Performing Reliable Lens Capsulotomy in the Presence of Corneal Edema With a Femtosecond Laser

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PURPOSE. To determine the effects of the Ziemer LDV Z8 liquid interface femtosecond laser platform during capsulotomy under different energy settings in the presence of corneal edema.

METHODS. Cadaveric porcine eyes (n = 36) employed at less than 6 and greater than 24 postenucleation hours to simulate clear/edematous corneas, underwent capsulotomy with the Ziemer LDV Z8 femtosecond laser (5-mm diameter, energy 90%, 130%, or 150%). Lens capsules were removed for evaluation by scanning electron microscopy and rupture strength determined by the single column universal testing system. Following ethical approval, 23 patients had lens capsules removed during routine cataract surgery following manual or Z8 capsulotomy and subjected to TUNEL assay.

RESULTS. There was no difference in edge morphology or rupture strength (120, 113, and 118 mN at increasing energy, P = 0.42) in the clear cornea. Only 50% of capsulotomies succeeded at 90% energy in an edematous cornea, improving with increased energy (75% completion at 150%, 100% at 150%). Rupture strength in edematous corneas was not significantly different at 112, 133, and 114 mN for 90%, 150%, and 150%, respectively (P = 0.3). In human samples, increased TUNEL-positive cells were seen at 130% energy, but not at 150% (0.0 manual vs. 0.2 [90%] vs. 2.1 [130%] vs. 0.6 [150%], P < 0.05).

CONCLUSIONS. Because of the low energy delivered by a femtosecond nanojoule platform, even incremental increases in energy appeared to have minimal effect on lens capsule morphology and strength and negligible influence on cell death. Furthermore, increasing energy appeared to enhance consistency and the ability to complete a capsulotomy in an edematous cornea.

Keywords: femtosecond laser, corneal edema, energy, capsulotomy

Endothelial dysfunction caused by conditions such as Fuchs’ endothelial corneal dystrophy (FECD) and bullous keratopathy result in visual impairment due to corneal edema and scarring.1–4 When corneal opacification occurs in addition to corneal edema, optimized visualization of the lens capsule by capsular staining or retroillumination may assist continuous curvilinear capsulorhexis (CCC),5,6 but an endothelial transplant is usually indicated at the same sitting. Surgical management of corneal disease is achieved by selective tissue transplantation by Descemet stripping automated (DSAEK) or Descemet membrane endothelial keratoplasty (DMEK).7,8 These interventions are usually undertaken following or combined with removal of cataract.7,9

Cataract surgery may contribute to corneal decompensation because of endothelial cell damage from phacoemulsification energy, however corneal edema may already be apparent. Central corneal thickness increases from healthy individuals (558 μm) incrementally from 586 μm in grade 1 FECD to 648 μm in grade 6 disease.10 However, the precise thickness that predicts the likelihood of decompensation during phacoemulsification in FECD is ill defined. Relatively thin corneas (such as those with grade 1 disease < 600 μm) may be relatively less edematous (thickened), despite frank evidence of decompensation and yet a greater central corneal thickness (CCT) may be seen in an optically clear cornea (e.g., greater than 640 μm following pre-existing high CCT).11

Femtosecond laser-assisted cataract surgery (FLACS) offers a potentially more consistent way of undertaking capsulotomy by creating a semiautomated anterior lens capsuleotomy.12–15 The effects of a thickened yet transparent cornea on successful FLACS is unknown. There is potential to use this technology to reduce disruption of endothelial cells by femto-fragmentation. This may have a specific role in cases where the endothelium is at high risk such as FECD.16,17 Despite these advantages, femtosecond laser firing patterns are vulnerable to enhance light scatter at deeper dissections, even in optically clear corneas. Increased variability is seen, for example, in LASIK flap creation in the peripheral (thicker) compared with central (thinner) cornea.18 This could potentially make femtosecond tissue cutting more challenging in the presence of corneal edema (e.g., penetrating keratoplasty).19,20 As FLACS relies on an optically clear interface, the potential advantage of laser
Capsulotomy and fragmentation could hypothetically be negated by the inability of the laser to fire successfully through a thickened cornea.

FLACS capsulotomy may induce capsular tags, leading to more serious complications such as anterior capsular tears.\textsuperscript{7,1-23} The strength of the capsulotomy in resisting rupture during manipulation of lens material is critical to success.\textsuperscript{24-29} It has recently been shown that increasing the laser energy pulse may adversely affect the strength of the lens capsulotomy.\textsuperscript{24} This effect was noticeable with a high-energy FLACS ('microjoule energy') platform. We have previously shown however that in a low-energy high-frequency system (operating in the nanojoule range) that a safe, smooth capsulotomy could be achieved.\textsuperscript{30} The utility of such a system in the presence of corneal edema is currently unknown, but microjoule energy platforms may penetrate edematous corneas more effectively. High-energy systems have been shown to induce increased apoptosis of the lens epithelium during femtosecond capsulotomy, however, suggesting an advantage for manual capsulotomy including minimal wound healing reaction and lens capsule cell death.\textsuperscript{31-33} By contrast, suction trephination during penetrating keratoplasty demonstrates less apoptosis than femtosecond laser.\textsuperscript{34} The potential for safely increasing energy in nanojoule steps to overcome corneal edema has yet to be resolved.

The aim of this study was to determine the efficacy of the low-energy Ziemer LDV Z8 liquid interface femtosecond laser platform during capsulotomy in the context of corneal edema and at different energy settings to compensate for thickened corneas.

**METHODS**

**Porcine Capsules**

Ex vivo cadaveric porcine eyes were sourced from a local abattoir, and capsulotomies performed at less than 6 hours or greater than 24 hours post enucleation and evaluated with the Ziemer LDV Z8 femtosecond laser (software version \times 5054; Ziemer, Port, Switzerland) for different laser energies (5-mm diameter, 0.8-mm height, cut speed 50 mm/s, energy 90%-150%), as previously described.\textsuperscript{30,35} Briefly, due to a tendency to cadaveric porcine corneal epithelium to slough, for consistency the corneal epithelium was debrided and the globe mounted in a suction stand prior to docking with a liquid patient interface followed by suction, liquid immersion, and attachment of the laser head. Corneas with impairment of subjective clarity (i.e., obvious scarring) opacities were excluded. Forty-four globes were used with \( n = 6 \) in each group for further experimentation (Table).

Lens capsules were removed and immediately placed on to a Millipore millicell culture insert (Merck Millipore, Billerica, MA, USA), photographed (×10 magnification with a Leica microscope; Leica Wetzlar, Germany) and anchored to the underlying membrane with 10-0 Ethicon overlay sutures. ImageJ 1.38X software (http://imagej.nih.gov/ij/) provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) was used to determine capsule/capsulotomy size by pixilation compared with a reference ruler and capsule circularity with the formula \( 4\pi \times \text{Area}/(\text{perimeter})^2 \) (value of 1.0 represented a perfect circle).\textsuperscript{36}

Capsules were washed twice in 1× PBS for 10 minutes each before being immersed in a fixative 2% solution of glutaraldehyde in PBS for 2 hours at room temperature, and then washed three times with distilled water before incubation in a 1% aqueous solution of osmium tetroxide (FMB, Singapore) for 1 hour at room temperature. Following this, the samples were dehydrated in increasing concentrations of ethanol (25%, 50%, 75%, 95%, and 100% ethanol, with 95% and 100% concentrations being performed twice). The samples were then critical point dried using Bal-Tec dryer (Balzers, Liechtenstein) and mounted on stubs secured by carbon adhesive tapes. They were then sputter coated with a 10-nm thick layer of gold (Bal-Tec) and examined using a JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). The edges of the extracted lenticules were then evaluated using scanning electron microscopy. The sample processing was performed as described previously. For each lens capsule, four micrographs, one in each quadrant, were taken (×140, ×750, and ×4000 magnification).

**Table.** Summary of Lens Capsule Completion at Different Energy

<table>
<thead>
<tr>
<th>Energy</th>
<th>Complete Cut</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0 h 90%</td>
<td>100% (6/6)</td>
<td>No complications</td>
</tr>
<tr>
<td>&lt;0 h 130%</td>
<td>100% (6/6)</td>
<td>No complications</td>
</tr>
<tr>
<td>&lt;0 h 150%</td>
<td>100% (6/6)</td>
<td>No complications</td>
</tr>
<tr>
<td>24 h 90%</td>
<td>50% (6/12)</td>
<td>Failed cuts (( n = 3 )), ripped edge (( n = 2 )), and significant tag (( n = 1 ))</td>
</tr>
<tr>
<td>24 h 130%</td>
<td>75% (( n = 8 ))</td>
<td>Failed cuts (( n = 1 )) or ripped (( n = 1 ))</td>
</tr>
<tr>
<td>24 h 150%</td>
<td>100% (( n = 6 ))</td>
<td>No complications</td>
</tr>
</tbody>
</table>

Lens capsulotomy strength was determined by the single column universal testing system (3443; Instron Corp., Canton, MA, USA) after removal of the lens en bloc with nucleus intact or following expression with an ophthalmic viscoelastic device (Viscoat, Alcon, Fort Worth, TX, USA). Two mushroom-shaped pins were placed posterior to the capsulotomy edge and the rate of pin displacement was set at 6 mm/min. Resistance of the capsulotomy to rupture was measured in meganewtons and the stretching ratio by (capsulotomy size mm + displacement mm)/capsulotomy size mm, as previously described.\textsuperscript{30}

**Human Capsules**

Increased cell death at the capsule edge may potentially correlate with a less smooth capsule edge morphology.\textsuperscript{31} In order to determine cell death at increasing energy during FLACS capsulotomy, we evaluated lens capsules ex vivo following routine cataract surgery. Human capsules were collected during routine phacoemulsification cataract surgery (MA, JSM) following informed consent and institutional review board approval in keeping with the tenets of the Declaration of Helsinki (CIRB Ref 2015/2565, Singapore). All patients’ corneal thickness were normal, in the range of 520 to 550 \( \mu \)m. Manual CCC was achieved with a 27-G needle with Viscoat ophthalmic viscoelastic device (Alcon) or capsulotomy with the Ziemer LDV Z8 (Ziemer) femtosecond laser (software version \times 5054) for different laser energies (5-mm diameter, 0.8-mm height, cut speed 50 mm/s, energy 90%-150%), as previously described.\textsuperscript{30,35} Mean patient ages (\( n = 6 \) for each group) were: manual 69 years (range, 49-75), 90% energy 74 years (56-85), 130% energy 71 years (56-76), and 150% 68 years (57-72) \( (P = 0.62) \). All cataracts were senile in origin and with no known trauma or coexisting disease.

After anterior capsule extraction, all specimens were immediately fixed in freshly prepared neutral buffered 4% paraformaldehyde in PBS (0.01M; First Base, Singapore) for 1 hour at 4°C. During fixation, the samples were carefully placed with epithelial side facing upward to prevent any mechanical damage to cells that might induce unnecessary apoptosis. After
fixation, the samples were carefully washed with PBS twice (10 minutes each) and subjected to TUNEL assay.

TUNEL reaction was performed using TMR-In Situ Cell Death Detection kit (Roche, Welwyn Garden City, England, UK), as previously described. Briefly, capsule specimens were permeabilized with 0.1% Triton X-100 (Sigma-Aldrich Corp., St. Louis, MO, USA) in 0.1% Na citrate (Sigma-Aldrich Corp.) for 20 minutes at room temperature (22°C) followed by complete TUNEL reaction. Negative controls were DNase-treated samples without TdT enzyme in TUNEL reaction mix. After incubation, all samples were washed three times with PBS and mounted with 4',6-diamidino-2-phenylindole (DAPI)-added fluoromount (FluoroShield; Sigma-Aldrich Corp.). Cells along the cutting edge were imaged under fluorescence microscopy (AxioImager Z1; Carl Zeiss, Oberkochen, Germany) at a magnification of ×10 (objective). Cells along the cutting edge were positioned in a rectangular field of 100 μm in width and quantified for the total number of cells and TUNEL-positive cells. At least six randomized regions along the cutting edge of each sample were analyzed and cells per millimeter squared and mean percentages of TUNEL cells from different energy settings were obtained.

TUNEL assays were not evaluated in the porcine model, as dead tissue would have been expected to demonstrate positive TUNEL staining irrespective of exposure to capsulorhexis/FLACS capsulotomy.

Statistical analysis was performed with the Wilcoxon paired test, Kruskal Wallis test with Dunn's post hoc test with Prism 5.0 for Macintosh (GraphPad, La Jolla, CA, USA).

RESULTS

Capsulotomy Formation

The effects of corneal edema can be seen in Figure 1A. Allowing the cornea to stand for 24 hours post enucleation resulted in a significant increase in CCT compared with less than 6 hours (1035 μm [range, 906–1140] vs. 766 μm [range, 651–866]; P < 0.05) (Fig. 1B). Successful capsulotomy was achieved in only 50% (6/12) attempts at 90% energy in an edematous cornea. Representative images showing unsuccessful cuts were illustrated in Figure 1C. This improved however with increasing energy (75% completion at 130% [6/8 attempts], 100% at 150% [6/6 attempts]). Six-hour cuts were all completed successfully without complication. These data are summarized in the Table.

Energy Effects on Circularity and Strength in a Clear Cornea

We tested the effects of energy on corneas at less than 6 hours post enucleation at three increasing energy setting—90%, 130%, and 150% (n = 6 in each group). There was a predictable shrinkage of the porcine capsule with a median difference of 0.56 (0.46–0.59), 0.44 (0.36–0.60), and 0.56 (0.52–0.59) mm, respectively (Fig. 2B, P = 0.42). Scanning electron microscopy images of capsule edge are shown at incremental energy increases (Fig. 2C). The consistent smoothness of the edge was reflected in the strength of the capsules when subjected to resistance with no significant difference in 117 mN (109–133) at 90% vs. 116 mN (84–140) at 130% vs. 118 mN (107–136) at 150% (P = 0.88) (Fig. 2D). The stretch ratio, however, increased with a commensurate increase in energy with a stretch ratio of 2.2 (2.0–2.4) at 90% vs. 2.4 (2.2–2.5) at 130% vs. 2.5 (2.4–2.7) at 150% (P < 0.01) (Fig. 2D).

DISCUSSION

The Ziemer LDV Z8 employs a high repetition femtosecond laser at low energy. We have previously evaluated the role of the Ziemer LDV Z8 nanojoule energy platform on lens capsule rupture, which had shown consistency and resistance to rupture with this system. Increasing energy in the microjoule range may adversely affect capsulotomy strength and increase cell death in high-energy platforms (5–15 μJ). But smaller increase in energy as a means of safely circumventing the effects of corneal edema with a low-energy laser (<100 nJ or 0.1 μJ) has not been previously determined. Therefore, in this study we found that the consistency of circularity and lens elasticity was seen both in clear corneas and at increased laser energy—from a conventional recommended setting of 90% to 150%. These observations were similar to our previous experimentation with porcine and human lens capsulotomies.

Energy Effects on Circularity and Strength in Corneal Edema

We retested energy on corneas at 24 hours post enucleation at three increasing energy setting—90%, 130%, and 150% (n = 6 in each group) (Fig. 3A). Circularity remained consistent at different energy settings (Fig. 3A) when capsulotomy was achieved (see above comment on capsulotomy formation), overall there was no difference between circularity at less than 6 hours and 24 hours (0.99 [0.95–1.0] vs. 0.99 [0.95–0.99]; P = 0.89). Predictable capsule shrinkage was seen following capsulotomy with corneal edema, with a median difference of 0.4 (0.26–0.58), 0.38 (0.24–0.62), and 0.52 (0.35–0.7) mm, respectively (Fig. 3B, P = 0.3).

Scanning electron microscopy images of capsule edge following femtocutting in corneal edema are shown at incremental energy increases (Fig. 3C). No gross difference in morphology was seen after successful cutting through an edematous cornea at different energies. Furthermore, capsulotomy rupture resistance showed no significant difference across energy settings 112 mN (61–142) at 90% vs. 133 mN (101–153) at 130% vs. 114 mN (100–143) at 150% (P = 0.27) (Fig. 3D). The stretch ratio was also unaltered at increased energy with a stretch ratio of 2.2 (1.8–2.7) at 90% vs. 2.4 (2.3–2.6) at 130% vs. 2.2 (2.1–2.5) at 150% (P = 0.27) (Fig. 3D). There was no significant difference in strength between nonedematous and edematous corneas at all energy levels or in stretch ratios at 90% or 130% (P > 0.05), but with a significant difference in stretch ratio seen at 150% energy (2.5 vs. 2.2; P < 0.05).

TUNEL Evaluation

Positive and negative controls are shown in Figure 4A. This demonstrated that DNase-treated samples as positive control showed 100% apoptosis; while negative control without TdT enzyme had 0%, confirming validity of the assay. Representative images from manual cut and LDV Z8 femtosecond cut capsulotomies are shown in Figure 4B. Percentage TUNEL-positive cells (n = 6 in each group) were 0% (0–0.5) (median, range) in the manual group, 0.2% (0–0.45) at 90% energy, 2.1% (0.3–4.1) at 130% energy and 0.6% (0–2.5) at 150% energy (P < 0.05).
at 5-mm diameter at 90% energy. However, in the presence of corneal edema, a successful lens capsulotomy was only achieved at increased energy settings (150%). The lower energy setting resulted in 50% failure in edematous corneas including irregular or incomplete cuts, which could lead to anterior capsular tears. This represents a potential challenge for patients who may have corneal edema associated with FECD undergoing FLACS.

The laser focus is anatomically more posterior to facilitate capsulotomy and successful cuts demonstrated that the edges of the capsulotomy appeared smooth and consistent across energy settings, despite edema. We have previously shown similar smooth capsulotomy edge morphology with the LDV Z8 using nanojoule energy, however, there has been shown to be significant variation in the appearance among microjoule energy laser platforms, especially when increasing the energy settings from 5 to 15 μJ. These changes include serrations and morphologic changes including coagulation of collagen fibers. Although increasing energy within one of the microjoule energy laser platforms (e.g., LenSx) showed increased disruption to capsule edge morphology, considerable variation exists in other platforms (e.g., Catalys, Victus).

**Figure 1.** Capsule retrieval, time to completion, and circularity. Color photographs with accompanying optical coherence tomography images showing the corneal clarity and thickness at less than 6 hours and greater than 24 hours post enucleation (A). Note the clear cornea seen in the left photograph and the presence of edema on the right. Thickness measurements are compared in (B) (n = 12; *P < 0.05). Representative photographs of lens capsules, which failed to cut in the presence of corneal edema, are shown in (C). These represent abnormal tearing of the capsule secondary to incomplete cutting. The left images shows a partially cut capsulotomy in situ, the middle panel shows the same capsule following removal with an extended crescent of lens capsule, and the right image shows an irregular, square edge.
**Figure 2.** The effects of energy alterations on capsulotomy circularity, edge, and strength in a nonedematous cornea. Representative images at increasing energy at less than 6 hours post enucleation with circularity (A). Predicted and actual capsule sizes are shown in (B). Scanning electron microscopy images of capsule edge taken at increasing energy are shown in (C). Lenses were removed with nucleus intact and resistance of the capsulotomy to rupture was measured in millinewtons and the stretching ratio by (size mm + displacement mm)/size. Two mushroom-shaped pins were placed posterior to the capsulotomy edge and the rate of pin displacement was set at 6 mm/min. Comparison between strength and stretch ratio at different energies are shown in (D). *P < 0.05, **P < 0.01.
FIGURE 3. The effects of energy alterations on capsulotomy circularity, edge, and strength in an edematous cornea. Representative images at increasing energy at greater than 24 hours post enucleation with circularity (A). Predicted and actual capsule sizes are shown in (B). Scanning electron microscopy images of capsule edge taken at increasing energy are shown in (C). Lenses were removed with nucleus intact and resistance of the capsulotomy to rupture was measured in millinewtons and the stretching ratio by (size mm + displacement mm)/size. Two mushroom-shaped pins were placed posterior to the capsulotomy edge and the rate of pin displacement was set at 6 mm/min. Comparison between strength and stretch ratio at different energies are shown in (D). NS, not significant. *P < 0.05. **P < 0.01.
FIGURE 4. Human lens capsule TUNEL staining following femtosecond capsulotomy. Positive controls pretreated with DNase and TdT enzyme and negative controls without TdT enzyme are shown in (A). Representative fluorescence microscopy images (×10) from manual cut and LDV Z8 femtosecond cut capsulotomies at 90%, 130%, and 150% are shown in (B). Percentage TUNEL-positive cells (taken from 6 randomized regions along the cutting edge of each sample in a rectangular 100-μm field) are shown in (C). *P < 0.05.
reflecting incrementally larger differences in energy delivery.21,23,31,38,39 We did not see this with the nanojoule energy laser used in clear, nonthickened, or edematous corneas in this study.

It is likely that the magnitude of the energy increase with a nanojoule energy platform (90% vs. 130% vs. 150%) does not induce collagen melting and denaturation seen at higher energy settings with a microjoule energy platform.2,25 Unlike previous studies evaluating increasing microjoule energy,25 we saw no significant difference in the strength of the capsulotomy at increasing energy in the nanojoule range (90% vs. 150% vs. 150%). This was unaffected by the presence or absence of corneal edema and consistent with the edge morphology demonstrated. This suggests that the 150% setting has incrementally lower energy than for platforms operating in the microjoule range.15 We cannot explain why the stretch ratio increased at 150% energy as intuitively as we might have expected this to reduce. The elastic recoil seen in the capsulotomy was consistent with our previous data and the behavior of porcine lenses.40 It would suggest that this was unaffected with the LDV Z8 laser and despite the personal observation of a temporary whitening of the capsulotomy edge at higher (150%) energy setting in patients (MA) compared with 90% energy.

TUNEL staining demonstrated low apoptosis across energy settings. DNase-treated positive controls showed 100% apoptosis; while negative control without TdT enzyme had 0%. This validated the assay and is important when considering energy disruption to the capsule itself, which was low, albeit at a statistically higher level in the 130% setting. It is unclear why this peaked at this energy level but it is possible that removal of the lens capsule may have been more traumatic in these capsules. At less than 2% TUNEL-positive cells however we postulate that this appeared to be clinically insignificant. Mayer et al.31 previously showed that by contrast there were significantly higher levels of cell death in the lens capsule at increasing energy, from 30 cells/mm² at 5 μJ to greater than 80 cells/mm² at 15 μJ laser pulse energy. Due to the possibility of underrepresenting TUNEL-positive cells by counting three randomized regions of the cutting edge,31 we quantified percentage of cell death along the whole edge and suggest that it is more representative than cells per millimeter squared. Nonetheless, counts were 7.5 cells/mm² for manual cut versus 13 cells/mm² at 90% energy, versus 68 cells/mm² at 150% energy versus 42 cells/mm² at 150% with the nanojoule laser. Even at 150% the nanojoule energy laser was focused with minimal collateral damage, showing the safety of increasing the energy setting in the context of corneal edema.

Limitations in this study include the absence of data on corneal gutter and/or scarring.31,41 The results are therefore more relevant for patients who have FEDC with the presence of corneal thickening. In conditions such as FEDC or viral endothelitis where guttae and/or scarring may coexist the influence of these parameters on laser scatter are unknown. Correlation between and porcine and human corneal edema is currently unknown, however the degree of edema is exaggerated in porcine corneas (1035 μm) compared with most human clinical situations. We were also unable to ascertain if TUNEL staining differed between the capsule edge examined or the residual capsular bag. We have previously shown there are similar cellular changes occurring either side of a femtosecond laser cut in the cornea and have no reason to believe this will be different on the anterior capsule.54 All other data however point to a consistent, smooth, and strong capsule edge in a clear or edematous cornea in our porcine model. Furthermore, energy effects of a femtosecond laser platform in an edematous cornea have not previously been explored in any model.

We have demonstrated that the LDV Z8 femtosecond laser was able to create a consistent, circular, and smooth capsulotomy that was capable of resisting stress at increased energy. Because of the low energy delivered by the Ziemer Z8 platform, even the incremental increases in energy appeared to have minimal effect on lens capsule morphology and strength and negligible influence on cell death. In eyes with FEDC and early decompensation, there are potential benefits of undertaking FLACS, and thereby reducing endothelial damage from phacoemulsification energy.41,42 Increasing energy in the nanojoule range appears to enhance consistency in the ability to complete a capsulotomy in an edematous cornea, which may be useful when performing FLACS in eyes with concurrent corneal pathology such as FEDC. We therefore advocate that greater energy should be used in an edematous cornea for FLACS-assisted capsulotomy.

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