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Mechanical Stability of Restorations Supported by Titanium Base, Zirconia, and Polyetherketoneketone Abutments on One- and Two-Piece Zirconia Implants

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Abstract: (max 350 words)

Purpose: Evaluation of survival, complication rates and bending moments of one- and two-piece zirconia implants restored with different abutment materials and lithium-disilicate crowns after aging, compared to titanium implants restored with titanium base-supported lithium-disilicate crowns. **Materials and Methods**: Sixty anterior crowns were either screw retained on two-piece titanium implants (C) and two-piece zirconia implants (T1, T2, T3) or cemented on one-piece zirconia implants (T4), resulting in 5 groups with 12 samples each. For the screw-retained crowns different abutment materials and implant connections were tested: titanium base with internal conical connection and horizontal joint component (C and T2), zirconia with internal hexagonal connection and horizontal joint component (T1) and Polyetherketoneketone with internal hexagonal connection and horizontal joint component (T3). After artificial aging with thermocycling (5° to 50° C,) and chewing simulation (1,200,000 cycles, 49 N, 1.67 Hz), the survived specimens were loaded until fracture and the bending moments were calculated. Survival rates and respective differences during aging were analyzed with Kaplan-Meier Log-Rank test, while complication rates were compared with Chi-squared tests. Bending moments data was evaluated using Kruskall-Wallis test (α=0.05).

Results: Survival rates after artificial aging ranged from 75% (T1) to 100% (C and T4) without significant differences between the groups ($p > 0.05$). Only 41.5% of the surviving specimens were free of complications, while the remaining presented screw-loosening, loss of retention of crowns or cracks on the crown or implant level. The complication rates varied significantly among the groups ($p < 0.05$). The mean bending moments (Ncm) were 173.7 ± 20.1 (C), 114.5 ± 20.1 (T1), 171.1 ± 46.1 (T2), 258.1 ± 147.4 (T3), 194.7 ± 30.9 (T4), and group T1 exhibited significant lower median bending moment values than the other groups ($p < 0.001$).

Conclusion: The zirconia one- and two-piece implants presented high survival rates after

aging, yet, the number of technical complications was high. New prosthetic solutions as titanium bases or polyetherketoneketone abutments may offer a comparable treatment option to restore two-piece zirconia implants.

Keywords: Ceramic implants, zirconia, titanium, two-piece implants, polyetherketoneketone, implant abutment

Introduction

Dental implants made of titanium have shown to be a valid and predictable treatment option to replace missing teeth (1-3). Despite its excellent mechanical properties, from an aesthetic point of view, titanium can lead to alteration of the colour appearance of the surrounding soft tissue (4-6). Moreover, the impact of released titanium particles on the peri-implant bone resorption has recently been questioned (7).

Striving for more aesthetic and corrosion-free reconstructions led to an increased demand for zirconia implants. Zirconia as a dental material shows a high level of biocompatibility without potential corrosion (8, 9). It also presents less plaque affinity and a reduced inflammatory integration which allows stabilization of surrounding soft tissues (10-12). Its optical characteristics as a tooth-like color and the possibility of staining with different colors contribute to the popularity of zirconia (13). Further, studies focusing on parameters like bone-to-implant contact, push-in forces and removal torque have demonstrated a capacity of osseointegration of zirconia implants comparable to the titanium ones (14-16).

The first introduced zirconia implants were designed as one-piece implants (17-19). From a biological point of view, one-piece implants copy the natural tooth model avoiding the micro-gap between the implant body and the supra-construction. However, this type of design presents some limitations. For instance, if the prosthodontic requirements for placing an implant are not respected, the correction of misalignment through an angulated abutment is not possible. The intraoral preparation of the supra-mucosal part of the implant to correct the shape becomes the only solution for those cases. However, it can affect the strength of the implant material negatively and increase the risk of failure (20, 21). This procedure can also lead to more biological complications due to the heat-development and the indirect damaging of the bone cells (22). Furthermore, as the implant is directly exposed to the oral cavity, forces

coming from the tongue or as a result of mastication cannot be avoided during the healing process (23, 24). For the prosthetic reconstruction, cementation appears to be the only method to restore these implants, and a higher risk for biological complications due to cement excesses is introduced (25). Especially in the anterior area where clinicians tend to place implants deeper in order to hide the prosthetic margins (9).

Due to these limitations, the first two-piece zirconia implants were presented into the market. First, systems used cemented abutments to the implant (8, 26-28), later screw-retained reconstructions became a possibility. Screw-retained implant-abutment solutions are appealing but difficult to develop as the thin-peaked zirconia fittings may be exposed to easy fracture (28).

Currently, different screw-retained solutions are available including zirconia abutments, high performance thermoplastic polymers such as Polyetherketoneketone (PEKK) abutments, or even titanium bases. Titanium base abutments have demonstrated good mechanical stability and promising results when used on titanium implants (29-32). However, its use on zirconia implants is still scarcely reported (33).

Therefore, the aim of this in vitro study was to evaluate the mechanical stability, including survival and complication rates, and the bending moments of different screw-retained and cemented reconstructions on zirconia implants after artificial thermo-mechanical aging, when compared to reconstructions on titanium implants. The null hypotheses were: 1) the type of reconstruction on zirconia or titanium implants does not influence the mechanical stability (survival and complication rates) after aging and 2) the type of reconstruction on zirconia or titanium implants does not influence the bending moment values.

Material and Methods

In this study, the mechanical stability and fracture load of four types of reconstructions supported by zirconia implants and one type supported by a titanium implant (control) were tested. To facilitate standardization and comparability of the data, this protocol followed the procedures of six previous studies (32, 34-38), including sample size calculation (n=12). The following groups were included (Figure 1) and the respective material details are presented in Table 1:

- Control group (C) two-piece titanium implants restored with screw-retained monolithic all-ceramic crowns using titanium base abutments.
- Test group 1 (T1): two-piece zirconia implants restored with screw-retained monolithic all-ceramic crowns using customized zirconia abutments.
- Test group 2 (T2): two-piece zirconia implants restored with screw-retained monolithic all-ceramic crowns using titanium base abutments.
- Test group 3 (T3): two-piece zirconia implants restored with cemented monolithic all-ceramic crowns on PEKK abutments.
- Test group 4 (T4): one-piece zirconia implants restored with cemented monolithic all-ceramic crowns.

Fabrication of specimens

Based on a clinical case with a missing central incisor, and with a two-piece implant already placed (CERALOG Hexalobe, Camlog Biotechnologies GmbH), the specimens of group T1 were created. The ideal shape for the customized abutment and the crown was virtually designed in a CAD software (3Shape dental system, 3Shape), and the respective standard tessellation language (STL) files were used for the fabrication of the customized zirconia

abutments by a central milling center (DEDICAM, Camlog Biotechnologies GmbH). To enable screw retention of the abutment/crown complex, a pit was created to mark the prospective access hole during the virtual design of the crown. The crowns were milled (inLab CEREC MC XL, Dentsply Sirona) from a lithium-disilicate block (IPS e.max CAD Ivoclar Vivadent AG). The finalization of the access hole was made by manual grinding directly after milling (inLab MC XL, Dentsply Sirona) and before crystallization of the crowns (Programat P500, Ivoclar Vivadent AG).

The final contour of specimens in group T1 was copied into all groups T2, T3, T4 and C, in order to achieve a similar specimen outer contour and allow comparability. The complex implant-abutment-crown of group T1 was inserted in a custom-made acrylic resin block using an auto-polymerizable acrylic (Technovit 4071, Kulzer GmbH) and leaving 3 mm distance from the implant shoulder to the top of the block (to mimic 3 mm of bone loss). Then, the complex was scanned (inEos X5, Dentsply Sirona), digitally superimposed (inLab CAD, Dentsply Sirona) and virtually transferred for the other implant-abutment conditions. In test group 4, as a one-piece implant with a classical cemented crown was previewed, the pit for the access hole was removed after the superimposition, allowing for a homogenous thickness of the crown.

Following the procedures described for group T1, the remaining crowns were milled and, with the exception of specimens of group T4, the access hole was created in the palatal surface as previously marked. The crowns were then polished, crystalized and glazed, following the manufacturer's recommendations.

The implants of the remaining groups were also embedded in the respective acrylic blocks, as it was performed for group T1. According to each group, the abutment surfaces were sandblasted: 50-µm aluminum oxide (Cobra Aluoxyd, Renfert GmbH) for groups C and T2; 30-µm silica-modified aluminum oxide (Rocatec Soft powder, 3M) for groups T1 and T3.

The specimens in group T4 were not sandblasted in accordance with manufacturer instructions. All lithium-disilicate crowns were etched for 20 seconds with 5% hydrofluoric acid (IPS Etching Gel, Ivoclar Vivadent AG), and then water-rinsed for 60 seconds. Consequently, all abutments and crowns were ultrasonically cleaned in an alcohol bath for 4 min (Micro 10+, Unident SA), and kept dry for cementation. The abutments were fixed to the respective implants following the manufacturer recommended torque, and the bonding surfaces of both crowns and abutments were conditioned with a universal primer for 60 seconds (Monobond Plus, Ivoclar Vivadent AG). After gentle drying, the crowns were cemented on the respective abutments using a dual-polymerizing resin-based cement (RelyX Unicem 2, 3M ESPE AG). Before setting, the cement excesses were removed and a light-polymerizing radiation (VALO LED curing light, Ultradent) was applied for 20 seconds per surface. The specimens were left undisturbed for 5 minutes in order to complete the polymerization. The screw access holes were closed with Teflon tape and resin composite (Tetric EvoCeram, Ivoclar Vivadent AG).

Aging

The restored specimens were aged by means of thermocycling (5° to 50° C, dwelling time 120 seconds) with simultaneous mechanical loading (1,200,000 cycles, 49 N, 1.67 Hz) (Chewing simulator CS-4.8, SD Mechatronik GmbH). A steatite ball (diameter of 6 mm) was selected as antagonist. The specimens were loaded 2 mm below the incisal edge at a 30° angle of the indenter to the palatal surface of the implant-abutment-crown complex. For each chewing cycle, the indenter made a 2 mm vertical movement.

Survival and complication rates

During aging, every catastrophic event, including location and cycle, was registered, and a survival rate was calculated. After the aging period, the surviving specimens were examined under an optical microscope (30x and 50x) (Olympus SZX9, Olympus Corp) for presence of cracks, debonding or screw-loosening. These events were considered as non-catastrophic events, and a complication rate was calculated based on those. Screw-loosening was assessed if a movement between abutment and the implant was detected. Crown debonding was recorded and assigned into 2 different categories: micro-movement or loss of retention. Micro-movement was registered under microscopic evaluation when a slight movement between the abutment/implant and the crowns was detected, while loss of retention was defined by the complete detachment of the crown from the abutment.

Fracture load test

In order to test the fracture strength with a sufficient sample size per group, the specimens with complications were retrieved whenever possible. A static load was applied to the specimens until failure (1 mm/min) using a Universal Testing Machine (Shimadzu AGS-X series, Shimadzu), and the fracture strength (N) was recorded using specific software (Trapezium X, V.1.4.4., Shimadzu). The load from the indenter was applied in a 30° angle to the palatal surfaces of the crowns/abutments as adapted from the ISO Norm 14801: 2016 (32, 34-38). To ensure even force distribution to the crown-abutment-implant complex, a 0.5 mm thick tin foil (Dentaurum GmbH) was placed in between the specimens and the indenter. Failure was defined either as visible fracture of the abutment/crown/implant or after a 20% decrease of the maximum load (F max) in case of no obvious fracture.

For comparison of the groups, the bending moments (M) were calculated in Ncm according to the formula $M = 0.5 \times F \times l$, with F being the load (N) and l being the vertical distance from the simulated bone level to the center of the load (cm) (32, 34-38).

Analysis of failure mode after the fracture load test

All specimens were analyzed with a digital microscope (30× and 50×) (VHX-6000, Keyence) to locate and determine the failure mode. Failures at crown, abutment and/or implant level were documented.

Statistical analysis

The data was statistically analyzed using a statistical software (IBM SPSS Statistics v20 software, IBM Corp). Survival rates and respective differences during aging were calculated applying a Kaplan-Meier Log-Rank test. Pearson's chi-squared tests were used to compare the complications rates after aging. The bending moments means were compared using non-parametric tests (Kruskall-Wallis). The level of significance was set at $\alpha=0.05$.

Results

Seven out of the 60 specimens (three in T1, two in T2 and two in T3) did not survive thermo-mechanical aging due to fractures at implant or abutment level (Table 2). Survival rates were 75% (T1), 83.3% (T2 and T3), and 100% (C and T4) without significant differences among the groups ($p > 0.05$) (Fig. 2). Most of the catastrophic events were seen at the abutment level (71.4%), and only two fractures (28.6%) were detected at the implant level, exclusively occurring in group T1 (Fig.4).

Of the surviving specimens, less than a half were free of complications (41.5%), while the remaining specimens presented screw-loosening, loss of retention of crowns (only in T4) or cracks on the crown or implant level. For these three types of complications the differences were significant among the groups ($p < 0.05$). Moreover, the complication rate also varied significantly among the groups ($p < 0.05$). Specimens which presented a loss of retention or a screw-loosening were re-bonded or re-tightened to the recommended torque before fracture loading test. The mean bending moments (in Ncm) were 173.7 ± 20.1 (C), 114.5 ± 20.1 (T1), 171.1 ± 46.1 (T2), 258.1 ± 147.4 (T3), 194.7 ± 30.9 (T4). A significant difference was only found between median values of T1 and all the other groups ($p < 0.001$).

The mode of failure of specimens in the groups using a titanium base abutment (C and T2) was seen solely at crown level, with no abutment or implant fracture or deformation (Table 3). Implant fractures were found mostly in groups using two-piece zirconia implant with zirconia or PEKK abutments (T1 and T3).

Discussion

In the present study no difference of the survival rates was found between the test and the control groups. Yet, the complications rates and the types of complications varied significantly among the systems. Therefore, the first null hypothesis was only partially rejected. A significant decrease in the bending moment values was found for the two-piece zirconia implant restored with a customized zirconia abutment. Hence, the second null-hypothesis was rejected.

Most of the catastrophic events that occurred during the aging happened at the abutment level. Failures at the implant level were less frequent and were only observed in the combination between two-piece zirconia implants and internally connected zirconia

abutments in group T1. These findings shed light on the importance of the material combination for the stability of the implant-abutment connection at zirconia implants, similar to what has been reported for titanium implants (35, 36, 39). As a brittle material, zirconia presents lower flexural strength and higher bending stiffness than titanium (40), which increases the risk for fracture when subjected to high masticatory forces. Even in anterior areas, where lower forces are expected, the non-axial direction of the forces may increase the risk of failures of zirconia components (33, 41). In addition, the material thickness and the design of the implant-abutment connecting parts may also play an important role in the fatigue resistance of the material (28, 30, 37, 42). Previous investigations applying thermo-mechanical aging protocols have reported high incidences of fractures for certain zirconia implant systems (27, 28, 33), while others reported no failures (21, 28, 29, 33, 39, 43-46). These differences are related to the different implant systems tested, but also to the specimen configuration and to the applied aging protocols (47).

A previous laboratory study (28) and a clinical study (48) revealed improved survival rates of a two-piece zirconia implant system using bonded implant-abutment connection when compared with screw-retained two-piece zirconia implant systems. However, the intraoral bonding protocol to the implant connection is technically demanding and operator dependent (49) and may impair the retrievability in case of complications (47). Hence, a reliable and stable screw-retention system for two-pieces zirconia implants is of high importance. In the present study, the number of catastrophic events of the two-piece zirconia implant systems was relatively low, which is a promising observation for its clinical application.

The incidence of complications after the aging affected all the tested systems, including the titanium implant. The one-piece zirconia implant group presented the highest number of complication events, with loss of retention of the crowns in all specimens. As

reported in the literature, the long-term bond stability on zirconia is highly dependent on the selection of the correct adhesive protocol, which should combine a mechanical treatment of the surface with appropriate monomer phosphate containing-bonding agents (50, 51, 52, 53). Despite the requirements for an adequate bond to zirconia, its effects on the mechanical properties have been questioned. Some in vitro studies showed higher flexural strength in airborne-particle abraded zirconia surfaces compare to untreated one (54-56). Other studies (21, 57, 58) report that roughening the zirconia surface will introduce deep surface flaws that act as stress concentrators and reduce the mechanical strength. Thus far, the majority of the manufacturers do not recommend any surface treatment at the abutment level of one-piece zirconia implants. Following the respective manufacturer recommendations, in this study, the absence of a mechanical surface treatment at abutment level might explain the number of adhesive failures. These issues, in addition to the short abutment height and the prosthetic limitation in terms of implant-abutment angulation, are reducing the clinical indications of one-piece zirconia implants for very selective cases.

Another frequent complication was the screw-loosening which affected half or more of the specimens in groups T3 and C. This could be related to the different implant-abutment connections tested (41, 42, 59, 60). Purely internal connected abutments might lead to an increased stability at screw-level. Supporting this assumption, the abutments with reduced horizontal joint component with internal connection zirconia implants in the present study showed either no (T1) or reduced (T2) screw-loosening. In contrast, the internal connections with a more extended horizontal joint component on the implant platform revealed higher screw-retention rates. Although this type of complication was frequent, it can be considered a minor complication in daily clinical practice, as it is simple to retrieve.

The two-piece zirconia implants with titanium base abutments presented the least number of complications after aging, while for the one-piece zirconia implant, all specimens presented complications (cracks at crown level and/or loss of retention). Even though very limited evidence is published regarding titanium bases on zirconia implants, their mechanical stability on titanium implants has been recently confirmed (29-32). The present findings agree with a previous investigation (33) that using titanium base abutments to restore zirconia implants may offer a comparable treatment option.

After the fracture load test, customized zirconia abutments on zirconia implants demonstrated the weakest mechanical stability, with the lowest bending moment values and failure mode at implant or abutment level. In contrast, customised zirconia abutment group was shown to have a stable implant-abutment connection with no screw-loosening events during aging.

Due to more elastic intrinsic properties and the presence of a horizontal joint component on the implant connection, PEKK abutments may absorb forces and transfer them into the implant connection in a different way. These mechanical characteristics may explain the large deviation found within this group. Nevertheless, PEKK abutments appear to be more fracture-resistant than internally connected zirconia abutments when used to restore the same type of zirconia implants.

The remaining groups showed similar behaviour on fracture testing, comparable to that of the titanium implant control group. One reason for these outcomes could be the failure mode exclusively at crown level for the groups with titanium bases. In contrast to previous studies (29-32), there was no elastic deformation on the titanium base and/or implant platform, as the crowns fractured beforehand, resulting in decreased bending moment values. Hence, the registered bending moments do not reveal the implant or abutment fracture

resistance and greater values could be expected for these components. As titanium base abutments with 3.5 mm height were used, it may be assumed that a greater abutment height could have resulted in better support for the ceramic crowns. The discrepancy in the crown and abutment dimensions may cause an increased force concentration in critical areas of stress and lead to a consequent premature fracture.

In a clinical setting there is no material performing without complications in the long term. In choice of materials, many aspects should be considered by balancing benefits and risks. Zirconia implants might have aesthetic (4-6) and biological (10-13) advantages, however mechanically titanium implants remain the gold-standard (1-3). In vivo studies reported maximal bite forces in the anterior regions ranging from 108 to 299N (61, 62). In this study, the tested specimens' resistance was revealed to be within this range. Thus, these types of reconstruction supported by zirconia implants should be used with caution. Complications like screw loosening or failures like crown fracture do not have the same impact as an implant fracture. If the fracture occurs at implant level, an explantation might be the only viable solution, which requires a new surgical procedure and may result in extended bone defects. For these reasons, patients not presenting increased occlusal forces might be clinically favourable for the use of two-piece zirconia implants in the anterior region.

One limitation of the present study was the reduced standardization of the study groups, due to the use of different types of implant connections, different abutments materials and designs, which resulted in different internal configurations of the crowns. On the one hand this allowed to compare different possible clinical combinations, but on the other hand it does not allow to assess the influence of each specific variable. Another limitation is the aging protocol used in this study. A longer period could reveal difference between the groups according to the different material behaviour to fatigue.

Further studies to assess the influence of each variable, as well as clinical trials, should be designed to validate the application of these new prosthetic solutions to zirconia implants.

Conclusions

The zirconia one- and two-piece implants presented high survival rates after aging. However, the number of technical complications was high. New prosthetic solutions as titanium bases or PEKK abutments show potential as future options to restore zirconia two-piece implants. More studies, including randomized controlled clinical trials, are needed to further elucidate the present observations.

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Table 1: Overview of the implant and abutment systems

Group	Implant material and specifications	Abutment	Abutment height*	Connection	Screw material	Torque
C	Two-piece titanium implant (RN 12 mm x 4.1 mm SP Straumann AG) Titanium Roxolid® (Ti-Zr Alloy)[1)]	Titanium Base Abutment (Variobase, Straumann AG) Titanium Ti-Al6-Nb7[1)]	4 mm	Internal conical connection with horizontal joint component	Titanium Ti-Al6-Nb7[1)]	35 Ncm
T1	Two-piece zirconia implant (12 mm x 4 mm Ceralog Hexalobe, Camlog Biotechnologies GmbH) >99% 3Y-TZP[1)]	Customized Zirconia Abutment (Ceralog, Camlog Biotechnologies GmbH) >99% 3Y-TZP[1)]	9.8 mm	Internal hexagonal connection with horizontal joint component	Titanium Ti-6Al-4V-ELI[1)]	20 Ncm
T2	Two-piece zirconia implant (RD 12 mm x 4.1 mm Pure Ceramic, Straumann AG) 100% 3Y-TZP[1)]	Titanium Base Abutment (CI RD PUREbase, Straumann AG) Titanium Ti-Al6-Nb7[1)]	3.5 mm	Internal conical connection with horizontal joint component	Titanium Ti-Al6-Nb7[1)]	35 Ncm
T3	Two-piece zirconia implant (12 mm x 4 mm Ceralog Hexalobe, Camlog Biotechnologies GmbH) >99% 3Y-TZP[1)]	PEKK Abutment (Ceralog, Camlog Biotechnologies GmbH) Polyetherketoneketone[1)]	8.4 mm	Internal hexagonal connection with horizonal joint component	Titanium Ti-6Al-4V-ELI[1)]	20 Ncm
T4	One-piece zirconia implant (RD 12 mm x 4.1 mm Pure Ceramic Straumann AG) 100% 3Y-TZP[1)]	-	4 mm	-	-	-

* Measured from the implant shoulder to the top of the abutment

1) Data according to manufacturers

580 Table 2: Overview of catastrophic and non-catastrophic events after aging, including survival

581 and complication rates (%).

	During aging				After aging						
Groups	Catastrophic events			Survival (%)	Non-catastrophic events						Complication-rate (%)
	Implant	Abutment	Crown		Cracks			Debonding		Screw-loosening	
					Implant	Abutment	Crown	Micro-mov.	Loss of retention		
C	0	0	0	100% (12/12)	0	0	0	0	0	58.3% (7/12)	58.3% (7/12)
T1	16.7% (2/12)	8.3% (1/12)	0	75% (9/12)	33.3% (3/9)	0	0	0	0	0	33.3% (3/9)
T2	0	16.7% (2/12)	0	83.3% (10/12)	0	0	0	0	0	10% (1/10)	10% (1/10)
T3	0	16.7% (2/12)	0	83.3% (10/12)	0	0	0	0	0	50% (5/10)	50% (5/10)
T4	0	0	0	100% (12/12)	0	0	25% (3/12)	0	100% (12/12)	n.a.	100% (12/12)

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585 Table 3: Overview of failure modes after fracture testing (%)

	After fracture load test			
Groups				
	Abutment fracture	Crown fracture	Implant fracture	Implant deformation
C	0/12 (0%)	12/12 (100%)	0/12 (100%)	0/12 (0%)
T1	3/9 (33.3%)	0/9 (0%)	6/9 (66.7%)	0/9 (0%)
T2	0/10 (0%)	10/10 (100%)	0/10 (0%)	0/10 (0%)
T3	1/10 (10%)	0/10 (0%)	9/10 (90%)	0/10 (0%)
T4	0/12 (0%)	9/12 (75%)	3/12 (25%)	0/12 (0%)

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595 Figure 1: Specimens according to each group, before placing the crown: (a) C, (b) T1, (c) T2,

596 (d) T3, (e) T4; and after placing the crown: (f) C, (f) T1, (h) T2, (i) T3, (j) T4.

597 (a) 

598 (b) 

599 (c) 

600 (d) 

601 (e) 

602 (f) 

603 (g) 

(h) 

(i) 

(j) 

615 Figure 2: Kaplan-Meier survival curve showing the cumulative survival considering

616 catastrophic events and respective cycle of failure.

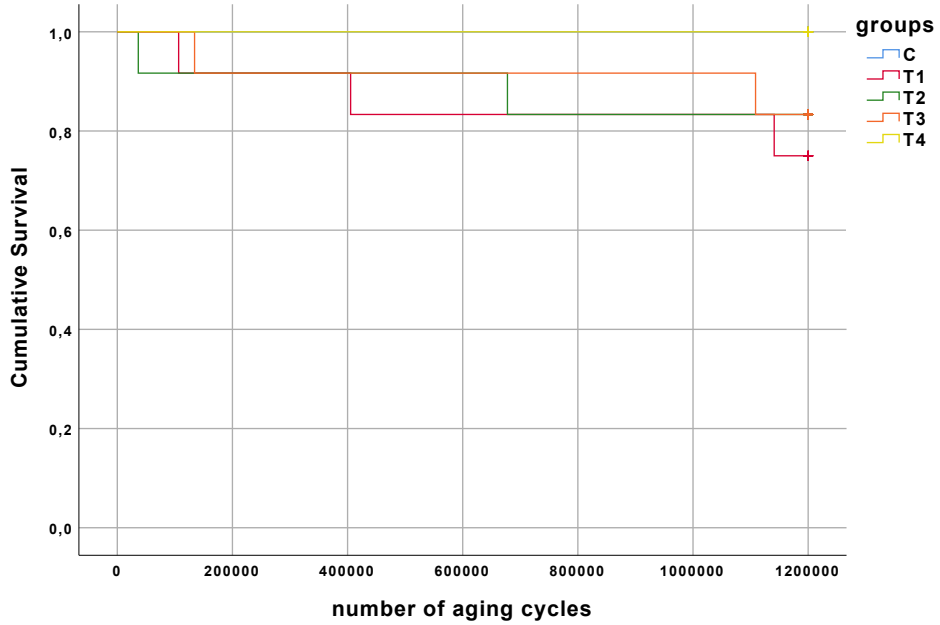

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Figure 3: Bending moments (Ncm) of all specimens that survived to thermo-mechanical aging, including the complications retrieved. Box plots illustrate medians and interquartile ranges. Statistically significant differences are indicated by an orange horizontal line below the box plot ($p < 0.05$), while º and * represent outliers.

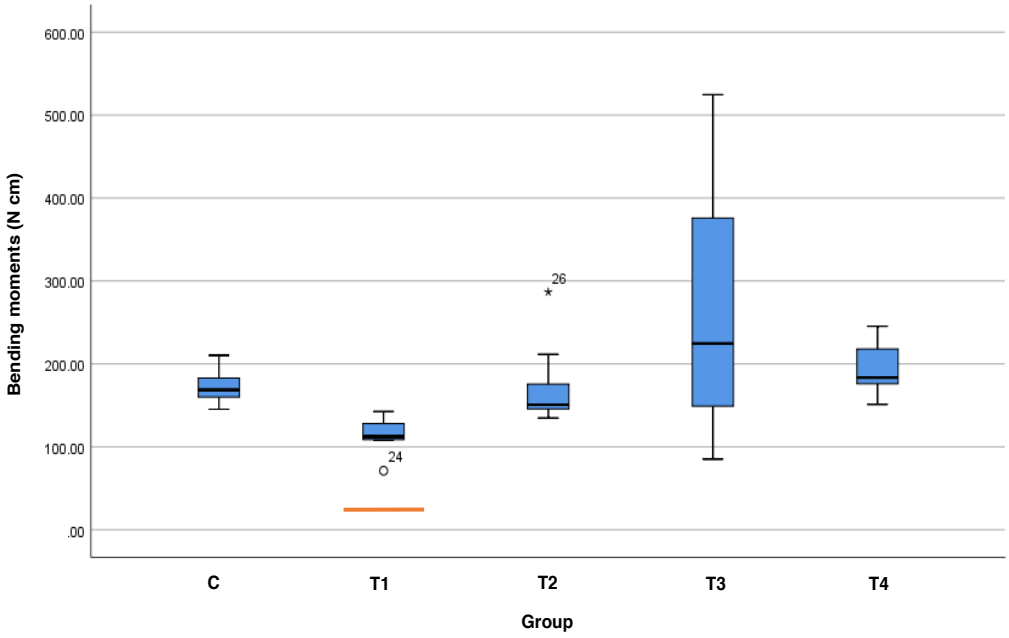
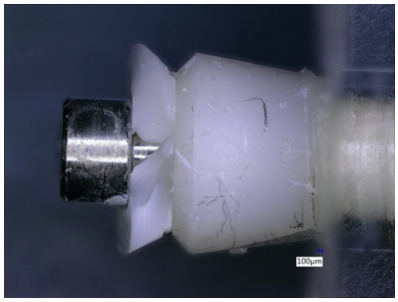
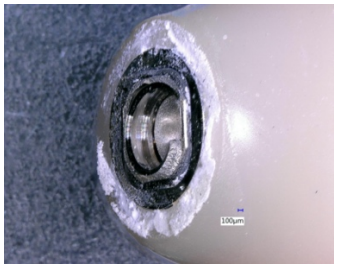
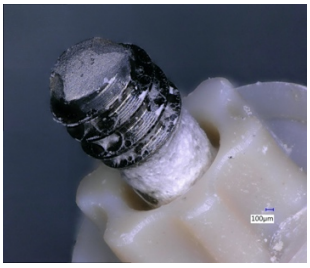


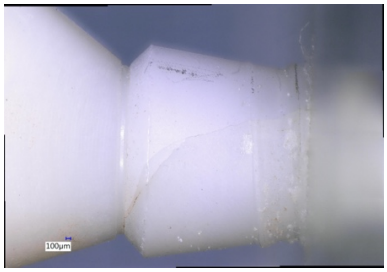
Figure 4: Representative images of catastrophic of (a, b) T1, (c) T2 and (d) T3; and non-catastrophic events of (e) T1 and (f) T4 under 30x light microscope magnification (VHX-6000, Keyence).

(a) 

(b) 

(c) 

(d) 

(e) 

(f)