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Comparison of *E. coli* Infiltration between New Synthetic Absorbable Sutures

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Purpose: The proper selection of suture is very important to minimize infection after gastrointestinal anastomosis and closure, which is one of the causes of postoperative complications such as leakage and stricture, etc, in the surgical field. Thus this study focuses on which suture can reduce bacterial infection after surgical operation by comparing *in vitro* microbial infiltration rates of three synthetic absorbable sutures and that of silk - a relatively absorbable material, using *E. coli*.
Methods: Four different, sterilized kinds of absorbable sutures were used for two experiments. In experiment 1, the cut-off suture was directly applied to the standard method agar plate and cultured for observation. In experiment 2, the cut-off suture was diluted with 1 ml of tryptic soy broth to be smeared and cultured in the standard method agar plate and counted using a spectrophotometer.
Results: The first experiment revealed that bacterial growth was not observed in the monofilament and antibiotic-coated multifilament sutures, while the other sutures of multifilament structure were invaded by bacteria. In the second experiment, counting and averaging the colony from five plates of each test showed that the number of *E. coli* of monofilament suture, antibiotics-coated polyglactin, polyglactin and silk were 0±0, 39.3±14.4, 208.6±76.6, 59.4±26.7, respectively.
Conclusion: Sutures of monofilament structure are believed to be a relatively safe material that can be used for gastrointestinal anastomosis and closure since it has lower bacterial infiltration rates than sutures of multifilament structure. **(J Korean Surg Soc 2009;77:1-6)**

Key Words: Microbial infiltration, Synthetic absorbable suture, Monofilament structure, *E. coli*

INTRODUCTION

The surgical site infection (SSI) following gastrointestinal anastomosis or closure is one of the most common causes of post-operative complications such as anastomotic leakage or stricture. It is crucial, therefore, to make appropriate selection of suture material to help minimize the likelihood of contracting the infections.(*1,2*)

A suture material used in gastrointestinal surgery is categorized based on several factors such as material, physical shape and absorbability: for instance, absorbable and non-absorbable materials depending on absorbability, and monofilament and braided multifilament sutures according to physical shape. The benefits of absorbable suture material over non-absorbable one include less resistance to the presence of a foreign body, greater safety to use with infection-affected tissues, no need for later removal of suture, lower biological reactions to the absorbed material, and strength that can be sustained only for a duration required. These have made absorbable material a popular candidate for gastrointestinal anastomosis or suture procedures.(*3*)

A recent advances in biomedical materials and engi-

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neering have led to the development of wide varieties of artificial absorbable sutures that are currently used in clinical settings. In the past, researchers examined correlations between the type of absorbable suture material and post-operative infections to help diminish the occurrence of post-operative complications. The same effort, however, has been rarely observed in recent comparative studies involving the latest versions of sutures currently in clinical use.

We examined potential ways of using suture material to help reduce the rate of post-operative infections following gastrointestinal suture or stapling. By using a colon bacterium (*E. coli* ATCC8739), we made *in vitro* comparison of the infiltration rate of microorganisms of 4 types of recently developed and clinically used artificial absorbable sutures: (1) a monofilament glycoside-ε-caprolactone-trimethylene carbonate (GCT) material; (2) a multifilament antibiotics-coated polyglactin suture; (3) a multifilament polyglactin suture; and (4) a silk - relatively absorbable, multifilament suture material.

METHODS

1) Materials

We used 4 types of recently developed and clinically used artificial absorbable sutures: (1) Monosynü® (B.Braun Melsungen AG, Germany), a monofilament glycoside-ε-caprolactone-trimethylene carbonate (GCT) material; (2) Vicryl-plus® (Ethicon, Sommerville, USA), a multifilament antibiotics-coated polyglactin suture; (3) Vicryl® (Ethicon, Sommerville, USA), a multifilament polyglactin suture; and (4) Silkam® (B.Braun Melsungen AG, Germany), a relatively absorbable, multifilament suture material. Using the same size 3-0 USP, the 4 samples were sterilized and stored at room temperature. We used standard agar and tryptic soy broth plates to compare the infiltration rate of *E. coli* ATCC8739 of the specimens via 2 experiments (standard methods agar vs. tryptic soy broth).

2) Methods

A single colony of *E. coli* ATCC8739, the bacterium used in this study, was removed from the master plate and then transplanted and subcultured on standard methods agar at 37°C for 12 hours. The subcultured bacterium was diluted (1×10^6/ml) to produce a 250 ml solution. The 4 suture specimens were tied to 4 sterilized clips and were immersed in the solution for 10 minutes (Fig. 1). After 10 minutes, a 1 cm portion at the end of each of the specimens was cut off to be used for the experiments (Fig. 2).

In Experiment 1, the sutures cut off from the 4 material specimens were placed onto standard methods agar plates and subcultured at 37°C for 12 hours for observation. In

<GCT> <Antibiotics-coated polyglactin> <Polyglactin> <Silk>

Fig. 1. Four sorts of sutures used in evaluation and process of microbial penetration.

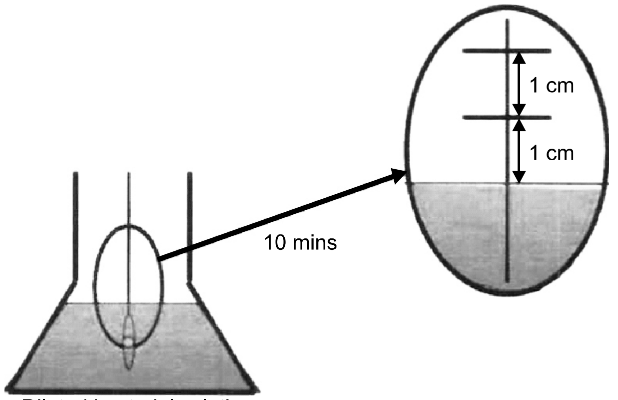

Fig. 2. Diagram in evalution method. Subcultured bacterium was diluted (1×10^6/ml) to produce a 250 ml solution. Suture specimens were immersed in the solution for 10 minutes. After 10 minutes, a 1 cm portion at the end of each of the specimens was cut off to be used for the experiments.

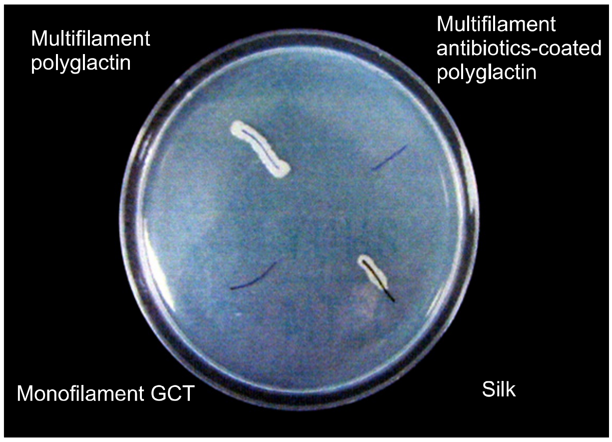

Fig. 3. Direct application to standard method agar plate. Bacterial growth was not observed in monofilament suture and antibiotic-coated multifilament suture, while the other sutures of multifilament structure were invaded by bacteria.

Table 1. Results from counting of microbial colonies

Suture	Mean number of microbial infiltration	Standard deviation	95% confidence interval	F-value	P-value
Monofilment GCT (Glycoside-ε-Caprolactone-Trimethylene carbonate)	.00	.000	.00～.00		
Multifilament antibiotics-coated polyglactin	39.25	14.367	37.25～41.25	3.930	0.024
Multifilament polyglactin	208.6	76.558	197.92～219.28		
Silk	59.4	26.719	55.67～63.13		

Experiment 2, the sutures cut off from the 4 material specimens were diluted with a 1 ml tryptic soy broth. A 100 μl of each of the diluted portions was then placed onto a standard agar plate and was subcultured at 37°C for 12 hours for later observation. For the quantification (counting colony), the spectrophotometry was used. Both experiments (standard methods agar and tryptic soy broth) involved 5 plates, respectively, for each of the 4 specimens.

3) Statistical analysis

The SPSS (version 14.0 for Windows, SPSS Inc, Chicago, IL, USA) was used for statistical analysis, and the ANOVA test was used to compare the 4 specimen groups. Statistical significance was defined to a P value less than 0.05.

RESULTS

1) Experiment 1

No microorganism infiltration and/or activities were observed in the sample of monofilament absorbable suture (GCT). All multifilament absorbable suture materials, except for the antibiotic-coated polyglactin, showed bacterial infiltration and growth in the samples (Fig. 3).

2) Experiment 2

An average number of colonies were calculated by spectrophotometer on the 5 plates of each of the 4 suture specimens, i.e., monofilament suture, antibiotics-coated polyglactin, polyglactin and silk. The number of infiltration by the microorganism was 0±0, 39.3±14.4, 208.6±76.6, and 59.4±26.7, respectively, showing statistically significant differences (P=0.024) (Table 1).

DISCUSSION

A suture material used in gastrointestinal surgery is categorized based on several factors such as material, physical shape and absorbability. An absorbable suture - material commonly used in the anastomosis of inner areas neighboring the luminal environment - has roughly 2 types depending on physical shape: braided multifilament suture and monofilament suture.(*4*) Since the first clinical use of catgut, chromic salt-sterilized and cleaned, in the 19th century, absorbable suture material has continued to see improvement.(*3*) In the 1960s, artificial absorbable suture was invented, including Dexon® (polyglycolic acid) and Vicryl® (polyglactin). Since the 1980s, greater varieties of artificial monofilament absorbable suture have been developed: PDS® (polydioxanone); Maxon® (copolymer of glycolic acid and trimethylene carbonate); Moncryl® (poliglecaprone 25); Biosyn® (glycomer 631), which consists of glycolide, dioxanone and trimethylene carbonate; and Monosyn® (glycoside-ε-caprolactone-trimethylene carbonate or GCT).(*5-8*) From 2000 and onward, a new models are being developed to help overcome the weaknesses of multifilament material, e.g., coated VICRYL Plus® (coated Polyglactin 910 suture with triclosan).(*2*) An artificial absorbable suture is widely used in most gastrointestinal, urological, gynecological and plastic surgeries, except for cardiovascular surgeries and prosthetic procedures which require non-absorbable material.(*9*)

The surgical site infection (SSI) following gastrointestinal stapling or suture is one of the most common causes of post-operative complications such as anastomotic leakage or stricture. The SSI is known to occur in close proximity of the suture site and to be closely related to the type of suture that was used. According to the 1992 statistic by the Centers for Disease Control and Prevention (CDC), 66% of all wound infections took place near wounded sites.(*10*) Aside from the technical problems associated with gastrointestinal stapling using suture material, there are other complications due to infection, such as leakage at the stapled site and abscess inside the abdominal cavity occurring at a 1 to 3% rate.(*11,12*) About a 1% stricture was reported resulting from the complications.(*13*)

During operations, the SSI is affected by both endogenous and exogenous factors. For the past 3 decades, many efforts have been made to help reduce the rate of SSI, with successful outcomes. Specific methods employed include the use of appropriate antibodies in the operating room, pre-operative antisepsis on the wound site, thorough operation, development of new surgical techniques, and pre-operative management of the physical state of patients. Nevertheless, various types of germs have been observed to breed not only in the wound site but also through the suture material being used. Recent advances in biomedical materials and engineering have led to a wide variety of absorbable suture that has consistent absorption duration, proper tension, the least amount of tissue reaction and lower probability of developing post-operative wound infection and the new inventions are currently in clinical use.(*2*)

Depending on physical shape, absorbable sutures are divided into two groups: monofilament type and braided multifilament type. In this study, we compared the infiltration rate of microorganism (*E. coli* ATCC8739) of both types of artificial absorbable suture materials, i.e., monofilament and multifilament models, via *in vitro* experiments.

A multifilament suture has a larger surface area than monofilament does because of its 3-dimensional structure which increases the probability of bacterial deposit and infection.(*14*)

To overcome the shortcomings, efforts had been made since 1980 to help develop antibiotic-coated suture material, but the progress was slow due to technical problems involving safety, stability and standardization. The development and subsequent use of Triclosan has begun since the 2000s. The antimicrobial, usually selected for local treatment, shows broad spectrum antibacterial activities against G(+) and G(−) bacteria.(*15*)

Previous *in vitro* studies involving Triclosan demonstrated its antimicrobial effects against several bacteria including Staphylococcus aureus, Staphylococcus epidermidis, MRSA, MRSE, vancomycin resistant Enterococcus faecalis, Pseudomonasaeruginosa, and *Escherichia coli*.(*16,17*) Though S.

aureus is the most frequently cited cause of SSI, *E. coli* and enterosbacteriacea are known to be the most commonly found bacteria after a clean contaminated or contaminated procedure. The majority of gastrointestinal stapling in surgical settings is a clean contaminated procedure.(*18*) So, we selected *E. coli* ATCC8739 for this study because it is one of the most common forms of intestinal bacteria as well as the most common cause of infections following gastrointestinal stapling, which is a clean contaminated (or contaminated) procedure, and is contained by Triclosan.(*3*) We carried out comparisons between monofilament and multifilament sutures, and between different models of multifilament sutures by using *E. coli*.

Compared with standard multifilament suture, Triclosan-coated multifilament suture is known to be stronger against *E. coli* infiltration, with a 35 to 40% less infiltration reported in *in vitro* comparative studies.(*14*) *In vivo* studies wherein the colon of pigs was stapled, and bacterial infiltration and colonization were examined for comparison reported the infiltration occurring at significantly lower rates.(*2*) This study found similar outcomes: (1) the antibiotic-coated multifilament suture used in Experiment 1 (standard methods agar) had a lower infiltration than the standard multifilament suture did; and (2) infiltration rates in Experiment 2 (tryptic soy broth) were also significantly lower (39.3±14.4, 208.6±76.6 (P=0.024)).

Advances in the development of monofilament suture have continued, with increased clinical demands. Compared with multifilament suture with braided structure, monofilament suture material is finer and is associated with less tissue damage and allergic reaction, limited tissue damage by absorption, gradual absorption during the healing process, excellent tension sustainability, and lower risk of wound infection.(*3*) This was confirmed by our findings: (1) monofilament suture showed a significantly lower level of bacterial infiltration and colonization in Experiment 1, as compared with that of the standard multifilament models; and (2) in Experiment 2, which compared monofilament suture, antibiotic-coated multifilament suture, standard multifilament suture and Silk, the monofilament material showed a significantly lower infiltration rate, i.e., 0±0, 39.3±14.4, 208.6±76.6, and 59.4±26.7 (P=0.024).

We assume that monofilament suture had lower infiltration rates due to smaller surface area,(*6*) while multifilament suture experienced more absorption of bacteria-contained solution due to capillary phenomenon and thus showed higher infiltration rates. Monofilament material's greater tolerance against microbial infiltration is also known to be associated with its antibacterial effects resulting from chemical properties of its final byproducts following hydrolysis.(*3*) We expect to see the same results with future *in vivo* studies and conclude that monofilament suture is a safer device to be used in clinical settings than standard or antibiotic-coated multifilament material because of its lower levels of microbial infiltration. Though the difficulty in handling and knotting of monofilament material did limit its clinical use in the past, recent advances in the development of biomedical materials and engineering as well as of surgical techniques and devices have improved monofilament suture's tensile strength, knot security and stability and flexibility to such a degree that they are on par with those of multifilament suture. The clinical use of monofilament material, therefore, has been on the rise.(*4*)

This study was designed as a sort of pilot study, in which bacterial infiltration rates of monofilament and multifilament suture materials were compared using only *E. coli*. Additional studies are believed to be necessary, but monofilament material has lower levels of bacterial infiltration because of its physical structure with smaller surface area and antibiotic effects of its final byproducts, and the advantage helps decrease the rate of infections following gastrointestinal anastomosis or closure, and the occurrence of post-operative complications such as leakage and stricture.

CONCLUSION

Monofilament suture, finer than multifilament material, is used in gastrointestinal anastomosis or closure, causing less tissue damage during the procedure. Monofilament models, a more popular type in clinical practices, are associated with low probability of infection, less foreign body reaction and lower rate of developing fistula. This was confirmed by the findings of this study, in which *E. coli* ATCC8739 was used to make *in vitro* comparison of the infiltration rate of the bacterium of 4 different kinds of artificial absorbable suture materials. The results showed monofilament suture had lower infiltration count than multifilament suture.

Based on the findings of this study, we assume that a monofilament absorbable suture is a safer device to be used in gastrointestinal stapling or suture, with lower risk of contracting post-operative infections. We recommend further *in vitro* as well as *in vivo* research using a greater variety of bacteria, and additional studies involving clinical use of the material.

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