

A comparison study of the degradative effects and safety implications of UVC and 405 nm germicidal light sources for endoscope storage



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ABSTRACT

Storage of flexible endoscopes under germicidal ultraviolet (UVC) light has been associated with degradation of device material leading to failure and increased risk to patients. 405 nm germicidal light presents a possible alternative, potentially providing effective bacterial inactivation without material damage. Samples of endoscope material were exposed to UVC and 405 nm germicidal light sources and a broad spectrum light source control. Material properties were monitored using FTIR, AFM, contact angle and confocal microscopy. Significant changes were observed with samples exposed to the UVC source, with variations in FTIR spectra indicative of side chain scission, a decrease in contact angle from 82.6° to 61.4°, an increase in average surface roughness from 2.34 nm to 68.7 nm and visible cracking of the surface. In contrast samples exposed to the 405 nm light source showed little to no changes, with any variations being comparable to those seen on samples exposed to the broad spectrum control. Bacterial adhesion tests on samples showed an 86.8% increase in *Pseudomonas aeruginosa* adhesion on UVC exposed samples and no significant increase in adhesion with samples exposed to the other light sources. 405 nm germicidal light therefore potentially represents a safer alternative to UVC light for use in flexible endoscope storage.

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1. Introduction

With the ever present issue of hospital acquired infections, decontamination and sterilisation technologies are of great interest in modern healthcare. A wide range of technologies play a role in sterilisation and infection control; in some cases, however, these methods can result in the degradation of underlying material [1–4]. As part of the infection control measures for flexible endoscopes, germicidal ultraviolet (UVC) light has been incorporated into some specialised drying and storage cabinets. These light sources have been implicated in the degradation of endoscope material, prompting warnings from manufacturers and regulatory bodies [5,6].

Infection control is important for flexible endoscopes [7–9]; they are commonly used, with more than 11 million procedures annually in the U.S. alone [10] and have been linked to more infectious outbreaks and pseudo-outbreaks than any other medical device [11]. Drying and storage cabinets contribute to control measures by ensuring the microbiological quality of endoscopes following disinfection, allowing safe storage for an increased period of time without repeat decontamination [12,13]. UVC light provides air and surface decontamination, promoting aseptic storage conditions; however any degradation of the endoscope material could be detrimental to device performance and patient safety.

UVC-light (200–280 nm) is known for its germicidal properties making it suitable for decontamination applications [14]. UV radiation is also absorbed by many polymers resulting in photodegradation: bond scission and chemical transformations creating structural heterogeneities [15,16], leading to the loss of material characteristics and properties. In endoscope storage, damage

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manifests as cracking of flexible components [5,6]. This presents major risks, as cracks have the potential to increase biofouling and inhibit adequate cleaning. Whilst it is thought this observed degradation is attributable to the UVC exposure, there is little direct supporting literature.

Recent studies investigating visible violet-blue light with a wavelength in the region of 405 nm have shown it to have broad germicidal efficacy [17], and due to the differing wavelengths, the mechanism of action is quite different to that of UV light. UV light is absorbed by thymine cytosine bases within microbial DNA, causing crosslinking and photoproducts that interrupt transcription and replication leading to mutations and cell death [14,18]. Microbial inactivation by violet-blue light in the region of 405 nm, is attributed to the absorption of photons in this region, termed the Soret band, by intracellular porphyrin molecules, which become photo-excited, resulting in the generation of reactive oxygen species, such as singlet oxygen and H_2O_2 , and oxidative damage to the microbial cells [19]. Literature suggests that light over 400 nm lacks the photodegradative effects associated with UV [20]. Therefore 405 nm light has potential as an alternative to UVC in flexible endoscope storage, providing the air and surface decontamination required for aseptic storage conditions without device damage or increased patient risk.

The aim of this study was to investigate whether UVC exposure does indeed degrade endoscope material, and verify the polymer safety of 405 nm light as an alternative. In the reported cases of flexible endoscope damage thought to be caused by storage under ultraviolet light, degradation manifested as cracking and blistering of the material surface [5,6]: cracking and changes in surface topography were therefore the primary manifestation of photo-degradation expected with germicidal light exposure of flexible endoscope material. Sample material exposed to each germicidal source was monitored for degradation using a number of techniques: changes in the polymer structure were monitored using Fourier Transform Infrared Spectroscopy (FTIR); changes in wettability were measured using Contact Angle Goniometry (CAG); and changes in surface roughness (R_a) and topography were monitored using Atomic Force Microscopy (AFM) and imaged using Confocal Microscopy. To examine the potential for increased biofouling, adhesion of *Pseudomonas aeruginosa* on exposed material was investigated. *P. aeruginosa* was selected for use due to it being commonly associated with endoscope contamination [21,22] and it has been implicated in more endoscopy-related outbreaks and pseudo-outbreaks than any other bacterial species [10].

2. Materials and methods

2.1. Material

A colonoscope flexible insertion tube (Order code D757-U5030-2) was purchased from Pentax Europe GMBH (Germany). The internal metal support material and mesh were removed leaving the outer elastomeric material required for this study. This was then cut into 5×5 mm samples, for degradation testing, and 10×10 mm samples for bacterial adhesion testing.

2.2. Experimental setup

Samples were exposed to: a 55 W germicidal UV fluorescent tube with a peak output at 254 nm (GBX55/UVC/2G11, GE Lighting, USA); a germicidal violet-blue light source consisting of an array of 9 LEDs (GE Lighting, USA) with a peak output at 405 nm; and, as a non-germicidal control, a broad-spectrum 55 W fluorescent tube (PL-L 55W/840/4P, Philips Lighting, Netherlands) (Fig. 1). Material samples were exposed for 400 h, in sealed enclosures at a distance

of 35 cm, and an irradiance of 2.6 mWcm^{-2} . Samples for degradation testing were exposed in increments of 100 h, with characteristics monitored every 100 h, samples for bacterial adhesion underwent a single 400 h exposure. During exposure, the surface temperatures never exceeded $36 \text{ }^\circ\text{C}$ which is within the safe operating temperature for endoscopes [23] and below the maximum temperature endoscopes are exposed to during the cleaning process [24].

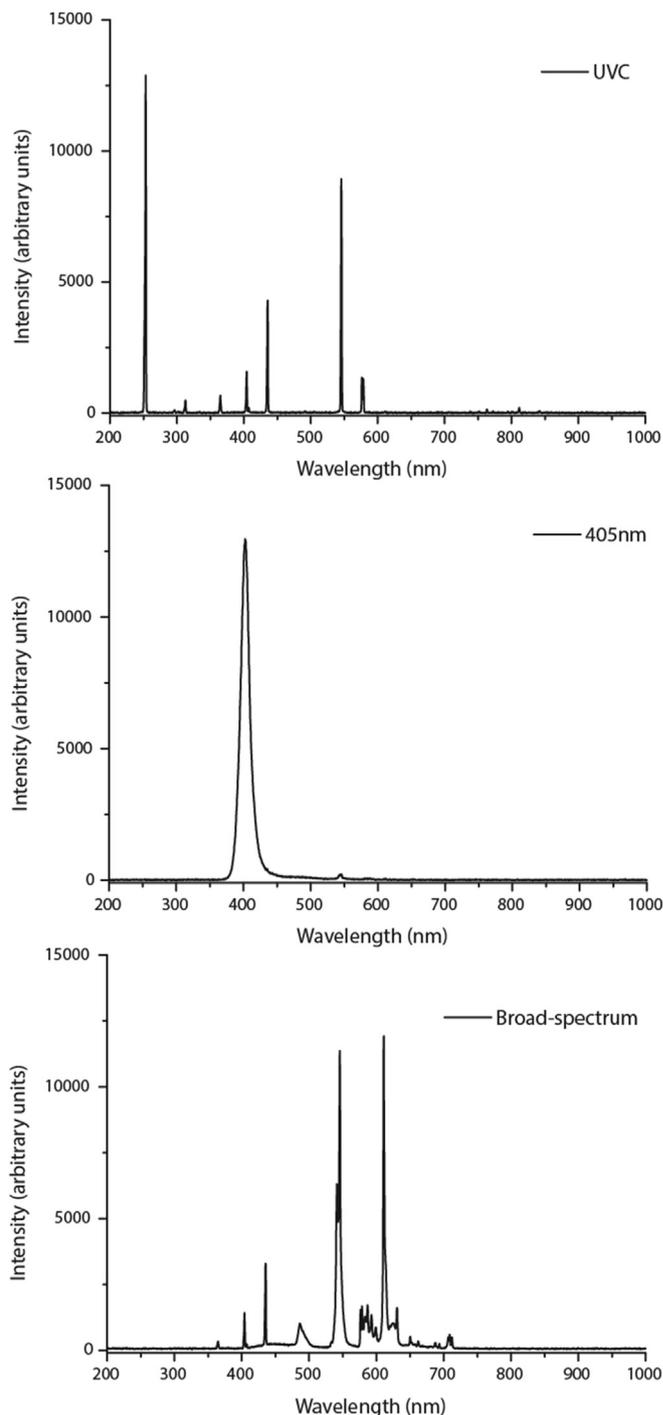


Fig. 1. Emission spectra for the UVC fluorescent tube, 405 nm LED arrays, and broad-spectrum fluorescent tube, measured using a high resolution spectrometer (Ocean Optics Inc, USA).

2.3. Characterisation methods

FTIR spectra were recorded, using the Attenuated Total Reflection (ATR) sampling technique, on a Nicolet iS10 (Thermo Scientific, USA) Smart iTR spectrophotometer (unexposed $n = 27$, exposed $n = 9$). The spectra were measured between 4000 cm^{-1} and 500 cm^{-1} over 16 scans at a resolution of 4 cm^{-1} and an interval of 1 cm^{-1} . They were processed using OriginPro 8.6 software (OriginLab, USA).

To monitor changes in wettability of the material samples, sessile drop contact angle for distilled water was measured by contact angle goniometry (unexposed $n = 18$, exposed $n = 6$), using a goniometer (Kruss G30, Germany) as described by Lamprou et al. [25].

The Roughness Average (R_a) of the material surfaces was measured using AFM to monitor the surface topography (unexposed $n = 27$, exposed $n = 9$). The R_a is calculated as the average height deviations from the mean plane. A Bruker Multimode 8 microscope (Digital Instruments, USA), with ScanAsyst-Air probes (Bruker, USA) was used in Peak Force QNM (Quantitative Nano Mechanics) mode, as described by Lamprou et al. [26]. A scan size of $5\text{ }\mu\text{m}$ was used for the measurements. R_a values were determined using Nanoscope Analysis software V1.40 (Bruker USA).

Images of the material surface were obtained using a confocal microscope incorporated into a Thermo Scientific DXR Raman Microscope (Thermo Scientific, USA) with a $20\times$ magnification objective lens. Images were captured using μView software (Thermo Scientific, USA).

2.4. Bacterial adhesion

P. aeruginosa LMG 9009 (Laboratorium voor Microbiologie, Universiteit Gent, Belgium) was cultured in nutrient broth (Oxoid Ltd, UK) at $37\text{ }^\circ\text{C}$ for 18 h at 120 rpm, centrifuged at $3939\times g$ for 10 min, and re-suspended in phosphate buffered saline (PBS; Oxoid Ltd, UK). Exposed and control material samples were cleaned with ethanol then immersed in $10\text{ ml } 10^7$ colony-forming units per millilitre (CFUml^{-1}) bacterial suspension for 30 min at room temperature to allow bacterial attachment. Samples were then dried for 20 min in a laminar flow cabinet to facilitate further adhesion [27]. Adhered bacteria were removed using a moistened cotton swab. The swab was then immersed in a suspension of 9 ml PBS and $1\text{ ml } 3\%$ Tween-80, vortexed for 30 s to re-suspend the bacteria, serially diluted in PBS and plated using the pour plate method. Plates were incubated at $37\text{ }^\circ\text{C}$ for 24 h and enumerated, with results reported as CFUml^{-1} ($n = 15$).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) with a confidence interval of 95% was used to test the statistical significance of variations in wettability, roughness and bacterial adhesion using OriginPro 8.6 software (OriginLab, USA).

3. Results

The average FTIR spectra obtained from unexposed samples was used to better identify the composition of the material samples, an exact identification of the sample material is challenging as it is likely a proprietary composition unique to the manufacturer. Comparison with a library of known materials (S.T. Japan USA LLC.) indicated that the material was likely poly(methyl methacrylate) (PMMA) based (93.8% similarity) with strong similarities to copolymers including poly(methyl methacrylate: butadiene) (95.1%), poly(methyl methacrylate-co-ethyl methacrylate) (93.7%) and

poly(butyl methacrylate-co-methyl methacrylate) (93.1%). The spectra also bore strong similarities with other poly(alkyl methacrylate)-based polymers [28–30] supporting these matches.

Comparing the spectra for samples exposed for 100 h to 405 nm and broad-spectrum sources with unexposed samples (Fig. 2A) showed no notable variation, indicating that the structure of the material was predominantly unaffected by either light source. Major changes to the material with 100 h exposure to the UVC source were, however, visible across the spectra. There were notable changes in the region between 2500 and 4000 cm^{-1} in particular (Fig. 2B). The group of peaks 2860 – 2950 cm^{-1} , corresponding to C–H bond stretching [31], decreased whilst the broad peak centred at 3390 cm^{-1} , corresponding to O–H bond stretching increased markedly with 100 h exposure.

Further exposure to both 405 nm light and the broad spectrum control caused no notable changes in the spectra; however 200–400 h exposure to the UVC source caused a decrease in peaks across the whole spectrum. This may in part be attributable to bond scission but is most likely caused by physical breakdown of the surface, reducing material contact with the spectrometer crystal. Whilst this is an indication of degradation it made the results from these exposure times difficult to interpret and less valuable for characterisation.

This physical breakdown of the surface can be seen in the AFM results (Table 1). Material exposed to the UVC source for 400 h showed a significant increase in R_a value, from $2.34 \pm 1.20\text{ nm}$ to $68.70 \pm 51.08\text{ nm}$, corresponding to an increase in surface roughness. Samples exposed to the 405 nm light source showed no significant change in R_a measurements, indicating no notable changes to the surface topography following exposure. The samples exposed to the broad spectrum control showed a slight decrease in average roughness following exposure. Whilst this change was statistically significant, it was a fraction of the change observed in material exposed to UVC. The R_a values for the samples exposed to the UVC source also had a large standard deviation; suggesting that exposure caused a decrease in the uniformity of the surface topography, which was previously relatively homogenous.

There was no significant change in contact angle following 400 h exposure to the 405 nm and broad spectrum sources, relative to the unexposed samples, indicating that exposure had no effect on the

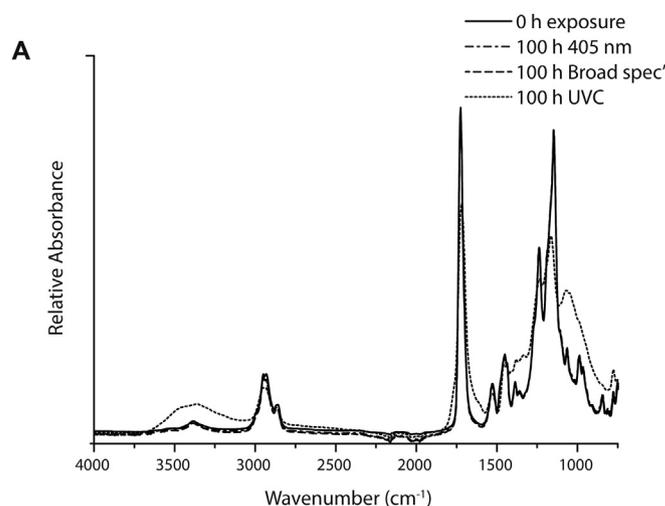


Fig. 2. FTIR spectra for endoscope samples before and after 100 h exposure. (A) Average spectra for unexposed samples and samples exposed to the 405 nm, broad-spectrum and UVC light sources, between 4000 cm^{-1} and 500 cm^{-1} . (B) Average spectra for unexposed samples and samples exposed to the UVC light source, between 4000 cm^{-1} and 2500 cm^{-1} . Unexposed $n = 27$, exposed $n = 9$.

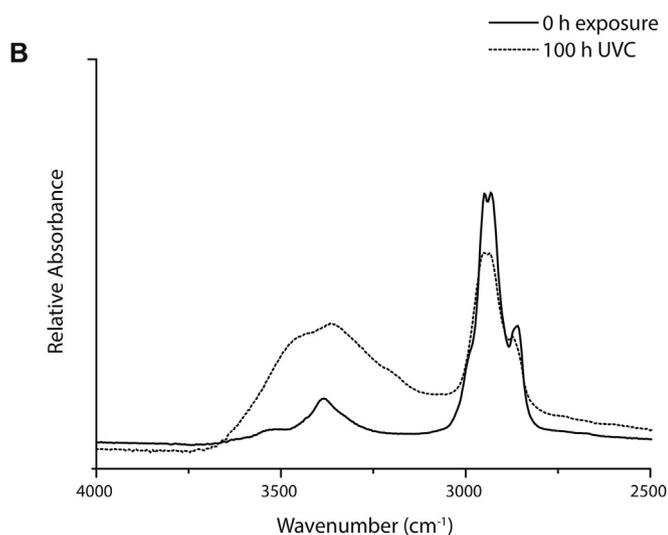


Fig. 2. (continued).

wettability of the material. Samples exposed to the UVC source, however, showed a significant change in wettability, with a significant decrease in the contact angle, from $82.6 \pm 4.6^\circ$ to $61.4 \pm 2.3^\circ$, after 400 h exposure.

The breakdown of the surface under the UVC source can also be seen in the optical microscopy images (Fig. 3), with visible cracking following 400 h exposure. As with the other material characteristics, no visible changes to the material surface were seen with 405 nm light exposure. Similarly the broad spectrum control appears to have caused no notable changes to the surface.

Microbiological results (Fig. 4) showed significantly increased bacterial adhesion on UVC exposed samples compared to the unexposed ($P = 0.001$), broad-spectrum exposed ($P = 0.002$), and 405 nm exposed ($P = 0.006$) samples. Bacterial counts corresponded to an increase of up to 86.6% in adhered *P. aeruginosa* on UVC damaged material. The level of bacterial adhesion on 405 nm light exposed surfaces was not significantly different to either the unexposed or broad-spectrum exposed control samples ($P = 0.170$ and 0.238 , respectively).

4. Discussion

The use of germicidal UVC-light in the storage of endoscopes as part of infection control measures has been implicated in the degradation of endoscope material, with increased risk of contamination and infection transmission. This study investigated the effect of exposing sample endoscope material to a UVC-light source as well as an alternative 405 nm germicidal light. Overall, results indicate that UVC-light is capable of causing notable degradation, whereas 405 nm light had little to no impact on

material properties. This degradative effect also encouraged increased bacterial attachment to the UVC damaged endoscope material.

The changes following UVC exposure seen in the FTIR spectra is congruent with photodegradation of similar polymers, suggesting these are good indicators of damage following exposure to this light source. In particular, the downward trend in peaks corresponding to C–H bond stretching at $2860\text{--}2950\text{ cm}^{-1}$, and the upward trend in the broad peak related to O–H stretching centred at 3390 cm^{-1} , correspond well with photodegradation of other poly(alkyl methacrylate) based polymers [28–30]. This change in spectra could be attributable to the scission of partial or complete side chains. Scission of ester side chains during photodegradation of PMMA can result in the generation of methyl formate and methanol which could account for the trends in C–H and O–H related peaks [15,30]. Additionally, if the material contains butyl methacrylate or ethyl methacrylate units, as suggested in the library match for the sample spectra, then scission within these longer ester side chains could result in the formation of methacrylic acid groups [29]. Again this would account for the apparent decrease in C–H bonds and increase in O–H bonds. Alternatively, if the material contains butadiene units, as indicated in the closest library match, the appearance of the broad O–H peak could be due to the degradation of these units. Butadiene units are highly susceptible to UV degradation and the appearance of a distinct hydroxyl peak at $3200\text{--}3600\text{ cm}^{-1}$ has been seen in the FTIR spectra of other butadiene containing polymers, such as Acrylonitrile Butadiene Styrene (ABS), following UV exposure [32].

The increased roughness and cracking of the surface, visible with AFM and optical microscopy, following UVC exposure is likely the precursor to the cracking and blistering described in warnings regarding the use of UVC sources in endoscope storage [5,6]. Increased roughness as an early indicator of photodegradation has been recorded with a number of polymers following UV irradiation including poly(methyl methacrylate) based polymers similar to that used in this study [33,34]. The loss of surface uniformity noted in this study has also been seen with the photodegradation of PMMA in other work, suggesting this irregular cracking of the surface is due to the degradation of the material [34].

The changes observed in surface topography are likely responsible, at least in part, for the loss of hydrophobicity seen with UVC exposure. The phenomenon of surface topography impacting on wettability is well documented [35]. It has been postulated that changes in surface area, localised contact angle and factors including air entrapment and wicking all result in roughness changes impacting on the wettability of a surface. For materials where $0^\circ \leq \theta \leq 90^\circ$ an increase in roughness results in an increase in surface hydrophilicity [36] as seen in this study.

It is clear that UVC-light caused notable photodegradation of the sample material, and that none of these changes were observed in the material exposed to 405 nm light under the conditions tested. The damage caused by the UVC-light would clearly affect the endoscope life-span; however, a greater concern is the potential

Table 1
- Contact angle and R_a values for endoscope material before and after exposure to UVC, 405 nm and fluorescent broad spectrum light sources. Results show the mean \pm SD. P-values show significance of variation between non exposed and exposed measurements calculated using one way ANOVA. Unexposed $n = 27$, exposed $n = 9$. *Significant change in measurement ($P \leq 0.05$).

	Contact angle		Roughness average	
	$\theta/^\circ$	P-value	R_a/nm	P-value
0 h exposure	82.6 ± 4.6	–	2.34 ± 1.20	–
400 h 405 nm	80.7 ± 3.2	0.353	2.32 ± 1.41	0.962
400 h broad spectrum	79.2 ± 2.2	0.090	1.25 ± 0.56	0.013*
400 h UVC	61.4 ± 2.3	$2.72 \times 10^{-8*}$	68.70 ± 51.08	$5.02 \times 10^{-8*}$

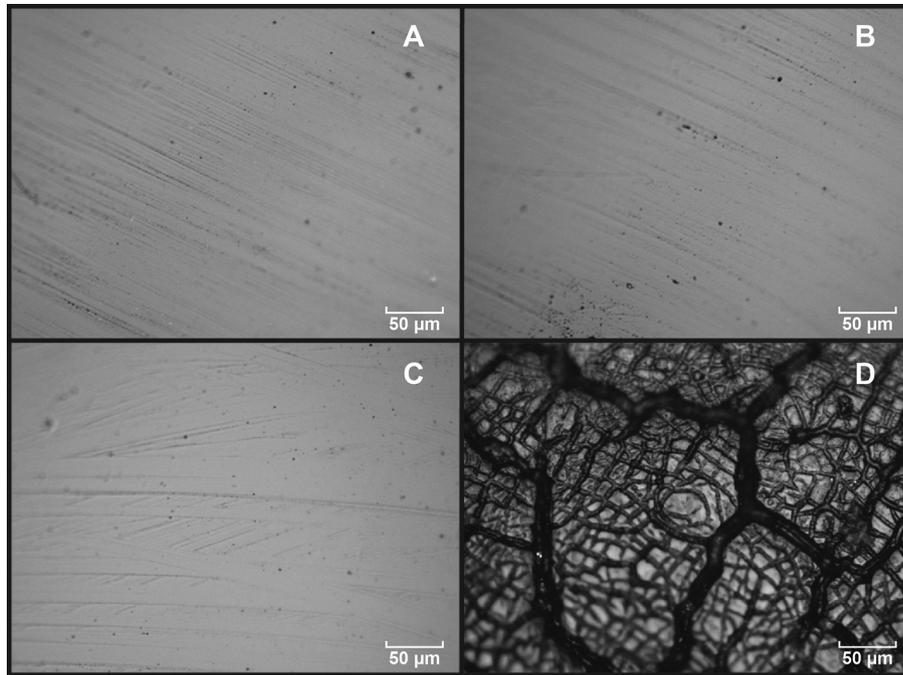


Fig. 3. Confocal microscope images of sample endoscope material surfaces before and after exposure to the light sources; (A) 0 h exposure, (B) 400 h 405 nm light exposure, (C) 400 h broad-spectrum exposure, (D) 400 h UVC exposure.

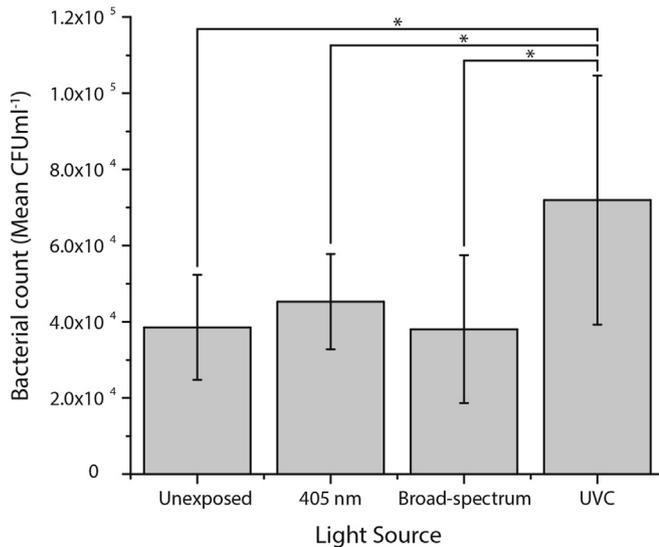


Fig. 4. – *P. aeruginosa* adhered to unexposed endoscope material samples, and samples exposed to 405 nm, broad-spectrum and UVC light sources. Error bars indicate SEM, $n = 15$. * Indicates statistically significant difference between sample exposure types ($P \leq 0.05$) calculated using one way ANOVA.

impact on relevant microbiological aspects.

Exposure to UVC-light increased the adhesion of *P. aeruginosa*: an effect not observed with 405 nm or broad-spectrum light. Factors including topography and wettability influence the adhesion of bacteria to a material surface [37]. Surface wettability can influence interactions with bacteria and affect the adsorption of proteins to the polymer surface, ultimately effecting bacterial adhesion [38]. Topographical changes can impact on bacterial adhesion not only by altering wettability but also by directly influencing adhesion. Increased surface roughness increases surface area and decreases

shear forces potentially increasing bacterial adhesion [39,40]. There are optimum values of surface roughness that can encourage bacterial adhesion and retention [41,42] and inhibit removal of bacteria [43] depending upon species and cell dimensions.

Increased adhesion could have a serious detrimental effect on infection control. An increase in bacteria on the device surface, and the potential inhibition of bacterial removal, would increase the chance of contamination surviving the disinfection process. Further degradation could compound this problem as larger cracks and defects could retain and protect higher bacterial populations.

In practical terms, storage cabinets incorporating 405 nm light could have significant operational benefits over designs using UVC by avoiding device photodegradation, and subsequent inefficient cleaning from contaminants residing in UV-induced microscopic cracks. Cabinets with 405 nm light would also have infection control benefits over non-UV endoscope cabinets by providing the added benefit of safe air and surface decontamination. The photo-oxidative reaction would facilitate the decontamination of any residual contaminants from handling/inefficient washing, and also inactivation of environmental contamination in the internal air and surfaces of the cabinet, without having sufficient energy to induce bond scission and chemical transformations of the polymer. Incorporation of 405 nm light into cabinets could potentially extend the safe storage duration, reducing the requirement for re-disinfection of unused endoscopes due to concerns of recontamination. Any decrease in the number of endoscopes having to be unnecessarily re-cleaned corresponds to a saving in both time and resources. This is particularly relevant with the burdened and cost conscious healthcare environment of today, potentially providing financial savings alongside increased patient safety. Further studies are required to ensure compatibility with other types of endoscope materials, and that 405 nm light can provide a sufficient germicidal effect within a storage cabinet, inactivating relevant contamination levels and organisms.

5. Conclusion

It is clear from this study that exposure of flexible endoscope insertion tube material to germicidal UVC-light caused substantial photodegradative damage. It is also clear that exposure of the same sample material to an alternative 405 nm light germicidal source under the same conditions had no notable detrimental effect on the material properties considered. The degradation caused by the UVC source could be detrimental to not only the device life-span but also to patient safety, with UVC exposed samples showing increased bacterial adhesion properties not seen on samples exposed to 405 nm light. 405 nm germicidal light therefore represents a promising alternative to UVC for inclusion in an effective, hygienic, endoscope drying and storage system. Further work will be required to investigate whether 405 nm light shows similar results with a range of other applicable endoscope materials and to determine if it is capable of effectively inactivating bacterial contamination relevant to endoscope storage.

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