgias de pequenos fragmentos, fornecidas por meio de sistema de consignação/comodato em um hospital brasileiro, foram selecionadas aleatoriamente durante quatro meses. Os parafusos mais utilizados (números 14, 16 e 18 – Grupo 1) e menos utilizados (números 10 e 38 – Grupo 2), portanto, os mais e menos expostos a agentes biológicos, químicos e físicos, foram aleatoriamente removidos e submetidos a inspeção visual (n=126), seguido de cultura bacteriana (n=6 parafusos/bandeja, 9 bandejas), teste de proteínas (n=6 parafusos/bandeja, 9 bandejas) e Microscopia Eletrônica de Varredura (MEV) (n=2 parafusos/bandeja, 9 bandejas). As culturas positivas foram submetidas a métodos automatizados de identificação bacteriana e suscetibilidade antimicrobiana.

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Conflicts to interest: none to declare.

Resultados: Foram detectadas ranhuras em 8,7% dos parafusos, predominantemente no Grupo 2 (8/11). Proteína residual foi detectada em 96,3%, e não houve diferença estatisticamente significativa na quantidade de proteína entre os grupos (P=0,07). Crescimento bacteriano foi identificado em 3/54 parafusos. Danos na superfície e presença de sujidade foram visualizados em todos os parafusos submetidos a MEV. Formação de biofilmes extensos foi detectada em oito parafusos, três do Grupo 1 e cinco do Grupo 2.

Conclusão: Recuperação de bactérias viáveis, acúmulo de biofilme e danos na superfície foram detectados nos parafusos prontos para uso. Os parafusos costumam permanecer nas bandejas cirúrgicas e serem submetidos a múltiplos processamento, sendo expostos a contaminação e danos repetidas vezes. Esses achados apontam para a necessidade de discutir e repensar a forma como esses implantes de uso único são atualmente disponibilizados para cirurgias.

Resumen

Objetivo: Evaluar la integridad de la superficie y las condiciones microbiológicas de tornillos listos para uso en bandejas ortopédicas después de múltiples procesamientos.

Métodos: Después del procesamiento completo, fueron seleccionadas aleatoriamente durante cuatro meses las bandejas utilizadas en cirugías de pequeños fragmentos, proporcionadas mediante el sistema de consignación/comodato en un hospital brasileño. Los tornillos más utilizados (números 14, 16 y 18 – Grupo 1) y menos utilizados (números 10 y 38 – Grupo 2), por lo tanto, los más y menos expuestos a agentes biológicos, químicos y físicos, fueron quitados aleatoriamente y sometidos a inspección visual (n=126), seguido de cultivo bacteriano (n=6 tornillos/bandeja, 9 bandejas), prueba de proteínas (n=6 tornillos/bandeja, 9 bandejas) y microscopía electrónica de barrido (MEB) (n=2 tornillos/bandeja, 9 bandejas). Los cultivos positivos fueron sometidos a métodos automatizados de identificación bacteriana y susceptibilidad antimicrobiana.

Resultados: Se detectaron ranuras en el 8,7 % de los tornillos, predominantemente en el Grupo 2 (8/11). Se detectó proteína residual en el 96,3 % y no se encontró diferencia estadísticamente significativa en la cantidad de proteína entre los grupos (P=0,07). En 3/54 tornillos se identificó crecimiento bacteriano. Se visualizaron daños en la superficie y presencia de suciedad en todos los tornillos sometidos a MEB. En ocho tornillos se detectó la formación de biopelículas, tres del Grupo 1 y cinco del Grupo 2.

Conclusión: Se detectó recuperación de bacterias viables, acumulación de biopelícula y daños en la superficie en los tornillos listos para uso. Los tornillos suelen permanecer en las bandejas quirúrgicas y son sometidos a múltiples procesamientos, donde están expuestos a contaminación y daños repetidas veces. Estos descubrimientos señalan la necesidad de discutir y repensar la forma como estos implantes de uso único se ponen a disposición para cirugía actualmente.

Introduction

Worldwide, reusable medical devices (RMD), especially those used in orthopaedic surgery, are sourced through loaner systems.[1] Orthopedic surgical boxes additionally include single-use implants, manufactured in stainless steel, such as plates and screws. Although classified as single-use, orthopedic plates and screws are packaged with the other surgical devices in the tray and are subject to multiple sterilization processes until they are implanted. Thus, multiple opportunities exist for exposure to physical, chemical and biological agents resulting in contamination and surface damage.[2]

The loaner system enables hospitals to access a wide variety of surgical devices in the face of rapid technological advances, but the high turnover of the surgical boxes between various healthcare facilities can hinders their proper management and reprocessing.[1-3] While the presentation of screws in racks within the surgical boxes, facilitates their use during surgery, it is not conducive to adequate reprocessing, especially the cleaning stage.[4]

One of the major problems resulting from improper reprocessing is the formation of biofilm, which consists of an aggregation of sessile cells adhered to a surface, encased in a matrix of extracellular polymeric substances (EPS).[5] Biofilms protect microorganisms against adverse conditions, such as exposure to detergents, disinfectants and sterilizers agents.[2,3,6] On RMD, biofilm develops gradually over successive rounds of exposure to fluids (patient use, precleaning, cleaning, disinfection/sterilization) and drying stages (packing and storage), and is named build-up biofilm.[6,7] This is more compact and adherent than the traditional biofilm,[6,8] which forms under constant wet conditions.[7] We aimed to assess the surface integrity and microbiological contamination of patient-ready orthopaedic surgical screws (least and most used cortical screws), provided through the loaner system.

Methods

This study was performed in the Central Sterilizing Service Department (CSSD) a public general teaching hospital (235 beds), in the Midwest region of Brazil, from August to November 2019. Loaned small fragments surgical boxes 3.5 (SFB 3.5) used

in orthopedic surgical procedures (fractures) of lower and upper limbs were evaluated. SFB 3.5 consist of surgical instruments and a tray containing cortical and cancellous screws of varying sizes ranging from number 10 to 50.

To prevent interference with hospital surgical routine, the supplying loaner company delivered an extra SFB 3.5 kit once a week. Upon delivery at the CSSD, SFB 3.5 were submitted to all processing steps (reception, cleaning, drying, integrity and functionality assessment, preparation, sterilization and storage) following the hospital protocol. The sterilization process was saturated steam under pressure (134ºC for 5 minutes - Baumer, Brazil). The routine sterilization quality control monitoring included: physical indicators for each and every cycle, Class I chemical indicators in all packages, Class II (Bowie Dick) daily, and Class V in all surgical boxes and third generation biological indicator daily. In the CSSD storage area, one of the SFB 3.5 was randomly selected (asset number registered), placed in a disinfected plastic box with a lid, and transported to the microbiological laboratory.

A total of nine SFB 3.5 were selected for the study, and 14 cortical screws were removed from each kit: seven screws sizes 14, 16 or 18 (Group 1 - the most frequently used, therefore, those that remain in the box for a shorter time and, consequently are less exposed to biological, chemical and physical agents during handling and multiple processing), and seven screws size 10 or 38 (Group 2 - less used and, therefore, more frequently submitted to the agents mentioned above).

Each SFB 3.5 has four units of cortical screws for each size, totaling 12 screws in Group 1 and eight in Group 2. Screws were randomly selected using the Random App (Mireia Lluch Ortola, Creations Apps). Selected screws were subjected to bacterial culture (n=6 screws/tray, 9 trays), quantitation of contaminating protein (n=6 screws/tray, 9 trays) and Scanning Electron Microscopy (SEM) (n=2 screws/tray, 9 trays).

All screws selected for the analytical tests were visually inspected with the aid of an eight-fold image amplification lens, with an attached light source (LED bench magnifier HL-500 8D, China).[9,10]

Protein was extracted from the screws using a modified alkaline hydrolysis.[11,12] Briefly, screws were individually immersed in 2mL (screws number 14, 16 e 18) or 4mL (screws number 38) of ice-cold 2M-Morpholino-ethane sulfonic acid (MES) (20 mM) in 0.9% saline, containing NaOH (30%), and subjected to sonication for 1 hour and vortexing for 2 minutes, prior to incubation at 30°C for 30 minutes followed by immersion in boiling water for 15 minutes. After cooling down, the extracted solution from each screw was transferred to individual plastic tubes and 32% HCl added, and then subjected to centrifugation (13,000 rpm for 5 minutes). A 1mL aliquot of the of extracted sample from each screw was used to quantify protein using 1mL mix of the Pierce® micro-BCA protein Assay test (Thermofisher, Waltham, USA), and incubated and read by spectrophotometry (Digital UV-VIS IL-593-S, Kasuaki), at a wavelength of 562nm, as per manufacturer instructions for use. The protein concentration was calculated (µg/mL), using a standard curve. The test sensitivity was 0.5µg/mL. The protein calculation per screw was: µg/mL X volume of extracted sample.

Individual screws (size 10, 14, 16, 18) were placed in 2.5mL of Tryptic Soy Broth (TSB) or 5.5mL for screw (size 38) and subjected to 10 minutes sonication (USC - 1400A, Unique, São Paulo, Brazil), and vortex for 2 minutes and then incubated at 35°C for up to 28 days.[13] A 10µL aliquot of the positive cultures were sown on Brain Heart Infusion agar, and bacteria colonies were subjected to Gram staining and cultivated on MacConkey or mannitol salt agar. Bacterial identification and antimicrobial susceptibility testing were performed using the Vitek 2 Compact system (BioMérieux, Marcy-l'Étoile, Carolina do Norte, USA). Antimicrobial susceptibilities were defined according to Clinical and Laboratory Standards Institute break points.[14] American Type Culture Collection (ATCC) strains were used as quality controls: *Enterococcus faecalis* (29212) and *Staphylococcus aureus* (25923). Antimicrobial sensitivity testing was not performed on *Micrococcus* sp., *Kocuria sp.,* Gram-positive bacilli and mycobacteria.

The screws were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH7.2, overnight.

Subsequently, the samples were washed in 0.1 M phosphate buffer pH 7.2; dehydrated in increasing concentrations of ethanol; rinsed in hexamethyldisilazane solution (HDMS); mounted on stubs with the aid of carbon painting; and subjected to gold coating (Denton Vacuum - Desk V; Morristown; New Jersey, USA). The whole screw was scanned using SEM (JSM-6610, JOEL, Japan). Screws were considered positive for biofilm if there were aggregated microorganisms immersed in EPS on their surface.[5] A new screw, subjected to one reprocessing, was also assessed by SEM under the same conditions. The Wilcoxon-MannWhitney test was used to analyze the amount of protein on the screws in Groups 1 (most used) and 2 (least used), using the R program. Values of $P<0.05$ were considered statistically significant.

The Research Ethics Committee from a *Universidade Federal de Goiás* approved the research (558.585) (CAAE: 26959614.0.0000.5078).

Results

Visual inspection, with the aid of an eight-fold image amplification lens, was performed on all 126 cortical screws. Damage, present as grooves, was detected on 11 (8.7%) screws, predominantly in group 2 (8/11). Two screws (1.6%) from group 2 showed signs of wear. Protein was quantified on 54 screws (27 from each group) and was detected in 52 (96.3%), 26 from each group. In group 1, the average of the amount of protein was 19.85µg (range from 5.03 to 56.3) and, in group 2, it was 26.95µg (range from 6.5 to 65.7). Most of the screws (22/26 and 24/26 from group 1 and group 2, respectively) had contaminating protein > 10µg. There was no statistically significant difference in the amount of protein between the groups ($P = 0.07$). Bacterial growth was assessed on 27 screws from each group, and was verified in three (5.5%) screws, one from group 1 (most used) and two from group 2 (least used). *Micrococcus luteus* was isolated from a screw from group 1, and *M. luteus* (screw A), *Kocuria rhizophila* and *Staphylococcus hominis* resistant to rifampin (screw B) from two screws from group 2. Soil and/or grooves were detected on all 18 screws subjected to SEM (Chart 1) (Figure 1), and also on the new screw. Biofilm was detected on eight screws, three from group 1 (most used) and five from group 2 (least used) (Figure 2).

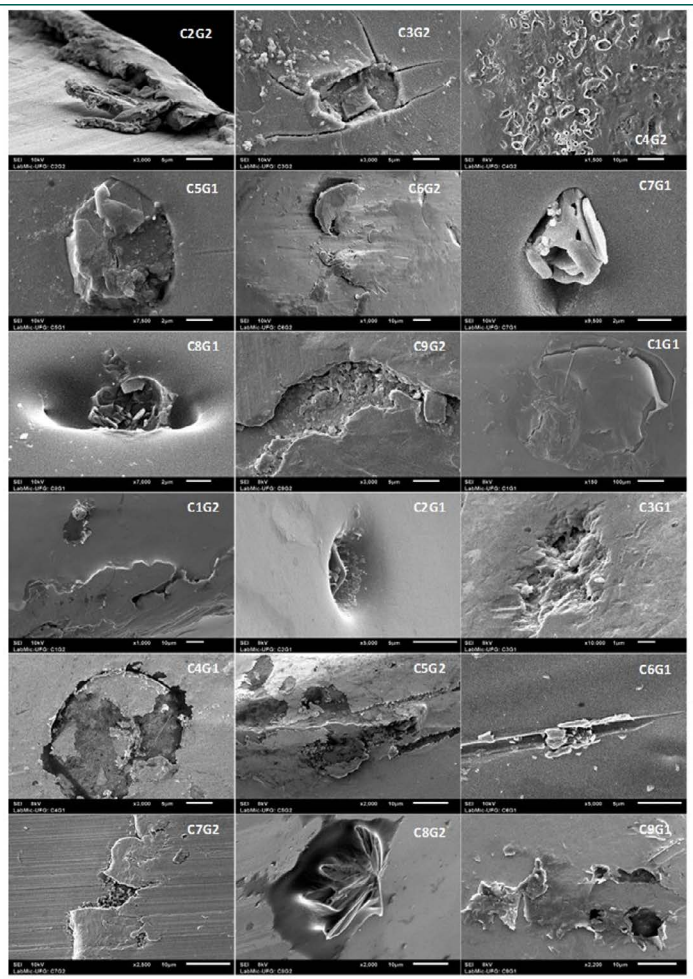
Chart 1. Surface damage, soil and biofilm detected on orthopaedic implants (cortical screws), from small fragments 3.5 surgical boxes acquired through loaner system

Box/ group	Screw number (size)	Surface damage (visual inspection)	Surface damage (SEM)	Soil	Biofilm
C1/G1	14	-	P	P	-
C1/G2	10	-	P	P	-
C2/G1	14	-	P	P	-
C2/G2	38	-	P	P	P
C3/G1*	14	+ (groove)	P	P	-
C3/G2	10	+ (groove)	P	P	P
C4/G1	16	-	P	P	-
C4/G2	10	+ (wear)	P	P	P
C5/G1	14	-	P	P	P
C5/G2	10	-	P	P	-
C6/G1	14	-	P	P	-
C6/G2*	38	-	P	P	P
C7/G1	14	-	P	P	P
C7/G2*	38	+ (groove)	P	P	-
C8/G1	14	-	P	P	P
C8/G2	38	-	P	P	-
C9/G1	16	-	P	P	-
C9/G2	10	-	P	P	P

*Screw from the same surgical box and group with positive bacterial culture; P – Positive; C - surgical box; G - group

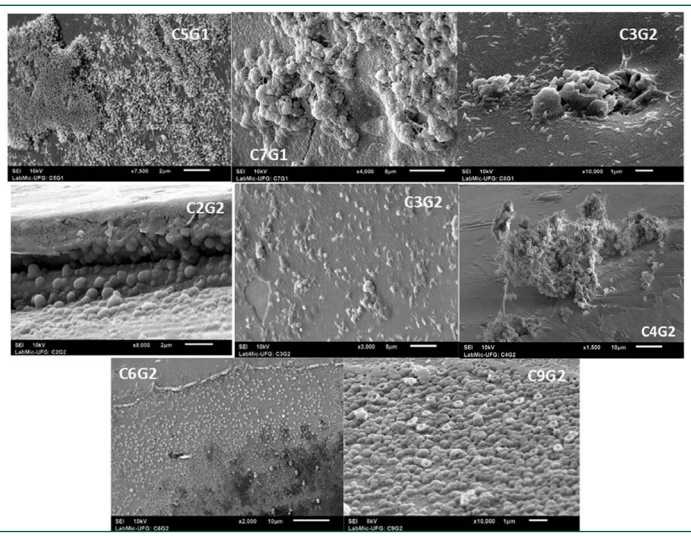
Discussion

Patient-ready single-use cortical screws, supplied in loaner orthopaedic kits were found to be contaminated with protein and biofilm, and all were structurally damaged by SEM. However, this damage was also detected by visual inspection in 3/63 and 8/63 screws of groups 1 and 2, respectively. Group 2 screws are likely to remain in the surgical kit for longer and are, therefore, subjected to more opportunities of damage during handling and reprocessing, this may result in "deeper"/"worse" damage in group 2 screws, which was able to be seen visual inspection (with the aid of magnifier lenses). Correlation between corrosion/deterioration on orthopaedic screws and number of reprocessing was reported by McAuley.[15] It is worthy highlighting that grooves were also detected on the new screw (reprocessed once only), revealing that surface dam-



C – Surgical box; G - Group; Group 1 – most used; Group 2 – least used

Figure 1. Micrographs showing grooves and soil on orthopedic implants (cortical screws), acquired through loaner system



C – Surgical box; G – Group. Cocci bacteria in C7G1, C2G2, C4G2, C6G2, and C9G2. Bacilli bacteria in C5G1 and C3G2

Figure 2. Micrographs of biofilm on the most used (Group 1) and least used (Group 2) orthopedic implants (cortical screws), acquired through loaner system

age may occur during the manufacturing process, transport of the screw and general handling. Surface damage favors the accumulation of organic matter, increasing the difficulty of reprocessing.

Protein was found on 96.3% (52/54) of the screws, reaching values six times higher (65.7µg) than recommended (5 µg per side of RMD[16] or 10µg per RMD), and the average of protein found in each group was 1.9 and 2.6 higher than this acceptable amount. Costa *et al.*[2] showed high levels of residual protein on surgical instruments/implants made of stainless steel and subjected to multiple reprocessing in Australia. In Costa *et al.*[2] study, all devices, including orthopedic screws, had protein level above the recommended (10µg per PPS), and one screw showed 24µg and 61% of the other surgical instruments had level of protein 10 times or more above the maximum acceptable quantity. Organic matter (carbohydrate, fat), soap and corrosion have also been reported on ready-to-use pedicle screw, used for spinal fusion, by Agarwal *et al.*[17] The current way screws are presented in the surgical box, on racks that are frequently not removable from the box, make their cleaning, the most important reprocessing step, difficult, as they are numerous (hundreds) and most are very small, increasing the difficulty of supplying sufficient manual friction to ensure adequate cleaning occurs. Most of the manufacturer's instructions for use in North America do not state how orthopaedic implants, such screws, should be reprocessed.[4] The high percentage of screws with above the acceptable amount of protein demonstrates this cleaning difficulty and suggests that orthopaedic screws should be presented in an alternate way. Furthermore, as reported by Agaral *et al.*[17] the recommended reprocessing for pedicle screws is impracticable (19 hours of reprocessing recommended by manufacturer's *versus* 1 hour 17 minutes in real-time observation).

Protein on ready-to-use surgical instruments/implants may result in inflammatory responses post-surgery and, in European countries, there is concern for transmission of variant Creutzfeldt–Jakob disease.[18]

The presence of organic matter decreases the efficacy of sterilization processes. Following processing viable biofilm-forming bacteria were isolated from 3/54 screws and extensive biofilm formation was visually confirmed on 8/18 screws subjected to SEM. This microbial consortium was also reported on loaned screws in previous studies,[2,19] and poses a challenge for infection control, as it protects the microorganisms against cleaning, disinfectants, biocides and, as reported by Almatroudi *et al.*,[20] *S. aureus* dry surface biofilm can survive autoclaving at 121ºC for 30 minutes. The presence of soil aids biofilm formation acting as a conditioning layer for bacteria to attach. Thus, biofilm can form quickly on reprocessed surgical instruments as demonstrated in Lopes et al *in vitro* study. Biofilm formed on medullary reamers and depth gauges biofilm within 20 cycles of use and reprocessing, including moist heat sterilization (134ºC for 3 min and 30 s).[3] Biofilm contaminated screws could release viable organisms into the surgical site and result in infection and loss of the implant.

Although classified as single-use, screws are subjected to multiple opportunities of contamination prior to implantation[21] and currently criteria for determining the maximum times a screw should be reprocessed or a way to track the number of times a screw is reprocessed is lacking. There is a trend towards changing to the use of individually packed screws.[17,22] For example, in Scotland reprocessing of implants is not allowed.[21] According to Litrico *et al.*,[23] infection rate was lower with presterile single packed screws (2%) compared with the reprocessed implants (6%). On the other hand, this practice also raises concerns about increasing the risk of contamination,[24] the length of surgery[25] and cost.[25,26]

In Australia and New Zealand,[27] RMD are subjected to high-level disinfection or sterilization before being returned to the loaner company. In Brazil, only cleaning is mandatory.[9] Cleaning reduces the microbial load and other organic and inorganic matter, however the remaining contamination favours biofilm formation until the surgical box is reprocessed/used at another healthcare service. Presence of blood has been reported on loaned surgical boxes upon delivery at the health-

care facilities in USA[28,29] and Brazil,[2] revealling the occupational risk for the loaner-company workers, which frequently do not have specific biological risk training.[2] Even though quality indicators regarding structure and work process for the management and reprocessing of loaned RMD and implants, such as screws, tent to be higher standard in high-income country than in middle or low-income country, failures are present in both countries category, highlighting the need to multifaceted loaner system key challenges need to be faced worldwide.[30]

Conclusion

Patient-ready cortical screws were found to be contaminated by viable bacteria, biofilm, and have grooves. Screws frequently remain in surgical trays for multiple reprocessing, thus they are repeatedly exposed to contamination and possible damage. These findings point to the need to discuss and review the way these single-use implants are currently made available for surgery.

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Collaborations

Pereira LA, Lopes LKO, Costa DM, Santos MA, Boff IMQ, Leão-Vasconcelos LSNO, Vickery K and Tipple AFV contributed to study, design, data analysis and interpretation, article writing, relevant critical review of intellectual content and approval of the final version to be published.

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