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The effect of commonly used sutures on inflammation inducing pathogens – An *in vitro* study

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ABSTRACT

Introduction: Sutures are a vital part of nearly every surgical procedure designed to close and stabilize wound margins consequently allowing undisturbed wound healing.
Aim: The aim of this study was to evaluate *in vitro* antimicrobial effect of 4 commonly used sutures.
Materials and methods: The Direct Contact Test was used to evaluate the antibacterial properties of 4 types of sutures: 2 absorbable and 2 non-absorbable braided sutures, immediately or after aging for 2 or 7 days. The tested bacteria were: *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Three-way ANOVA, two-way ANOVA, one-way ANOVA and Tukey multiple comparison were used for statistical analysis.
Results: The absorbable Vicryl Plus exhibited a bactericidal effect against the Staphylococcus strains, which was unaffected by aging. With *P. aeruginosa*, there was only an initial delay in bacterial growth. All other tested sutures did not have antibacterial effects against any of the tested bacteria ($p < 0.001$).
Conclusions: Vicryl Plus had sustained bactericidal effect against the Staphylococcus strains but not against *P. aeruginosa*. None of the other sutures presented any antibacterial properties.

1. Introduction

Surgical site infection is a major concern. It is relatively frequent, after head and neck oncological surgery and is associated with significant morbidity and mortality (Cunha et al., 2012). Sutures are a vital part of nearly every surgical procedure, designed to close and stabilize wound margins and allow undisturbed wound healing (Wikesjö et al., 1992; Minozzi et al., 2009). Most surgical infections potentially impairing wound healing are intimately related to sutures (Horan et al., 1992). In the oral cavity, sutures are placed within tissues of high vascularity in a moist bacteria rich environment with infectious potential. Bacteria and necrotic debris lodge on the suture material and invade the suture track (Selvig et al., 1998), retain infection and delay the healing cascade (Kim, 2002; Morrow and Rubinstein, 2002).

The physicochemical characteristics of a suture material influence its ability to attract bacteria and consequently promote wound infection (Chu and Williams, 1984). Bacteria adhere to various types of sutures with different affinities (Katz et al., 1981). It has been shown that silk sutures, which are multi-filamentous and braided, produce a greater inflammatory reaction in the oral mucosa than monofilament sutures (Lilly et al., 1973; Racey et al., 1978; Leknes et al., 2005; Merritt et al., 1999). This reaction was attributed to the presence of bacteria in the interstices of the sutures (Morrow and Rubinstein, 2002; Parirokh et al., 2004). Therefore, it is not surprising that abscess formations have been reported more frequently with multi-filamentous, braided sutures than with monofilament sutures that elicited only a mild inflammatory tissue response (Morrow and Rubinstein, 2002).

Bacteria enclosed in the interstices of the braided suture may be protected from the phagocytic activity of leucocytes, thus sustaining and prolonging an infection (Selvig et al., 1998; Österberg and Blomstedt, 1979; Österberg, 1983).

Edinger and Luhr (1986) tested the influence of absorbable versus non-absorbable sutures on nerve healing indicating the advantages of absorbable sutures. Absorbable sutures, which are made of materials that are either digested by body enzymes or hydrolyzed by tissue fluids, are also susceptible to bacterial attachment and colonization (Minozzi et al., 2009; Kim, 2002).

Hospital-acquired infections and antibiotic-resistant bacteria particularly methicillin-resistant *Staphylococcus aureus* (MRSA) can cause severe soft tissue, bone or implant infections (Warnke et al., 2009).

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Transcutaneous maxillo-facial surgery can lead to infections caused by bacteria that are commonly found on the skin such as *Staphylococcus epidermidis* (Spaey et al., 2005).

Pseudomonas aeruginosa is a common nosocomial contaminant although it was found to be associated with severe necrotizing infection of the eyelid (Bodey et al., 1983; Steinkogler and Huber-Spitzy, 1988). Microbial contamination of sutures has been shown not to be effected by daily use of 0.2% chlorhexidine solution (Sortino et al., 2008). Although controversial, the use of systemic broad-spectrum antibiotic drug therapy can significantly reduce bacterial contamination of the suture (Leknes et al., 2005).

This susceptibility of sutures to infection led to the introduction of sutures treated with triclosan, a broad-spectrum antibacterial agent, which are claimed to have antibacterial properties (Rothenburger et al., 2002).

The aim of this study was to evaluate *in vitro* the antimicrobial capacity of 4 commonly used sutures and to test whether such activity is sustained when the sutures are aged for up to 7 days in phosphate-buffered saline.

2. Materials and methods

2.1. Sutures

Four commercially available suturing materials were tested: (a) braided Vicryl Plus absorbable suture (coated polyglactin 910 with triclosan, Ethicon Inc., Johnson & Johnson, New Brunswick, NJ); (b) monofilament plain gut absorbable suture (Look, Surgical Specialist Corporation, Reading, PA); (c) Polyviolene, a braided-polyester, uncoated, white, non-absorbable suture (Look, Surgical Specialist Corporation); and (d) Mersilk, a non-absorbable silk suture (Ethicon, Johnson & Johnson). The braided Vicryl Plus suture contained triclosan (2,2,4-trichloro-2-hydroxy-diphenyl ether) at a concentration of up to 150 μg/m. The other sutures did not contain any reported antibacterial agents.

2.2. Bacteria and growth conditions

The three bacteria that are found most frequently in infected wound cultures (Selvig et al., 1998) were tested: *S. aureus* (ATCC 9144 known as the 'Oxford Staphylococcus'), *S. epidermidis* (RP62A) and *P. aeruginosa* (ATCC 17933). The microorganisms were grown aerobically from frozen stock cultures in brain–heart infusion (BHI) broth (Difco Laboratories, Detroit, MI).

2.3. Suture samples

The suture samples were prepared in the shape of a flat surface continuously covered by the suture material (Fig. 1A). The suture was wrapped tightly around a small flat polypropylene plate to form a uniform layer of suture with an area of 20 ± 0.3 mm^2 (Fig. 1A). These suture-covered plates were then placed along the wall of the wells (see below) positioned to keep the suture samples away from the perpendicular light path of the plate reader, thus allowing for turbidity measurements in the wells (see below).

The suture samples were tested either unaltered or after aging for 2 and 7 days while immersed in phosphate-buffered saline (PBS) that was kept at 37 °C and replaced every 24 h. Each plate included 4 groups of 8 independent wells ($n = 32$). Each experiment was repeated 3 consecutive times.

2.4. Direct Contact Test

The Direct Contact Test (DCT) was performed as previously described (Weiss et al., 1996; Matalon et al., 2004). This assay is based on bringing bacteria in close contact with the tested material, followed by determination of microbial growth from bacteria that survived this contact.

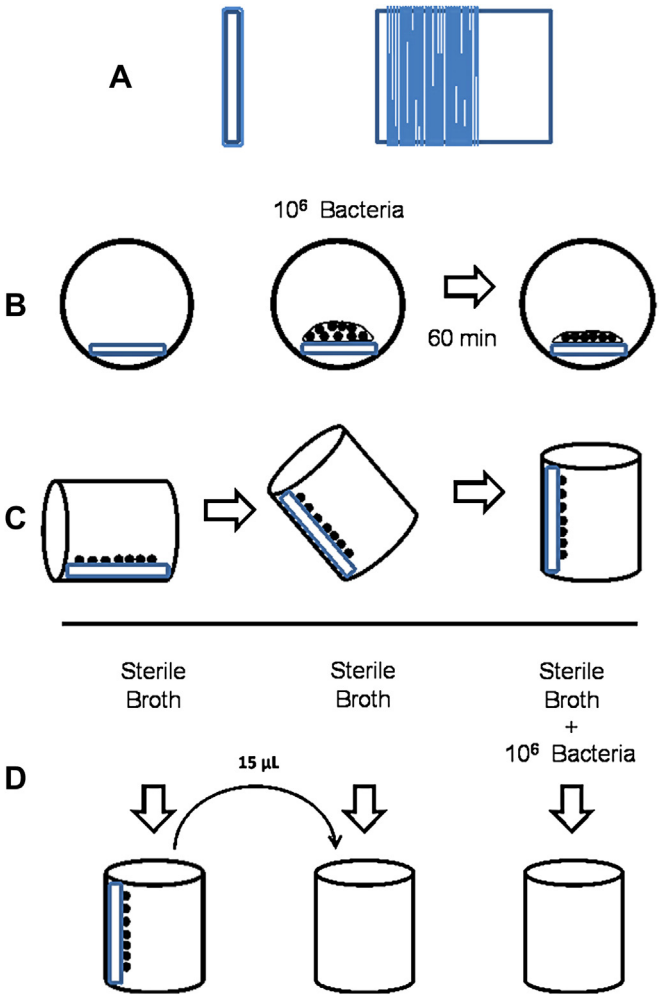

Fig. 1. The experimental setup. (A) The suture sample: polypropylene plate wrapped with the suture. Left: Cross-section. Right: View of the flat surface. (B) The microtiter plate is held perpendicular with the wells horizontal. Left: a well with the suture sample. Center: The bacterial cell suspension is placed on the suture sample surface; the suture is in contact with the bacteria for 60 min. Right: Evaporation of the water brings bacteria into intimate contact with the suture. (C) The plate is placed horizontally, bringing the wells back to an upright position. (D) Left: The well of the experimental group A well filled with BHI broth, then vortexed gently for 2 min. Center: 15 μL from the experimental group well is then transferred into a group B well to determine the bactericidal vs. bacteriostatic properties. Right: A positive control well.

A 96-well, flat-bottom microtiter plate (Nunclon, Nunc, Copenhagen, Denmark) was held vertically, on its side, with the walls of the wells parallel to the floor (Fig. 1B). The suture samples were placed in the wells with the flat surface of the sample at a horizontal orientation. Ten microliters of the bacterial suspension, optical density of 650 nm, containing approximately 10^6 of *S. aureus* (5.3×10^7), *S. epidermidis* (2×10^8) and *P. aeruginosa* (1.2×10^7) bacterial cells in BHI were placed on the flat surface of the suture sample. The plate remained in this position for 1 h at 37 °C. During this time the suspension had evaporated, thus ensuring direct contact between the microbes and the surface of the suture sample (Fig. 1B).

The plate was then re-positioned horizontally with the wells in the upright position (Fig. 1C), and 235 μl of sterile BHI were added