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Detecting Enteral Nutrition Residues and Microorganism Proliferation in Feeding Tubes via Real-Time Imaging.

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Abstract

Background:

Enteral nutrition (EN) residues that persist in feeding tubes provide substrates for microorganisms to proliferate and occlude the tubes. Visible EN residues in tubes are easily identified, but smaller residues can persist. We developed a new imaging technique to visualize EN residues and proliferation of microorganisms in feeding tubes.

Materials and Methods:

Feeding tubes containing EN labelled with fluorescent dye and EN with or without various types or amounts of thickeners were flushed once with water and then the tubes were seeded with *Pseudomonas aeruginosa* Xen5 with recombinant luciferase DNA. Because EN fluoresces intrinsically, EN in the feeding tubes without fluorescent dye was repeatedly flushed until the intrinsic fluorescence levels reached background levels. Fluorescent images of EN residues and bioluminescent images of microorganisms were acquired using an optical imaging system.

Results:

Fluorescence images showed that the amount of EN residues increased at various sites in tubes depending on EN viscosity and the thickening agent, and bioluminescence images showed that microorganism proliferation was associated with a commensurate increase in EN residues. The intrinsic fluorescence of EN also enabled the detection of EN residues in tubes even in the absence of fluorescence dye. Higher EN viscosity required more flushes to reach undetectable levels.

Conclusion:

EN residues and microorganism proliferation in enteral feeding tubes were detected on fluorescence and bioluminescence images, respectively. This simplified approach allowed the real-time visualization of EN residues and microorganisms in feeding tubes.

Introduction

Enteral nutrition (EN) residues persisting in enteral feeding systems not only facilitate bacterial contamination but also obstruct the systems. As EN is supplied prepackaged in sealed bags, the risk of bacterial contamination originating from containers is relatively lower than the risk of contamination from EN feeding tubes[1) 2)].

Such tubes are repeatedly re-used in medical practice, and they are flushed and/or sterilized as required[3)]. The interiors of indwelling tubes that are used for the long-term management of enteral feeding are difficult to dry and thus become susceptible to bacterial contamination[4) 5)]. In contrast, the flow of even highly viscous EN can be increased through the use of wider-bore tubes. The administration of high-viscosity EN to prevent gastro-esophageal reflux seems paradoxical[6) 7)] because it could produce an environment that is conductive to bacterial contamination due to an increase in EN residues remaining inside EN tubes[8)]. Once EN is exposed to contamination, bacteria rapidly proliferate and infusion with contaminated EN can cause sepsis, fever and gastrointestinal dysfunction[9) 10)]. For instance, patients given contaminated EN developed gastrointestinal symptoms within 24 h 10.5 times more frequently than those given non-contaminated EN[9)]. The infusion of EN contaminated with $>10^3$ CFU/mL of gram negative bacteria causes severe infection[11)]. Furthermore, the growth of bacteria lowers the pH inside EN tubes, leading to the denaturation of compounded protein in EN that leads to curd production and tube occlusion[12)].

Flush the tube with 20 to 100 mL of water before and after feeding will reduce the possibility of tube occulusion[13)14)]. However, 100mL water flushing is thought to be too much and may lead to fullness and thus intolerance of feeding. Instead, flushing the tube with 20-30ml water before and each feeding and visually monitoring EN residues responsible for bacterial contamination and tube occlusion have gained wide acceptance in the routine clinical setting. However, these practices cannot detect microscopic amounts of residues and those diluted with the water used for flushing. This might explain why infectious complications arising due to handling EN tubes do not significantly decline. Due to these issues, the development of EN products that do not remain attached to enteral tubes and help to suppress bacterial proliferation remains clinically important.

The balance of EN remaining after flushing must be weighed to quantify EN residues inside EN tubes. However, the amount of water remaining in flushed tubes cannot be ignored. The dry weight of EN inside tubes comprises residues from administered EN. Additionally, the assessment of bacterial contamination requires the incubation of tube contents and bacterial counts. These approaches are complex, time-consuming and not suitable for routine medical practice.

Fluorescence and bioluminescence imaging technology has become more widespread because it enables real-time and spatial imaging of target substances in a non-destructive and non-contact manner[15) 16)]. We speculated that this technology could visualize relatively small amounts of EN residues inside tubes as well as bacterial proliferation and biofilm formation on the inside attributed to the residues. We initially hypothesized that fluorescence imaging of EN labelled with fluorescent dye would reveal EN residues that are invisible to the naked eye and that bioluminescence imaging

would show that the residues affect the proliferation of the model bacterium, *Pseudomonas aeruginosa*. In addition, we hypothesized that the intrinsic fluorescence of EN can enable the detection of EN residues inside tubes even in the absence of a dye that emits fluorescence. We suspect that these imaging techniques could demonstrate the effects of repeated flushing upon EN residues with different degrees of viscosity as well as the amounts and types of thickening agents.

Materials and methods

EN with thickening agents

The viscosity of the Hine® formulation (×××× Inc., ××××, ××××) was increased using different concentrations of a xanthan gum-based thickener (EN-KG (L), (M) and (H), 3.5, 4.5 and 6 g/dL, respectively). Hine® jelly (EN-A) is representative of viscous EN with an agar-based thickener. Hine® (EN) is a standard EN formula (1 kcal/mL) that does not contain fiber and was therefore used as a negative control without additives. Each EN was mixed beforehand with the fluorescent dye 0.01% indocyanine green (×××, Ltd., ××××, ××××) to detect EN in feeding tubes. The viscosity of EN, EN-A, EN-KG(L), EN-KG(M) and EN-KG(H) measured at 12 rpm at 25°C using a Brookfield viscometer was 10, 6,000, 1,350, 4,530 and 9,300 mPA·s, respectively.

Fluorescence and bioluminescence imaging of EN residues and bacterial proliferation

The silicon catheters of gas barrier gastrostomy tubes (length, 255 mm; inside diameter, 8 mm) were filled with an EN and left for 5 min. The EN then passed through the tubes in free fall and the tubes were washed once for 10 seconds with 30 mL of distilled

sterile water, which is within the recommended range for flushing in medical practice[17]. The distribution of fluorescence inside flushed tubes was monitored using the IVIS® Spectrum live imaging system (Perkin Elmer Inc., Waltham, MA, USA) with excitation and emission at 745 and 840 nm, respectively. The flushed tubes were loaded with 1×10^6 CFU/mL of the bioluminescent bacterium[18] *Pseudomonas aeruginosa* Xen05 (Caliper Life Sciences, MA, USA) that had been incubated at 35°C in Mueller–Hinton Broth medium overnight and resuspended in sterile distilled water. The tubes were sealed and cultured at 35°C for 4, 8, and 24 h after spiking them with *P. aeruginosa* and then the distribution of bioluminescence inside the tubes was photographed in real-time using the IVIS®. All tubes that were incubated for 24 h were washed twice with 30 mL of distilled, sterilized water for 10 seconds to remove floating bacteria and allow bioluminescence imaging of adherent bacteria. Regions of interest (ROI) were placed over the catheter at locations excluding the funnel and balloon to determine fluorescent and bioluminescent signals. Photon emission in ROI was standardized in terms of exposure duration, binning and f/stop.

Imaging intrinsic fluorescence of EN residues

By changing excitation and emission wavelengths, we similarly detected EN, EN-A, EN-KG(L), EN-KG(M) and EN-KG(H) residues after several washes with water in the absence of the fluorescent dye. Briefly, all types of EN were allowed to free-fall through 20 Fr silicon tubes (length, 30 cm) for 5 minutes. The tubes were flushed with 30 mL of water for 10 seconds and the intrinsic fluorescence of EN was visualized at excitation and emission wavelengths of 430 and 500 nm, respectively. The water was then repeatedly flushed until the emitted fluorescence reached <10 % of the loaded

amount. Regions of interest were placed around the catheters to determine photon signals from the tubes. Interactions between EN (variate) and the number of washes (covariate) were tested by covariance analysis.

Results

Fluorescence and bioluminescence imaging of EN residues and bacterial proliferation

Figure 1 shows the distribution of fluorescence inside tubes containing EN with various viscosities and thickening agents after a single wash, and the distribution of bioluminescence emitted at 4, 8, 24 h after spiking the tubes with *P. aeruginosa* Xen05. Figure 1D-F shows that a single wash resulted in more residual fluorescence intensity of EN-KG with a xanthan gum-based thickener. The bioluminescence intensity of the bacteria inside tubes containing EN-KG also increased in parallel with the residual fluorescence (Figure 2). The amounts of fluorescence residues left by EN-A with an agar-based thickener and EN without a thickener were similar, even though the viscosity of EN-A was between that of EN-KG (M) and of EN-KG (H). Furthermore, biofilm formed inside tubes containing EN-KG (M) and of EN-KG (H) at 24 h after spiking with bacteria (Figure 3).

Intrinsic fluorescence imaging of EN residues

Excitation of ENs at a wavelength of 430 nm results in the emission of various amounts of fluorescence (Figure 4). In addition, EN residues in tubes were detected based on their intrinsic fluorescence (Figure 5). Residues of EN and EN-A fell below the detectable limit after a single wash. In contrast, significantly more washes were required to remove EN-KG due to increased viscosity (Figure 6).

Discussion

Accumulating EN residues inside feeding tubes create an environment suitable for bacterial growth and tube occlusion. Fluorescence and bioluminescence imaging allowed the detection of invisible EN residues as well as the growth of a bioluminescent bacterium and biofilm formation inside tubes.

Fluorescence imaging can identify and locate a target of interest in a non-destructive and non-contact manner. This is the first study to visualize EN residues remaining inside feeding tubes using fluorescence imaging rather than visual assessment or weighing tubes. Concentrated EN residues can easily be visualized during free-fall through the tubes, but EN residues diluted by several washes with water are too small to see with the naked eye. We found that the amount of EN residues remaining inside tubes increases with increasing viscosity, which is in agreement with the findings of a previous study showing that viscous EN frequently occludes tubes[19)20)]. As bacteria proliferate in EN systems immediately after contamination, open system EN should be administered within eight hours[21)]. The number of bacteria challenged was similar to the number found in EN prepared and administered in hospital or in the home[21)22)]. Our methodology can be used for infection control where EN is administered. For example, formulations could be designed to suppress the adhesion of residues, or a new method of washing tubes could be considered.

The fluorescence reagent used in this study is not immediately available for clinical application, because it is not permitted as a food additive and it is not approved for medical use. However, EN has intrinsic fluorescence that allows the detection of residues without the need for a fluorescent reagent. Foodstuffs comprise vitamins, amino acids and natural trace components, each of which have an individual

fluorescence peak wavelength and intensity. Therefore, using the same wavelengths for excitation and for emission revealed differences in the intensity of intrinsic fluorescence generated by each EN. In EN with similar compositions, the intrinsic fluorescence is also similar. As a result, it is easy to compare EN using the same default levels. The amount of residues inside EN tubes increases as viscosity increases. Even though the viscosity did not significantly differ when compared to EN with gum-based thickener, the amount of residues remaining after washing viscous EN with agar-based thickener was similar to that of EN without a thickening agent. This finding suggests that thickening agents significantly alter the effect of washing EN tubes. Thickening agents contain either agar, which promotes gelation, or gum, which increases viscosity by promoting sol synthesis. A gelled matrix resembles a solid more than a sol and maintains its shape against natural aeration or flushing water, implying that it can pass through an EN tube with minimal distortion due to air and water pressure. In contrast, a sol is highly elastic and easily distorted by these pressures. Water and air therefore pass through EN tubes leaving more solid residues inside.

Powdered EN is difficult to dissolve at relatively higher concentrations. For example in terms of density (1.5 kcal/mL), the viscosity of the EN formulas based on raw materials that are identical to the EN used herein (1.0 kcal/mL) was 356 mPa･S. Furthermore, commercially available EN (1.0, 1.5, 2.0 kcal/mL) was more viscous at 1.0 kcal/mL compared with others in which the viscosity was increased using various concentrations of thickeners[23]. Flushing tubes containing condensed EN and thickened EN at various energy densities is effective and might be very practical on a daily basis. Further investigation is warranted.

Flushing EN tubes is an effective measure to prevent tube occlusion. However, the type of EN affects the outcomes of simple flushing with water. Fluorescence imaging can be used as a high-throughput method of monitoring the efficiency of flushing tubes.

Conflict of interest

All authors are employees of Otsuka Pharmaceutical Factory, Inc. and have no other conflicts to declare.

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Figure legends

Figure 1. Representative images of EN residues and proliferation of bioluminescent bacteria in percutaneous endoscopic gastrostomy tubes.

A, B, C, D, E, and F represent tubes without treatment, tubes containing EN, EN-A, EN-KG (L), EN-KG (M), and EN-KG (H), respectively.

Figure 2. Regression analysis of EN residue vs. proliferating bioluminescent bacteria in tubes.

Correlation coefficients were 0.93, 0.97, and 0.92 at 4, 8, and 24 hours, respectively (n = 3).

Figure 3. Representative images of biofilm-forming bioluminescence bacteria.

A, B, and C represent tubes containing EN-KG (L), (M), and (H), respectively.

Figure 4. Intrinsic fluorescence emitted by EN.

Commercially available EN (EN A-E) emits intrinsic fluorescence, and almost all EN samples emitted varying amounts of fluorescence when visualized at excitation and emission wavelengths of 430 and 500 nm, respectively.

Figure 5. Representative images of EN residues in tubes.

Agar- (A) or gum- (B) based semi-solid EN with similar viscosity were poured into PEG tubes (number: 0) that were subsequently washed with water. Intrinsic fluorescence was assessed by imaging (numbers 1 - 7).

Figure 6. Effect of washing frequency on photon count inside tube.

Same amounts of EN were poured into tubes and then repeatedly washed until EN-associated photon counts reached background level. Slopes of photon-count curves significantly differed among EN ($p < 0.001$).

Figure 1

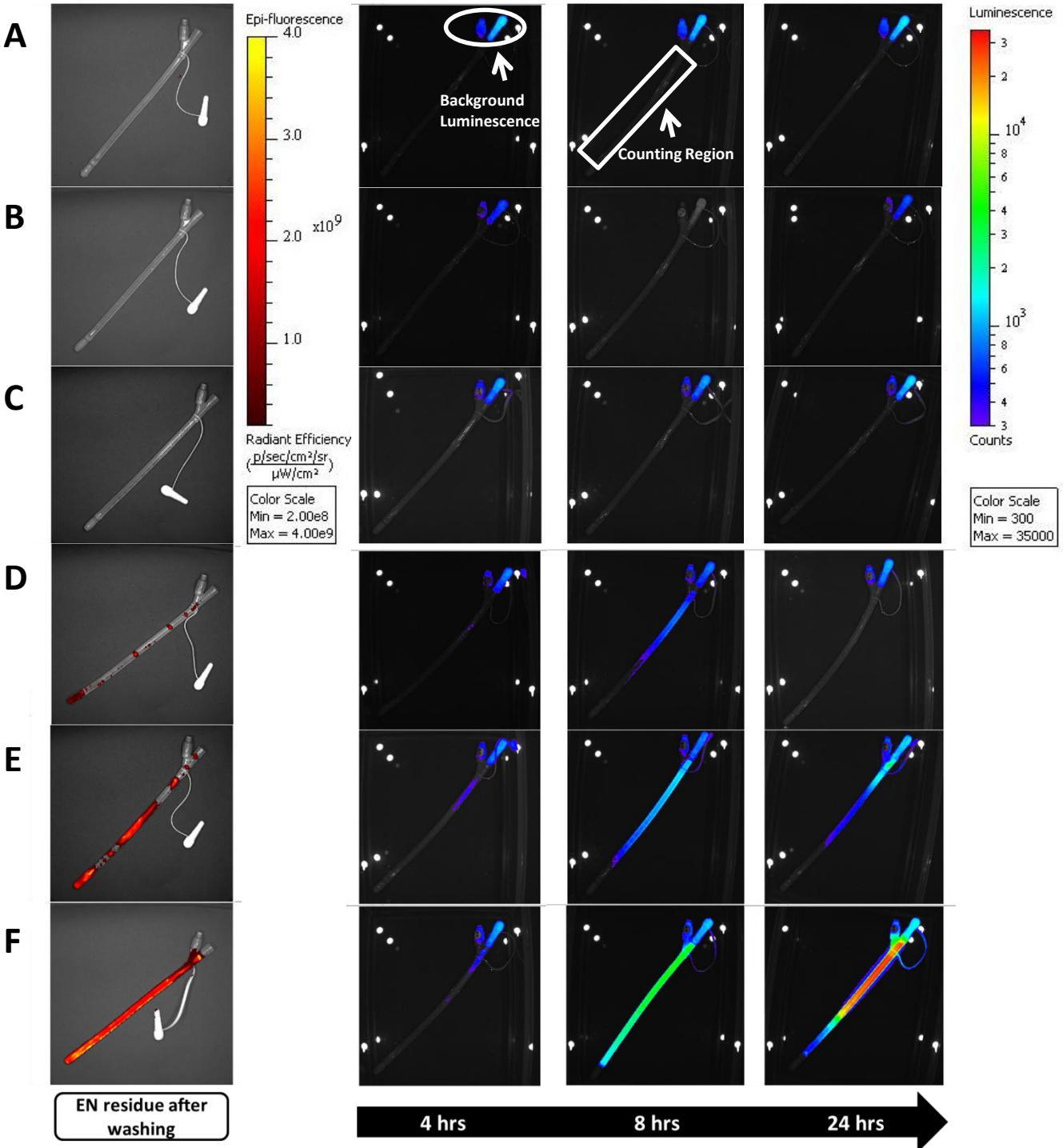
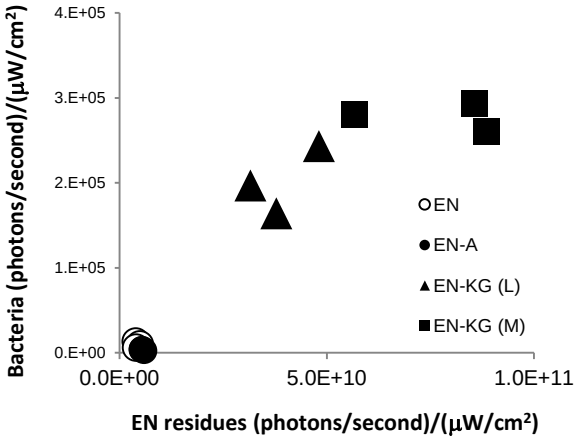
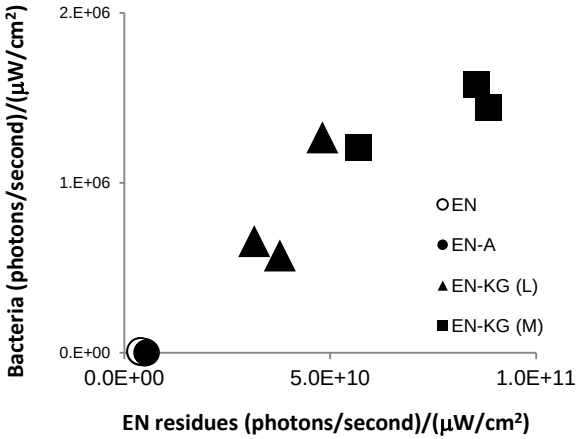

A
B
C
D
E
F
Epi-fluorescence
4.0
3.0
2.0
x10^9
1.0
Radiant Efficiency
(p/sec/cm²/sr / µW/cm²)
Color Scale
Min = 2.00e8
Max = 4.00e9
Background Luminescence
Counting Region
Luminescence
3
2
10^4
8
6
4
3
2
10^3
8
6
4
3
Counts
Color Scale
Min = 300
Max = 35000
EN residue after washing
4 hrs
8 hrs
24 hrs

Figure 2

A


4.E+05
3.E+05
2.E+05
1.E+05
0.E+00
0.0E+00
5.0E+10
1.0E+11
Bacteria (photons/second)/(μW/cm²)
EN residues (photons/second)/(μW/cm²)
EN
EN-A
EN-KG (L)
EN-KG (M)

B


2.E+06
1.E+06
0.E+00
0.0E+00
5.0E+10
1.0E+11
Bacteria (photons/second)/(μW/cm²)
EN residues (photons/second)/(μW/cm²)
EN
EN-A
EN-KG (L)
EN-KG (M)

C

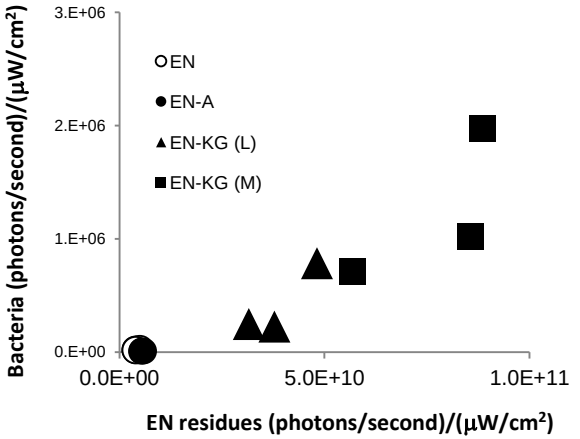

3.E+06
2.E+06
1.E+06
0.E+00
0.0E+00
5.0E+10
1.0E+11
Bacteria (photons/second)/(μW/cm²)
EN residues (photons/second)/(μW/cm²)
EN
EN-A
EN-KG (L)
EN-KG (M)

Figure 3

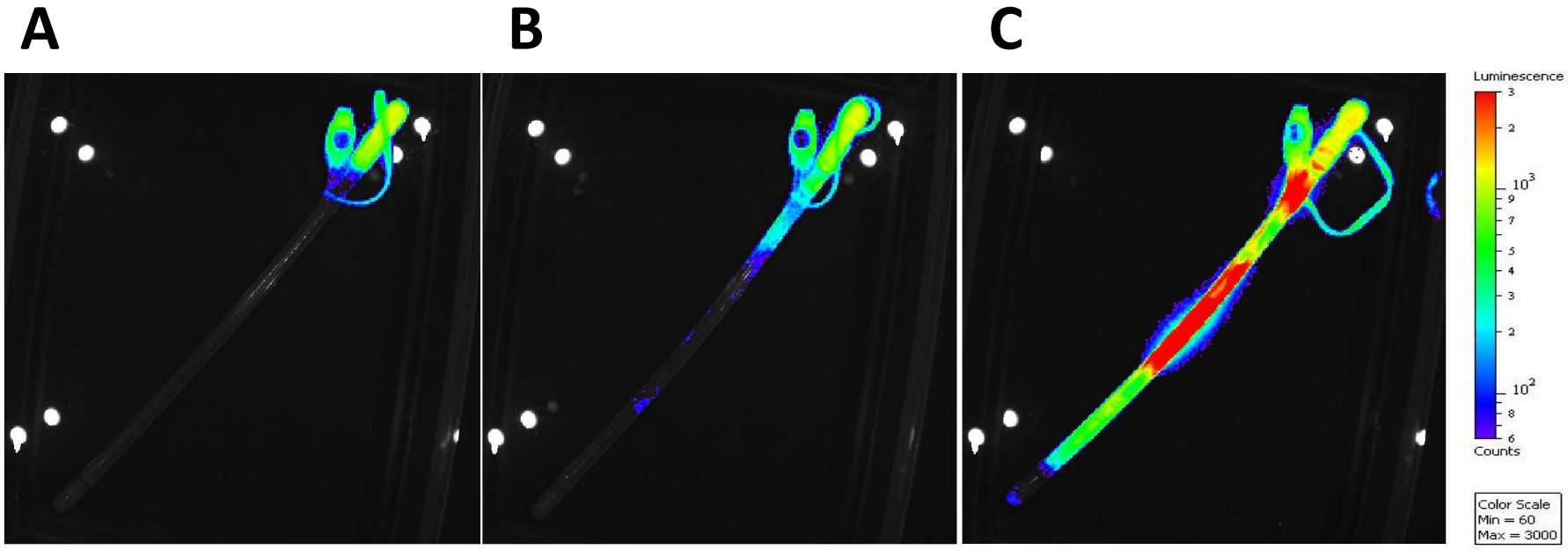

A
B
C
Luminescence
3
2
10^3
9
7
5
4
3
2
10^2
8
6
Counts
Color Scale
Min = 60
Max = 3000

Figure 4

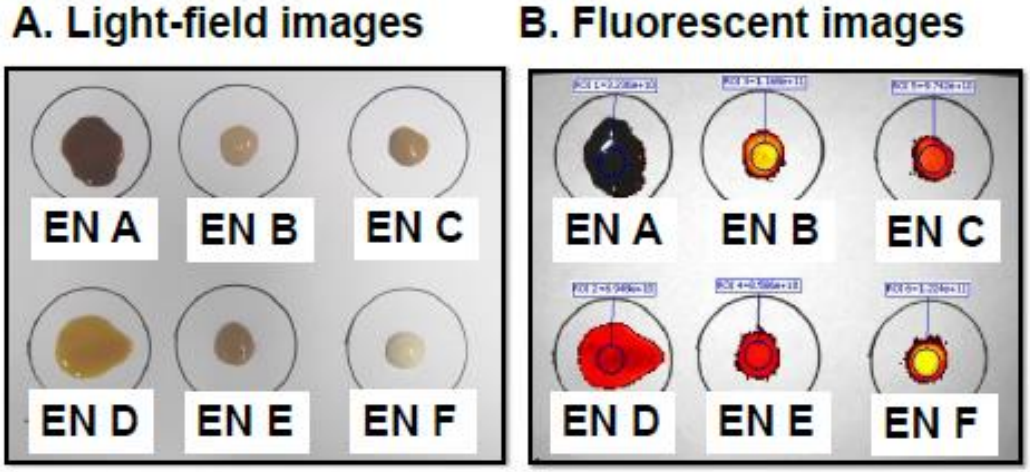

A. Light-field images
B. Fluorescent images
EN A
EN B
EN C
EN D
EN E
EN F
EN A
EN B
EN C
EN D
EN E
EN F

Figure 5

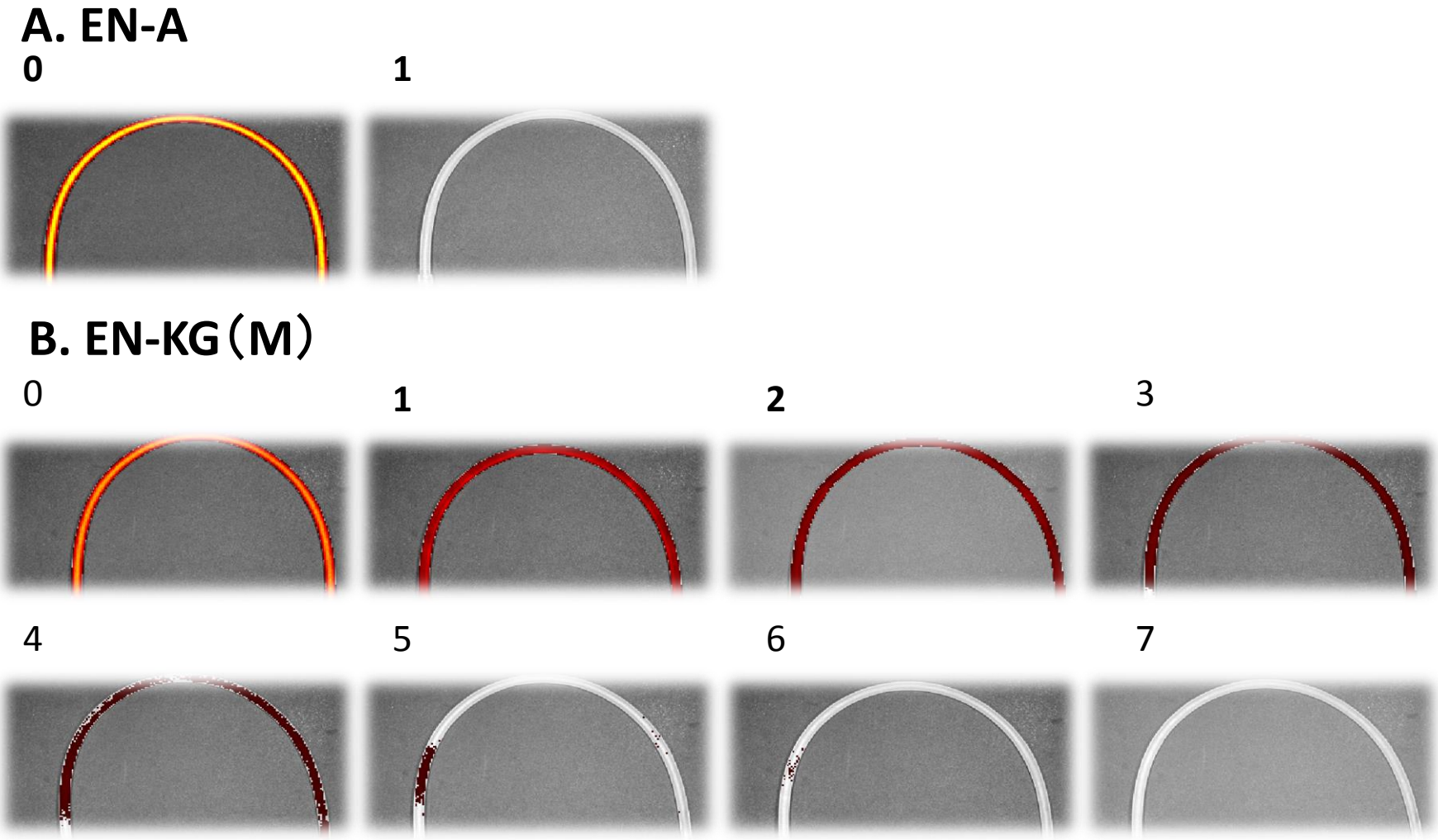
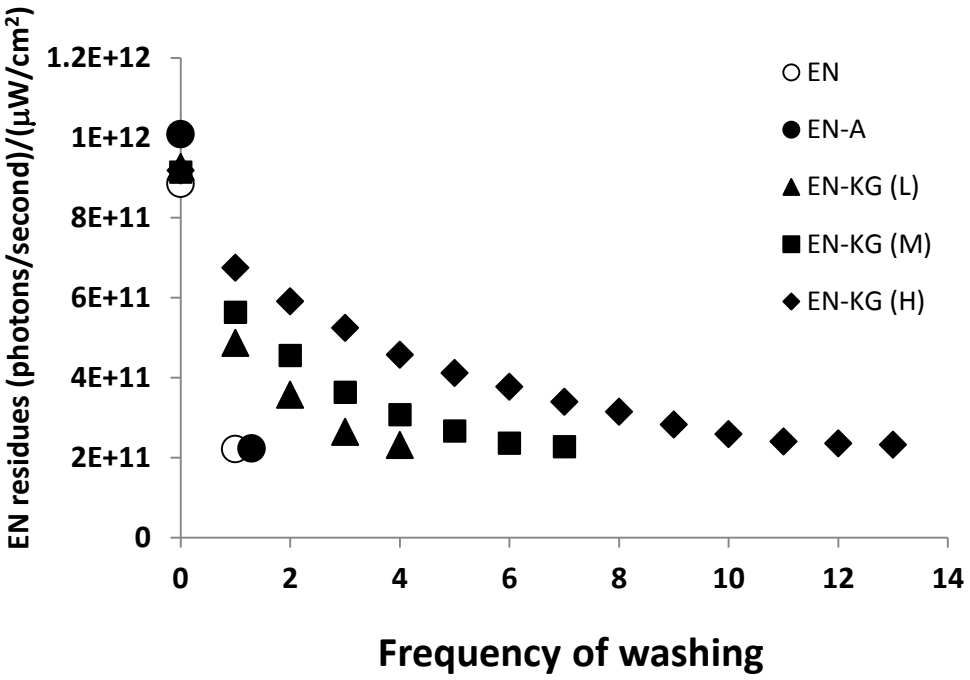


Figure 6


EN residues (photons/second)/(μW/cm²)
1.2E+12
1E+12
8E+11
6E+11
4E+11
2E+11
0
0
2
4
6
8
10
12
14
Frequency of washing
EN
EN-A
EN-KG (L)
EN-KG (M)
EN-KG (H)