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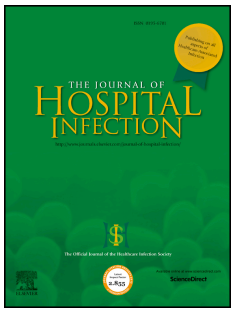

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Challenging the Six-Hour Recommendation for Reprocessing Sterisable Medical Equipment

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Reprocessing, Surgical instruments, Holding time, Protein residue, Corrosion

Abstract

Background

At present, reprocessing of sterilisable medical equipment is recommended to be initiated within six hours after completion of surgery, to ensure that the quality of the instruments do not deteriorate. A literature search showed a lack of evidence for consequences that may occur if medical personnel deviate from the standard six-hour sterilisation protocol.

Aim

This study evaluates the six-hour recommendation for reprocessing sterilisable medical equipment. We investigated whether residual protein increased proportional to holding time before reprocessing was initiated, and likewise whether an increase in corrosion was present on surgical scissors proportional to holding time.

Method

Residual protein was identified on surgical instruments contaminated with human blood after different holding times and before washes using the o-phthaldialdehyde (OPA) method. Corrosion was identified on surgical scissors contaminated with human blood after different holding times and before reprocessing using light stereomicroscopy and scanning electron microscopy.

Results

Protein residues ranged between 14.0 µg and 51.9 µg and thus below the accepted threshold of 100 µg per instrument surface. Corrosion corresponding to 0.05% of the surface was identified on 22 of 30 scissors. Pitting corrosion was seen on four of 30 scissors.

Conclusion

No association was identified between residual protein and holding time as well as between incidence of corrosion and holding time. The study thereby challenges the relevance of upholding the recommendation of a maximum wait of six hours prior to reprocessing. The findings will potentially have an impact on the organisation of reprocessing of surgical instruments in Denmark and internationally.

Introduction

At present, the reprocessing of sterilisable medical equipment is recommended to commence no later than six hours after completion of surgery, according to National and International guidelines for infection control in the health care sector [1-3]. The concern is that a longer holding time may result in deterioration of the instruments i.e., the instruments may not become clean using standard protocols for reprocessing and may consequently be more susceptible to corrosion. The recommendations for reprocessing of sterilisable medical equipment are described using the term 'should', and medical personnel are advised that reprocessing must be initiated 'as soon as possible'. However, a literature search in Embase, Web of Science and PubMed revealed a lack of evidence regarding the potential consequences regarding infection control and risk of corrosion if there is a deviation from the standard six-hour reprocessing window.

The most recent recommendations from a working group representing manufacturers of instruments, disinfectants, cleaning and care agents, washer-disinfectors and sterilisers states:

> "Because of the corrosion risk and the cleaning factors, long intervals between instrument use and reprocessing (e.g. overnight or over the weekend) should be avoided, irrespective of the disposal methods used (i.e. wet or dry). Field experience has shown that in the case of dry disposal, intervals of up to 6 hours pose no problem" [1, p 30].

This recommendation is found in the 10th edition, from 2012. Since publication of the first edition, in 1979, editions have been widely disseminated in 19 languages, reflecting the working group's international relevance. Its recommendations build on guidelines, procedural descriptions and consensus about 'best practices' [1]. Additionally the ISO 17664:2017 standard describes "The

time between medical device use, the initial treatment and/or the next step of the process" [3 p 7], to be information that the medical device manufacturer *shall* provide, where applicable.

Research-based knowledge indicates that there is an increase in residual protein, bacterial load and prion amyloid proportional to holding time before reprocessing is initiated [4-7]. However, the work by Lipscomb et al demonstrated that "... all pre-soaks significantly reduce (by up to 96%) the prion-infected tissue contamination, and that controlling the temperature whilst in transit between theatres and cleaning facilities may allow an increase in time before high protein adsorption levels occur" [4]. No research to date has tested the association between holding times exceeding six hours and the cleanliness of the instruments after reprocessing. Additionally, based on reported incidents associated with the unsuccessful decontamination of reusable surgical instruments, the risk of cross-transmission of infection and exposure appears to be very low [8]. These results call for further studies challenging the recommendation of a maximum wait of six hours before reprocessing. With this in mind, this study evaluates the six-hour recommendation for reprocessing sterilisable medical equipment. We investigate whether an increase in residual protein content is proportional to holding time before reprocessing is initiated, and whether an increase in corrosion is present on surgical scissors proportional to holding time before reprocessing is initiated.

Methods

Simple instruments, such as scissors and knife shafts, and more complex instruments with cavities, such as puncture cannulae, were tested. Defibrinated human blood was donated by consenting, voluntary, unpaid and anonymous blood donors in accordance with Danish rules [9]. The instruments were contaminated with the blood and then left to dry for 0, 3, 6, 9, 12, 24 and 36 hours at room temperature before washing (i.e. dry storage). A sterile cotton swab was soaked in undiluted blood and used to lubricate all surfaces of knife shafts and scissors, and puncture cannulae were flushed with blood. The instruments were washed in the washer-disinfector using the standard protocol for the Sterile Centre at Aalborg University Hospital, Denmark, Appendix 1. After washing, but before disinfection, the instruments were examined for protein residue using the o-phthaldialdehyde (OPA) method (Annex C, C. 2 to ISO 15883-1: 2006 (E)).

OPA analysis

The OPA analysis was based on EN-ISO 15883-1: 2009 [10]. For elution the puncture cannulae were placed in sterile 15 mL tubes with 5 mL 1% SDS, and rinsed using a 5 mL syringe by filling and emptying 5 times. Items were left in an orbital shaker (200 rpm) at room temperature for 30 minutes, after which they were rinsed additionally 5 times. Finally, the tubes were exposed to vortexing for 5 seconds, prior to transfer of 100 µL per well, within a microplate.

Scissors were eluted with 10 mL 1% SDS in stomacher bags. Scissors were rubbed for 30 seconds and placed in an orbital shaker (200 rpm) at room temperature for 15 min. The items were turned and again placed in the orbital shaker for a further 15 minutes. The scissors were again rubbed for 30 seconds. The eluate was transferred to 15 mL tubes, exposed to vortexing for 5 seconds prior to transfer of 100 µL per well, within a microplate.

Knife shafts were eluted with 10 mL 1% SDS in stomacher bags. The bags were placed in de-gassed ultrasonic baths where they were sonicated at 40 kHz for 5 min at room temperature. Then the items were left for 20 minutes, after which sonication was repeated. The eluate was transferred to 15 mL tubes, exposed to vortexing for 5 seconds prior to transfer of 100 µL per well.

In Denmark the consensus acceptable level for surface protein residues is a maximum of 100 µg/instrument [11]

Corrosion analysis

Corrosion resistance was tested using two qualities of surgical scissors, in order to include metals of different composition. Analyses showed that one quality of scissors had a chromium content of 16%, and the other 12.5%. Fifteen scissors of each type were lubricated with blood on all sides using a sterile cotton swab and left to dry for 6, 12 and 24 hours (i.e. dry storage), following which, they were washed, disinfected and autoclaved. After washing and disinfection, the scissors were inspected for visible signs of corrosion before being autoclaved. The process from contamination to end autoclaving was repeated in the same way 50 times. Pairs of scissors of each quality subjected to each of the three holding times were tested for corrosion after 25, 35 and 45 reprocessing cycles, respectively. The remaining two scissors of each quality and holding time were retrieved after 50 reprocessing cycles. The individual scissors had the same holding time before reprocessing throughout the test period. The scissors were examined and evaluated using light stereomicroscopy and scanning electron microscopy (SEM). The degree of corrosion was assessed according to the ISO 4628-3 standard [12]. The number of pictures defines the degree of

corrosion from Ri 0 to Ri 5 where Ri 1 for example corresponded to 0.05 % of the instrument surface. Corrosion above 50 % corresponded to Ri 5 (see Table I). The OPA protein assay, stereomicroscopy and SEM analyses were conducted by the Danish Technological Institute, Aarhus, Denmark.

Data analysis

We investigated whether there was an association between holding time and the amount of protein residue for the three instrument types separately using a linear regressions. During the analysis, the protein residues were converted into µg on the basis of the linear function of the standard series. The equation used to determine linearity for puncture cannulae was $y = 1.494*x + 8.928$ ($R^2 = 0.999$). For knife shafts $y = 1.589*x + 7.168$ ($R^2 = 0.998$) and for the scissors $y = 1.525*x + 7.749$ ($R^2 = 0.998$).

We investigated whether there was a difference in the distribution of corrosion with respect to the number of reprocessing cycles completed for the two qualities of scissors using a Fisher's exact test for each type separately. We included a spine plot for visualisation of the proportion of scissors with and without corrosion in terms of the proportion of scissors within each number of reprocessing cycles. To compare the same number of observations per number of reprocessing cycles, we took the average in cases of repeated observations, as we expected the variations to be minimal.

The study was designed as a small-scale study, and hence no power calculation was used to determine sample size.

Results

Protein residue

Table II shows protein residues identified on puncture cannulae, scissors and knife shafts. The negative controls (instruments that were not contaminated but washed) had the same amount of protein residue as the instruments with holding times prior to washing. Regardless of holding time and instrument type, all protein residues were below the consensus-accepted threshold of 100 µg per instrument surface, with the lowest value at 14.0 µg and the highest value at 51.9 µg. Only three out of 42 values were above 50 µg; the remaining 39 values were ≤40 µg. The room temperature in the Sterile Centre during the trial fluctuated from 22.1 °C to 25.7 °C, with the highest temperatures in the late afternoon and during the night. Humidity ranged from 26.4 to 42.4 %. However, the fluctuations in temperature and humidity are not considered relevant to the results.

No correlation between holding time and the amount of protein residue was identified for the puncture cannulae. A non-significant slope of -0.37 (p = 0.09, 95% CI = (0.07, 0.81)) was identified, and R2 = 0.216. The amount of protein residue on the contaminated puncture cannulae varied from 14.0 µg to 50.9 µg. One of the lowest and the highest values (14.3 µg, 50.9 µg) were obtained from the samples with a holding time of 36 hours. The 6-hour values of 16.2 µg and 18.5 µg were higher than the 12-hour values of 14.0 µg and 15.6 µg.

Likewise, there was no correlation between holding time and the amount of protein residue remaining on the scissors. The observed slope was -0.21 (p = 0.11, 95% CI = (0.47, 0.06)), and R2 = 0.196. Protein residue values for the scissors ranged from 33.7 µg to 51.9 µg. The two highest

values were identified on the scissors with holding times of 0 hours and 6 hours, 51.9 µg and 50.3 µg, respectively. We found a weak correlation between holding times and protein residue for the knife shafts, with a slope of -0.08 (p = 0.01, 95% CI = (-0.13, -0.02)), and R2 = 0.431. The protein residue on the knife shafts ranged from 31.0 µg to 35.9 µg.

Corrosion

Stereomicroscopy showed surface areas with corrosion of the degree Ri 1 (Tables I and III). The corroded areas were identified as those with red-coloured deposits. In addition, lighter discoloration was observed on the scissors; we considered this to be caused by detergents and the drying process. The scissors were examined in two areas, as illustrated in Figure 1. The analyses were made only for Area 2, because corrosion grade Ri 1 was observed for both types of scissors for all holding times and for any number of reprocessing cycles in Area 1.

A comparison of the two qualities of scissors showed that the surface structure of scissors with 12.5% chromium was not entirely as smooth as the surface of scissors with 16% chromium. The scissors with 12.5% chromium also appeared to have small silicon embeddings (3 µm in diameter) and were therefore 'born' with small impurities in the surface within which pitting can develop. Examples of silicon embedding and pitting corrosion are illustrated in Figure 2. A higher incidence of corrosion was identified on scissors with 12.5% chromium, where 12 out of 15 scissors were affected, compared to 10 out of 15 scissors with 16% chromium.

Figure 3 illustrates a spine plot for the proportion of scissors with and without corrosion in relation to holding time. In the plot, holding times are illustrated by a colour code and the occurrence of corrosion by 0 and 1, where 0 is no corrosion and 1 is the corrosion degree Ri 1. Similarly, Figure 4 illustrates the proportion of scissors with and without corrosion in relation to the number of reprocessing cycles they have been through. The differences in occurrence of corrosion are shown by the size of the coloured areas in Figure 3 and 4

The light stereomicroscopy showed a weak tendency (no clear signs) toward less corrosive activity on scissors with 16% chromium and holding times of 6 or 12 hours, compared to scissors with a holding time of 24 hours. There was no clear tendency for the scissors with 12.5% chromium, where the same degree of corrosion was observed on the scissors with holding times of 6, 12 and 24 hours. Pitting, indicating severe corrosion attack, was observed in Area 2 on four scissors with 12.5% chromium. These were the scissors with holding times of 12 hours and 35 reprocessing cycles, 12 hours and 50 reprocessing cycles and two scissors with holding times of 24 hours and 50 reprocessing cycles. It is possible that this pitting corrosion had already begun at inclusion and was caused by the quality of the scissors, not the holding time before reprocessing.

Discussion

Substantiated by recommendations from the Instrument Preparation Working Group [13] and the Medical Devices Agency [14] Danish guidelines for the health care sector strongly recommend that reprocessing of sterilisable medical equipment commence no later than six hours after the completion of surgery [2 p. 32]. However, the present study questions whether a longer holding time actually results in increased protein residue contamination and a heightened risk of corrosion. Lipscomb et al. [4] found that at 22 °C all adsorption of protein to the surface of an instrument will have occurred after approximately 40 minutes. Furthermore, that at higher temperatures, the speed of adsorption will increase.

Secker et al. [5] showed that different types of storage for surgical instruments prior to reprocessing could reduce the adsorption of proteins to surgical steel surfaces. They observed an increase in biological contamination proportional to increased drying times whether preserved dry and uncovered or dry and covered. By contrast, independent of the drying time, they observed a minimal increase in contamination if the steel was stored in a humid environment. According to these studies, the amount of protein adsorbed to the instrument reaches a maximum after 40 minutes. Even with the use of enzymatic softeners and environmental and temperature control during transportation, full adsorption will have already occurred when the instruments reach the cleaning facilities.

Hence, these studies do not provide evidence that can support the recommendation of a maximum wait of six hours before reprocessing. The objective of the present study was to imitate current reprocessing practice at the Sterile Centre at Aalborg University Hospital and therefore we

only tested instruments stored dry and uncovered. Therefore, this study does not clarify whether sterile services should consider using commercially available wetting agents or processes to maintain moisture and improve cleanability as suggested by Secker et al [5] and Lipscomb et al [4]. However, the present study provides evidence for no association between the presence of protein residue on three different types of surgical instruments and the holding time before reprocessing was initiated. Thus, the cleanliness of instruments after dry and uncovered storage seems to be independent of holding time before reprocessing. Furthermore, the study demonstrates that instruments can have holding times up to 36 hours before reprocessing is initiated without exceeding the accepted upper limit of protein residue of 100 µg.

The literature search revealed two recent experimental studies addressing augmented bacterial growth on instruments not reprocessed within the recommended six-hour period [6,7]. In both studies, the instruments were contaminated with three common pathogenic bacteria with a quantification of bacterial growth at room temperature at various time points. A notable increase in the numbers of colony forming units per square centimetre was reported for a 12-hour holding time, compared with a six-hour period. It is not surprising that bacterial growth occurs over time on unwashed contaminated instruments. However, these studies do not answer the question of whether there is a difference in cleanliness after the reprocessing of instruments left with bacterial growth for less than six hours, compared to those left for more than six hours.

Instead of a surrogat (“test soil”) we have used defibrinated human blood. The National Committee on Health Research Ethics have endorsed use of human blood for research purposes conditional of the blood donor’s informed consent. Thereby use of a substitute for human blood is

avoided. The addition of Enterococcus faecium to “test soil” is not relevant in this context. The human blood used in the study was not pre-treated or cleaned, which means it potentially may have been contaminated with both bacteria and medicine residues. This fact contributes supporting evidence that the cleanliness of instruments seems to be independent of holding times before reprocessing.

The study revealed distinct differences in the surface structure of the two qualities of scissors. The surfaces of the scissors with 12.5% chromium were not entirely as smooth as the scissors with 16% chromium and had small silicon embeddings (3µm in diameter) within which pitting corrosion could potentially develop. This finding emphasises the higher incidence of corrosion identified on the scissors with 12.5% chromium and that pitting corrosion attacks were only observed in the scissors with 12.5% chromium. These findings are in concordance with Rosenberg’s [15] and Kaiser et al.´s [16] claim that the corrosion resistance of the steel depends on the amounts and composition of its specific components.

Finally, this study demonstrated no clear signs for either an association between the incidence of corrosion and holding time before reprocessing was initiated, or the number of reprocessing cycles. Unfortunately, no research-based knowledge has been identified that can challenge or support this finding.

Our study have several limitations. The number of test units for both protein residue and corrosion is a restricting factor. In comparison to protein residue, inclusion of more test units could have enhanced the statistical analysis. In relation to corrosion, a higher number of

repetitions of the reprocessing cycle could provide additional knowledge. Likewise, only three different instrument types were included in the study; perhaps the inclusion of other instruments, which may be more complex, could have strengthened the study.

Conclusion

In conclusion, this study found no evidence that a longer holding time results in deterioration of reusable instruments. The three different instruments (scissors, knife shafts and puncture cannulae) tested in this study all become clean using a standard protocol for reprocessing, and their levels of identified residual protein ranged from 14.0 µg to 51.9 µg, below the accepted threshold of 100 µg. Furthermore, the study revealed no evidence that two different qualities of scissors are more susceptible to corrosion when holding times exceed six hours. The study clearly challenges the relevance of upholding the recommendation of a maximum wait of six hours before reprocessing.

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Figure legends

Figure 1. Scissors with markings of Area 1 and Area 2

Figure 2. Example of silicon embedding and pitting corrosion

Figure 3. Scissors with and without corrosion in relation to holding time

Figure 4. Scissors with and without corrosion in relation to the number of reprocessing cycles

Table legends

Table I. Corrosion adjustment scale

Table II. Protein residues

Table II. Corrosion data

Table I. Corrosion adjustment scale [12]

Degree of corrosion	Area [%]
Ri 0	0
Ri 1	0,05
Ri 2	0,5
Ri 3	1
Ri 4	8
Ri 5	40-50

Table II. Protein residues

Treatment	Protein µg/ puncture cannulae	Protein µg/scissors	Protein µg/knife shafts
Positive Control*	>500 (794)***	>1000 (2720)***	>1000 (2200)***
	>500 (1010)***	>1000 (2730)***	>1000 (2290)***
Negative control**	12,7	41,4	36,6
	16,0	43,5	37,3
0 hours holding time	14,3	39,2	35,0
	14,3	51,9	32,6
3 hours holding time	15,0	35,2	35,2
	14,8	35,0	33,4
6 hours holding time	16,2	36,3	33,1
	18,5	50,4	33,0
9 hours holding time	20,4	40,0	35,5
	25,9	38,2	35,9
12 hours holding time	14,0	35,1	33,5
	15,6	34,6	33,6
24 hours holding time	15,3	33,7	33,0
	13,8	37,7	32,4
36 hours holding time	50,9	35,4	31,7
	14,3	34,5	31,0

*Positive controls - instruments that were contaminated but not washed.

**Negative controls - instruments that were not contaminated but washed.

*** The signals for positive controls were out of range for the standard series. The values in the parentheses were found by extrapolation of the linear function for the standard series.

Table III. Corrosion data

Scissor 16% chromium					Scissor 12.5% chromium				
Cycles	Number of scissors with no corrosion	Number of scissors with corrosion	Total	p-value (Fisher's exact)	Cycles	Number of scissors with no corrosion	Number of scissors with corrosion	Total	p-value (Fisher's exact)
0	1	0	1		0	1	0	1	
25	2	1	3		25	1	2	3	
35	3	0	3		35	1	2	3	
45	0	3	3		45	1	2	3	
50	0	6	6	0.05	50	0	6	6	1.00

Figure ~~3.~~ 1 Scissors with marking of area 1 and area 2

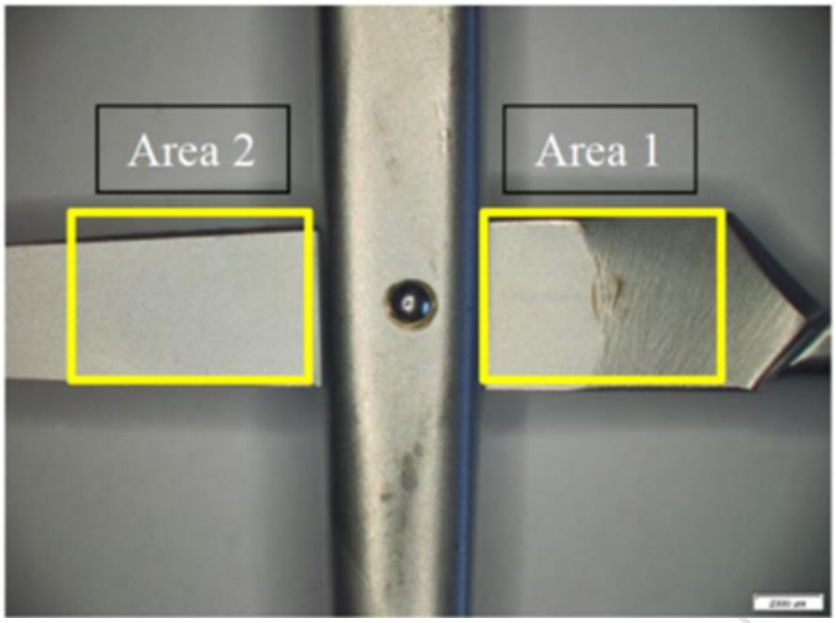
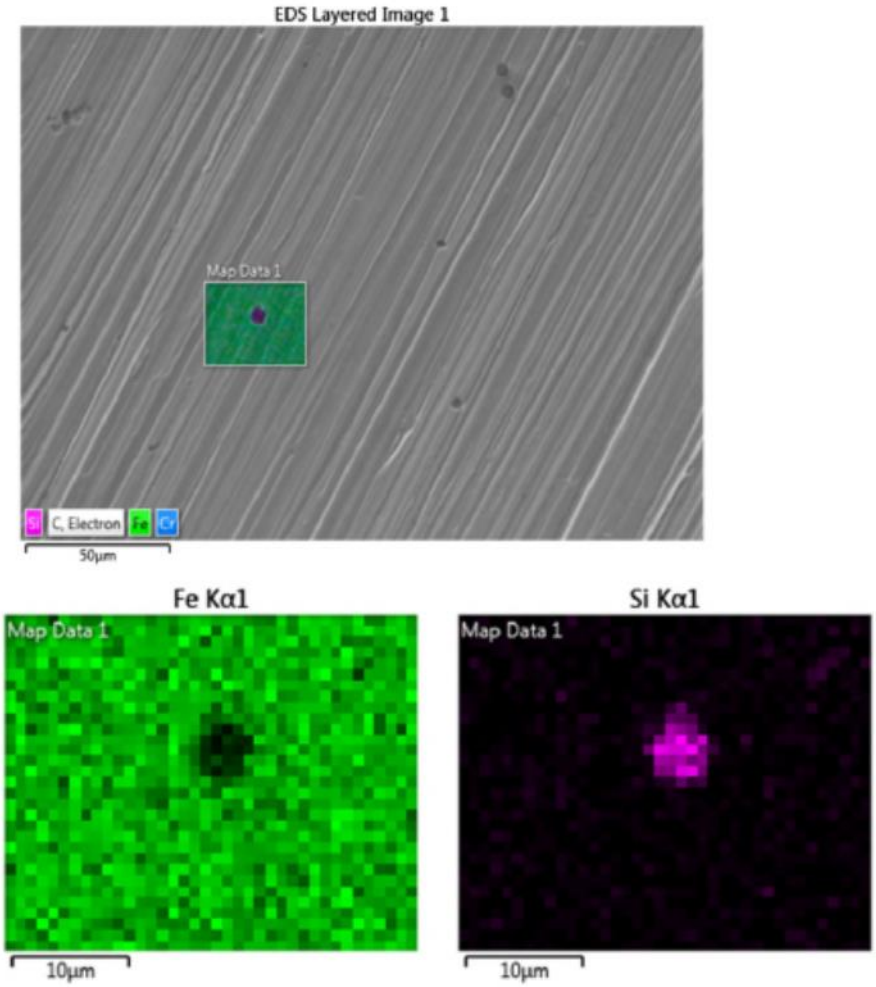
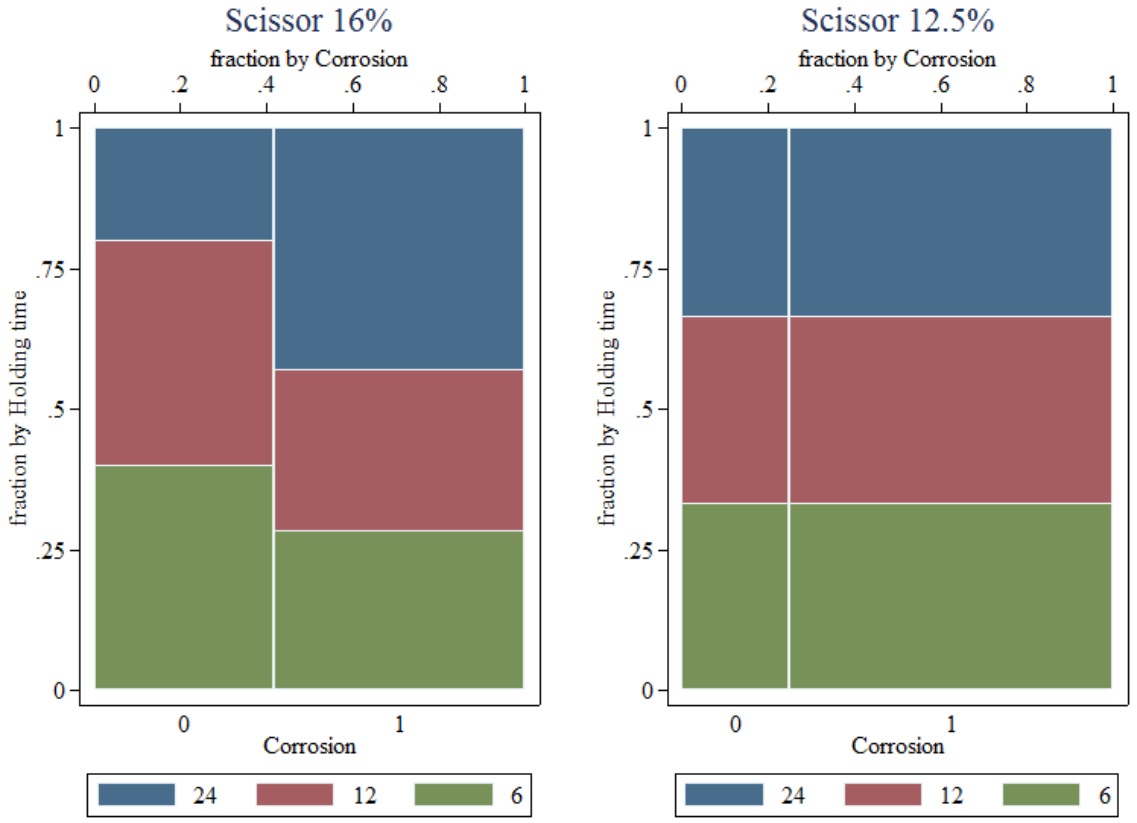
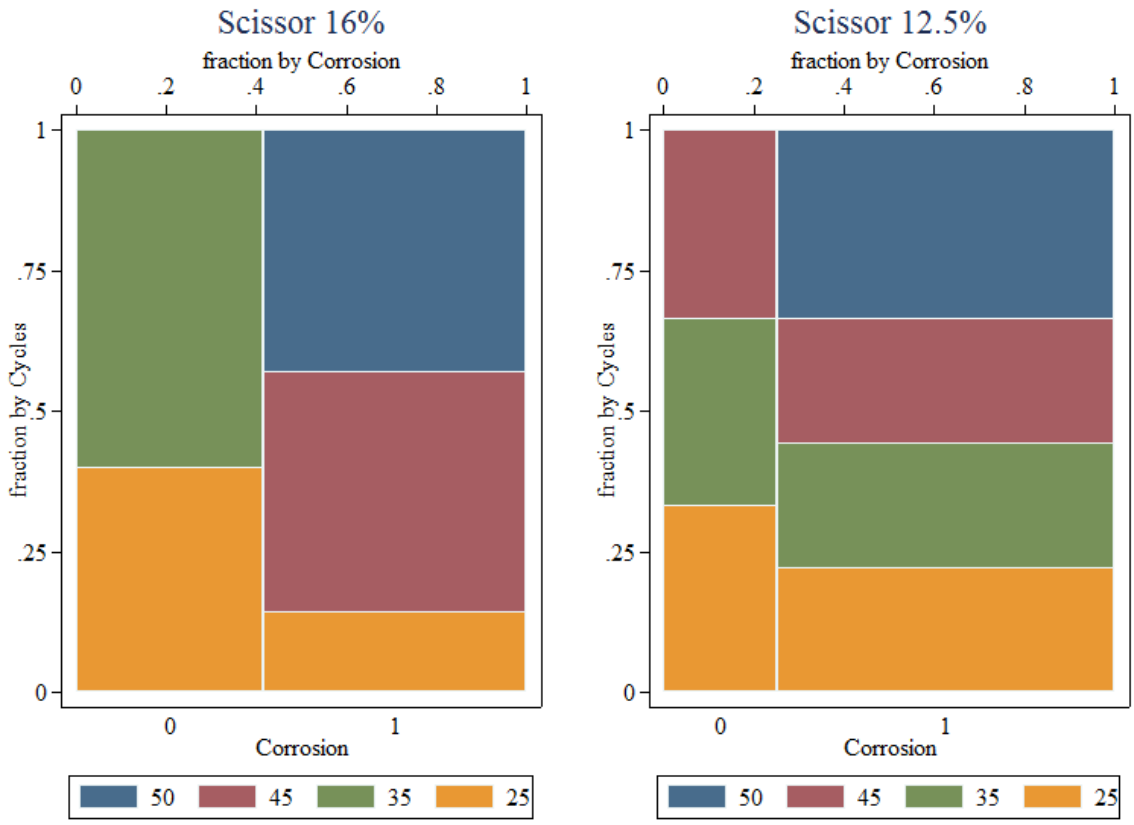


Figure ~~4.~~ 1 Example of silicon embedding and pitting corrosion




Scissor 16%
fraction by Corrosion
0
.2
.4
.6
.8
1
fraction by Holding time
.75
.5
.25
Corrosion
24
12
6
Scissor 12.5%
fraction by Corrosion
fraction by Holding time
Corrosion
24
12
6


Scissor 16%
fraction by Corrosion
0
.2
.4
.6
.8
1
fraction by Cycles
.75
.5
.25
Corrosion
50
45
35
25
Scissor 12.5%
fraction by Corrosion
fraction by Cycles
Corrosion
50
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