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Antimicrobial Effect of Medical Adhesive Composed of Aldehyded Dextran and ε-Poly(L-Lysine)

Lee, Jeong-Hyun[1,2], Hye-Lee Kim[1,2], Mi Hee Lee[1], Hideaki Taguchi[3], Suong-Hyu Hyon[4], and Jong-Chul Park[1,2]*

[1]*Department of Medical Engineering, and* [2]*Brain Korea 21 for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea*
[3]*Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, Japan*
[4]*Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan*

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Infection of surgical wounds is a severe problem. Conventional tissue reattachment methods have limits of incomplete sealing and high susceptibility to infection. Medical adhesives have several advantages over traditional tissue reattachment techniques, but still have drawbacks, such as the probability of infection, low adhesive strength, and high cytotoxicity. Recently, a new medical adhesive (new-adhesive) with high adhesive strength and low cytotoxicity, composed of aldehyded dextran and ε-poly(L-lysine), was developed. The antimicrobial activity of the new-adhesive was assayed using agar media and porcine skin. In the agar diffusion method, inoculated microorganisms that contacted the new-adhesive were inactivated, but this was not dependent on the amount of new-adhesive. Similar to the agar media results, the topical antimicrobial effect of new-adhesive was confirmed using a porcine skin antimicrobial assay, and the effect was not due to physical blocking based on comparison with the group whose wounds were wrapped.

Keywords: Medical adhesive, ε-poly(L-lysine), aldehyded dextran, antimicrobial effect

Traditional tissue reattachment methods do not meet all surgical needs. Leakage of body fluids is one of the problems of conventional tissue reattachment techniques such as sutures and staples. It is well known that the presence of suture or staple materials in surgical wounds increases susceptibility to infection [3, 7]. According to the National Nosocomial Infections Surveillance system reports, surgical site infection is the third most frequently reported nosocomial infection among hospitalized patients. Furthermore, surgical site infection is the most common nosocomial infection among surgical patients, accounting for 38% of all infections [8]. Surgical site infection may not only retard wound healing and cause wound chronicity, but also threaten the patient's life [2, 5, 10]. Therefore, it is important to prevent infection of surgical wounds because hospitalized patients tend to be more susceptible to infection owing to their underlying disease conditions.

Medical adhesives, glues used for tissue reattachment, can completely seal the wound site and keep the edges together until repair is complete. However, conventional medical adhesives such as fibrin glue, cyanoacrylate, and aldehyde-based adhesives have the disadvantages of viral infections, delayed wound healing, chronic inflammation, and high cytotoxicity [11, 15, 17]. To address these problems, a medical adhesive (new-adhesive) composed of ε-poly(L-lysine), a food additive that has antimicrobial activity, and aldehyded dextran was recently developed [11]. ε-Poly(L-lysine) was found to be nontoxic at high level in acute animal studies and was not mutagenic in bacterial reversion assays to be categorized into "Generally Recognized As Safe" (GRAS) by the US Food and Drug Administration (FDA)[6]. Moreover, Nakajima *et al.* [11] reported that aldehyded dextran and ε-poly(L-lysine) have low cytotoxicity; IC_{50} of ε-poly(L-lysine) and aldehyded dextran were 6.019 mg/ml and over 10 mg/ml, respectively. IC_{50} indicates the concentration of materials in the culture medium that depresses cell viability down to 50%, and high values of IC_{50} mean a lower toxicity. Furthermore, Araki *et al.* [1] examined *in vivo* histotoxicity for a biocompatibility assessment of biodegradable hydrogel glue composed of two solutions of aldehyded dextran and ε-poly(L-lysine) and concluded that the biocompatibility of the glue was sufficient for clinical use.

*Corresponding author
Phone: +82-2-2228-1917; Fax: +82-2-363-9923;
E-mail: parkjc@yuhs.ac

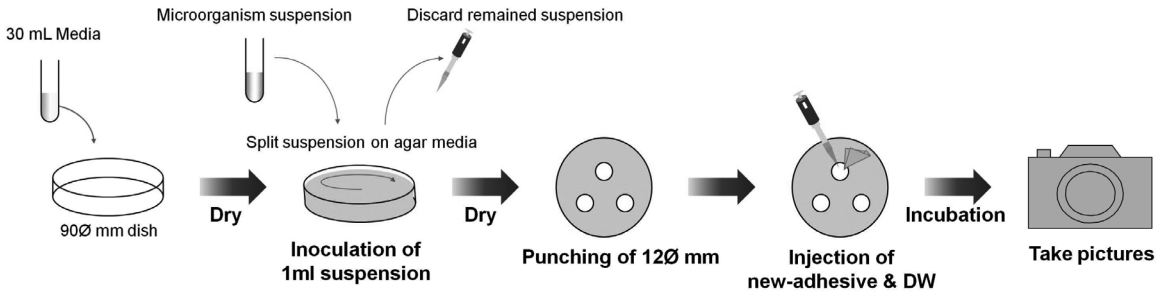

Fig. 1. A schematic of the agar diffusion antimicrobial assay for new-adhesive.

It is presumed that new-adhesive keeps the antimicrobial effect of ε-poly(L-lysine). To evaluate the topical antimicrobial effect of new-adhesive, an agar diffusion experiment was conducted. Among surgical sites, the skin is at particularly high risk for microbial infection because of its high exposure to the external environment. It is well established that porcine skin is a good model for human skin because of many similarities in properties [4, 9, 13]. Therefore, the antimicrobial effect of new-adhesive was investigated on agar media and porcine skin in this study.

Materials and Methods

Materials

To evaluate the antimicrobial effect of new-adhesive, four different bacteria and one kind of yeast were tested. The experimental bacteria were *Escherichia coli* (*E. coli*, ATCC 8739), *Salmonella* Typhimurium (*S.* Typhimurium, ATCC 13311), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 9027), and *Staphylococcus aureus* (*S. aureus*, ATCC 6358p). The experimental yeast was *Candida albicans* (*C. albicans*, NBRC 1388). Bacteria were incubated on standard method agar, which is also known as plate count agar, and tryptone glucose yeast agar (Becton, Dickinson and Company, Franklin, Lakes, NJ, USA) at 37°C, and yeast was incubated on potato dextrose agar medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 25°C.

New-adhesive changed its powder form rapidly into gel form on the basis of Schiff base formation and started to acquire adhesive characteristics after distilled water was added to it. Porcine skins were cut into 20 mm × 20 mm squares and sterilized by gamma irradiation (Gammacell ELAN, MDS Nordion, Ottawa, Canada) with 258.75 Gy. Gauzes and LLD-PE wraps were cut and sterilized by gamma irradiation.

Antimicrobial Assay of New-Adhesive by the Agar Diffusion Method

A schematic of the agar diffusion antimicrobial assay is shown in Fig. 1. A microorganism suspension was prepared in 0.9% NaCl in distilled water. The density of microorganisms in suspension was approximately 10^6 colony-forming units (CFU)/ml for bacteria and 10^4 CFU/ml for yeast. One ml of the suspension was spread onto each agar plate uniformly, and the remainder was discarded. Bare agar plates without microorganism were prepared as controls. After drying the inoculated plate, three holes (12 mm) were punched in each agar plate, and the prepared new-adhesive was injected into each hole. The plates were incubated for 7 days at 37°C in the case of bacteria and 25°C in the case of yeast, and pictures of all plates were taken using a digital camera (Powershot SX20 IS; Canon. Inc., Tokyo, Japan).

Antimicrobial Assay of New-Adhesive Using Porcine Skin

A schematic of the antimicrobial assay using porcine skin is shown in Fig. 2. Sterilized gauzes were placed in a culture dish, and prepared porcine skins were placed on it. To prevent new-adhesive and the microorganisms from drying up, distilled water was distributed to the gauzes. Each microorganism was inoculated as parallel lines onto the porcine skins. The porcine skins were treated by wrapping or by new-adhesive, or left untreated. The control group was bare porcine skins without any microorganism. After each treatment, the skins were incubated for 7 days at 37°C for bacteria and 25°C for yeast. The skins were then stained with crystal violet solution, and pictures taken.

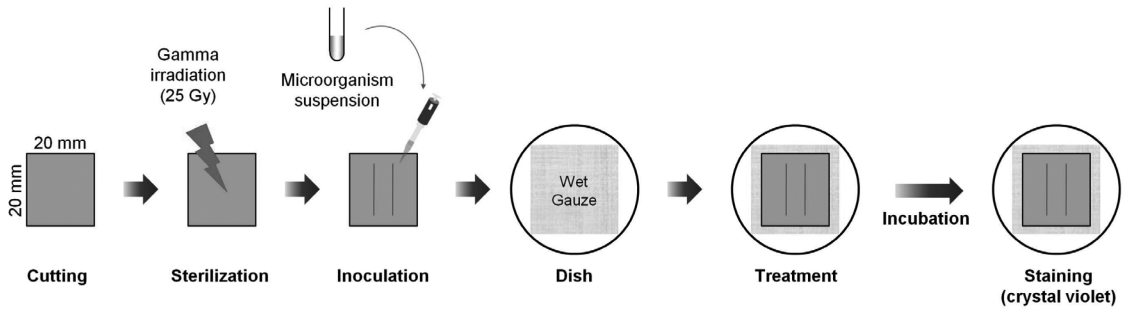

Fig. 2. A schematic of the antimicrobial assay for new-adhesive using porcine skin.

RESULTS AND DISCUSSION

Antimicrobial Assay of New-Adhesive by the Agar Diffusion Method

Images of agar plate antimicrobial assay results varying the amounts of new-adhesive are shown in Fig. 3. New-adhesive treatment had no influence on the bare agar media. On microorganism-inoculated agar, however, a transparent zone was observed around the new-adhesive treated parts, in contrast to the opaque parts over the zone where bacteria or yeast were alive. The transparent inhibition zone showed that the inoculated bacteria and yeast in contact with new-adhesive were inactivated by new-adhesive. It is presumed that the ε-poly(L-lysine) component of new-adhesive accounts for the antimicrobial effect of new-adhesive. Shima *et al.* [14] reported that ε-poly(L-lysine) has a wide antimicrobial spectrum, which supports our assumption.

The size of the zone was similar with different amounts of new-adhesive (5 mg vs. 50 mg). This result indicates that the antimicrobial activity of new-adhesive was affected partly by dextran derivatives, because aldehyded dextran was not dissolved well in water and therefore new-adhesive was not diffused much. New-adhesive would have to be effective topically on the contact region to avoid side effects caused by the diffusion of the adhesive components. It is well known that the antimicrobial activity of ε-poly(L-lysine) results from adsorption of its positively charged amino groups onto the negatively charged microbial cell walls on the basis of its polycationic property [14, 19]. Attached ε-poly(L-lysine) inhibits formation of the cell membrane by stripping the outer membrane and distributing the cytoplasm abnormally. Therefore, the positively charged amino groups of ε-poly(L-lysine) would be exposed externally after reaction with aldehyded dextran, and this cationic nature results in the antimicrobial effect of new-adhesive. Some trials to add antimicrobial activity by mixing or coating antibiotics with some medical adhesives were presented; however, it needed an additional process to make it antimicrobial [16]. Unlike these methods, the new-adhesive has antimicrobial activity without any supplementary process.

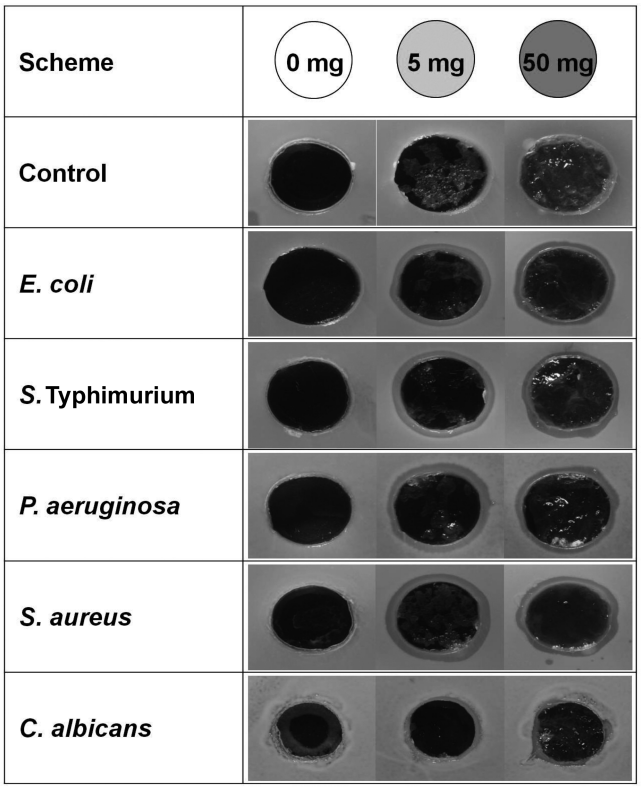

Fig. 3. The punched agar plates inoculated with each microorganism and incubated for 7 days after treatment with new-adhesive (0, 5, and 50 mg, respectively).

Antimicrobial Assay of New-Adhesive Using Porcine Skin

Images of the results of porcine skin antimicrobial assay for new-adhesive are shown in Fig. 4. Using crystal violet, a well-known dye for staining live bacterial cell walls and yeast [12, 18], the skins inoculated with bacteria were stained violet in the untreated and wrapped groups. Skins treated with new-adhesive were not stained by crystal violet. Skins inoculated with yeast showed the same tendency. The violet staining of the porcine skins meant that the inoculated microorganisms were not sterilized or inactivated. Since no bacteria or yeast were inoculated in the control group, the skins of the control group were not stained violet. Based on the results in the untreated and wrapping groups, physical protection was not sufficient to prevent infection by microorganisms, so the antimicrobial effect of new-adhesive did not come from physical protection of the site.

New-adhesive is easily hydrated by body water and activated to attach to the skin. After activation, new-adhesive could completely seal the surgical wound, preventing leakage of body fluids. Our co-workers examined the bursting pressure before and after application of new-adhesive and fibrin glue, and new-adhesive showed better sealing effect than fibrin glue after application [1]. According to this study, the amount of new-adhesive required for sealing also showed antimicrobial effect. In conclusion, new-adhesive could be a good substitute to conventional medical adhesives because it has high adhesive properties and topical antimicrobial effect and therefore can protect surgical wounds from infection until repair is complete.

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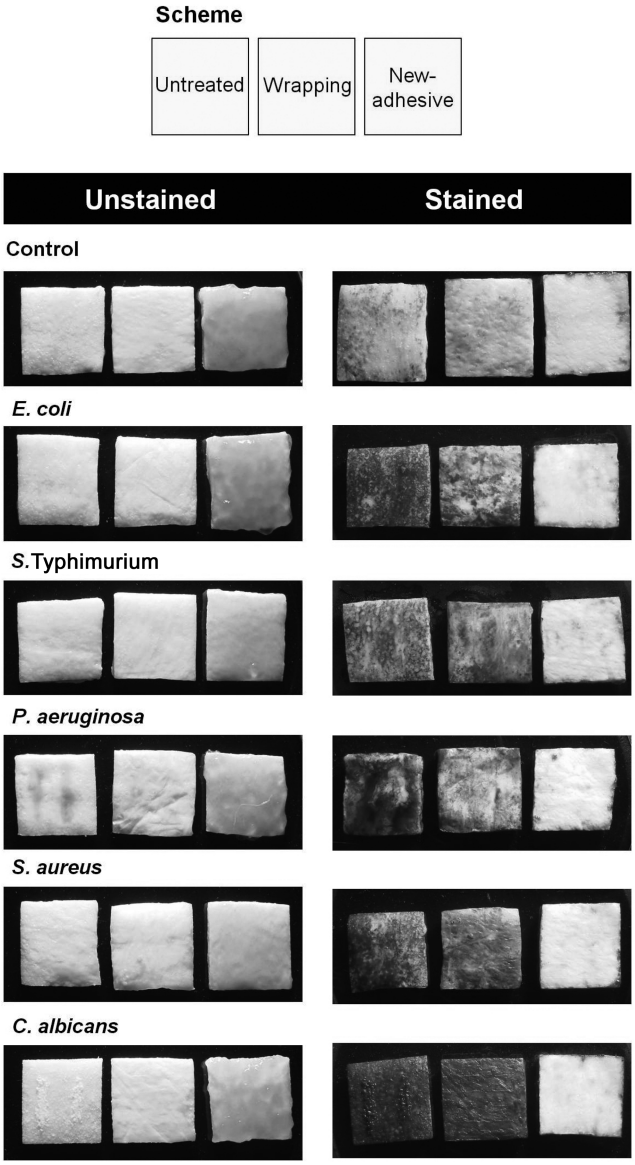

Fig. 4. Stained porcine skins inoculated with each microorganism and incubated for 7 days after wrapping, treatment with new-adhesive, or left untreated.

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