injury. According to a previous methodology,[6b] the dorsal skin of mice was exposed for 4 s to water heated to 70 °C through a Walker–Mason template to induce SDB. Histological observations showed that the epidermis of dorsal skin that had received SDB-induced injury was defective in comparison with normal dorsal skin (Figure S5 in the Supporting Information). Next, the suspension of the fragmented nanosheets was simply dropped onto the region of the SDB and then dried for 5 min. SEM observations clarified that the fragmented nanosheets perfectly wrapped the site of burn injury (Figure 3Dii, compared to Figure 3Di). This finding indicates that the flexible fragmented nanosheets adhere not only onto flat interfaces such as a SiO_2 substrate and membranes but also onto uneven interfaces such as skin, resulting in a perfect patchwork. Next, we tested the effectiveness of the seal by carefully dropping a suspension of *P. aeruginosa* onto the region of nanosheet-patchwork. Skin samples were then taken from the mice 3 days after treatment of fragmented nanosheets for histological observations. Interestingly, the *P. aeruginosa* stained in blue-purple with hematoxylin and eosin (H&E) remained on the dermis and did not migrate into the dermis of nanosheet-patchwork-protected dorsal skin with SDB-induced injury (Figure 3Div). In the case of the negative control (dorsal skin without a coating of fragmented nanosheets), the *P. aeruginosa* migrated into both the dermis and subcutaneous layer, meaning *P. aeruginosa* infection (Figure 3Diii). Six days after treatment with fragmented nanosheets, however, the skin was infected with *P. aeruginosa* despite the nanosheet patchwork (Figure S6, Supporting Information). In fact, there were some pores and cracks in the nanosheets and their partial detachment in almost all cases even before *P. aeruginosa* was applied suggests that the nanosheets may be degraded by tissue fluid exuded from the burn wound (Figure S7, Supporting Information). On the other hand, the surface morphology of the nanosheets was maintained for at least 3 days. Consequently, the single patchwork of the fragmented nanosheets could act as an excellent barrier for burn wound treatment to prevent *P. aeruginosa* infection for at least 3 days.

Next, we tested whether the barrier effect could be maintained for longer periods. To this end, we proposed the repeated patchwork treatment of the fragmented nanosheets as follows: skin with SDB-induced injury was sealed with fragmented nanosheets (1st patchwork). On day 3 after treatment with fragmented nanosheets, the region of nanosheet-patchwork was sealed or not with the nanosheets again (2nd patchwork), and then a suspension of *P. aeruginosa* was applied to the same region. The histological observations on day 6 revealed that the burn skin treated with the 2nd patchwork certainly prevented the infection, whereas the skin without the 2nd patchwork treatment was significantly infected with *P. aeruginosa* as expected (degradation manner over 3 days in Figure S7, Supporting Information) (Figure S8, Supporting Information). Consequently, the repeated patchwork treatment with fragmented nanosheets repaired the degraded 1st patchwork. This suggests that an additional patchwork treatment on day 3 could potentially prevent infection for a longer period. Moreover, we were also able to demonstrate that the fragmented nanosheets were sufficiently coated on the fingertips of a mouse by simple dipping and lifting only (Figure 2Dv and vi). This patchwork technique using fragmented nanosheets shows immense potential as a novel burn wound therapy for both relatively flat dermal skin and skin with an irregular surface shape.

In conclusion, 25 freestanding PLLA nanosheets with a thickness of 60 ± 6 nm were successfully obtained in one pot by a simple combination of a spin-coating-assisted multi-layering process of PVA and PLLA and a novel peeling technique. The nanosheets were then fragmented to form a stable suspension that could be reconstructed to provide a single continuous film that firmly attaches to various interfaces without the need for any adhesive reagents ("patchwork"). The patchwork of fragmented PLLA nanosheets displays excellent barrier ability to prevent infection by *P. aeruginosa* during the treatment of burns for 3 days. Moreover, an additional patchwork treatment on day 3 would effectively prolong the period of wound protection against infection. Hence, this material shows great promise as a novel wound dressing for burn therapy and would certainly improve the quality of life by reducing the need for dressings to be changed daily. We are also planning to fabricate fragment nanosheets carrying antibiotics and growth factors to aid the process of wound healing more effectively.

Experimental Section

Fabrication and characterization of fragmented PLLA nanosheets: All fabrication processes for fragmented PLLA nanosheets were conducted in a clean room (class 10000 conditions) to avoid contamination. Silicon wafers (SiO_2 substrate, KST World Co., Fukui, Japan) cut to an appropriate size (typically 40 mm × 40 mm) were treated with a piranha solution (96% H_2SO_4: 30% H_2O_2 = 4:1 (v/v)) at 120 °C, followed by rinsing with distilled water. PVA (Mw: 22 kDa, 99% hydrolyzed, Kanto Chemical Co., Tokyo, Japan) was dissolved in distilled water at a concentration of 100 mg mL^{-1}. As shown in Figure 1A, the solution (approximately 1 mL) was dropped onto the SiO_2 substrates, and then the substrates were spin-coated at 4000 rpm for 20 s (Spin Coater IH-D3, Mikasa Co. Ltd., Tokyo, Japan), followed by drying at 70 °C for 90 s. Next, a solution of PLLA (Mw: 80–100 kDa, Polysciences Inc., Warrington, PA) dissolved in methylene chloride at a concentration of 10 mg mL^{-1} (approximately 1 mL) was also dropped onto the SiO_2 substrates. The substrates were then spin-coated and dried as described earlier. For one-pot preparation of many PLLA nanosheets, the multi-layering process of PVA and PLLA was repeated with multiple rounds of spin-coating (4000 rpm, 20 s) and drying (70 °C, 90 s). The resulting substrates were immersed in distilled water at room temperature (RT) overnight to obtain a suspension of freestanding PLLA nanosheets. The dissolved PVA was completely removed by repeated cycles of centrifugation (1600 *g*, 10 min, RT, 5 times) and washing with distilled water. Next, the resulting PLLA nanosheets were homogenized at 30000 rpm for 1–10 min (Physcotron NS-51, Microtec, Co. Ltd., Chiba, Japan). After centrifugation (1600 *g*, 10 min, RT, 5 times), we obtained the fragmented PLLA nanosheets.

Using the above procedure, we also prepared nanosheets labeled with hydrophobic octadecylrhodamine B chloride (Invitrogen Co., Eugene, OR). The dye (20 μM) was dissolved in the methylene chloride solution of PLLA (10 mg mL^{-1}) and the multi-layering process of PVA and the PLLA was repeated with multiple rounds of spin-coating (4000 rpm, 20 s) and drying (70 °C, 90 s). The resulting substrates were immersed in distilled water at RT overnight and the PVA and free rhodamine were removed by centrifugation (1600 *g*, 10 min, RT, 5 times). The nanosheets were homogenized (30000 rpm, 10 min) and then centrifuged (1600 *g*, 10 min, RT, 5 times), yielding fragmented PLLA nanosheets labeled with octadecylrhodamine.

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The macroscopic morphologies of the freestanding PLLA nanosheets before and after fragmentation (Figures 1B and C) were photographed using a digital camera Olympus C-5050 Zoom (Olympus Co., Tokyo). In order to measure the average single-side surface area and concentration of the fragmented nanosheets, 50 μL of a suspension diluted with distilled water at an optimal concentration was dropped onto the bare SiO_2 substrate. The substrates were dried in a desiccator overnight and then analyzed using a stereomicroscope (SZX7, Olympus Co.). The average single-side surface area of the fragmented nanosheets and the number of fragmented nanosheets in a 50 μL suspension (i.e., nanosheet concentration) were subsequently calculated using an image analyzer (DP2-BSW, Olympus Co., Tokyo). Each experiment was performed at least three times. Values are given as the mean ± SD.

Formation of a patchwork of fragmented PLLA nanosheets on several surfaces: Suspensions of the fragmented PLLA nanosheets (50 μL, average single-side surface area of one fragmented nanosheet: 0.24 ± 0.08 mm^2) at a concentration range from 1×10^3 to 3×10^4 sheets mL^{-1} were dropped onto a part (6 mm × 6 mm) of the SiO_2 substrate and then dried in a desiccator overnight (Figure S4, Supporting Information). Next, we examined whether the fragmented PLLA nanosheets could be reconstructed into one continuous nanosheet. The fragmented nanosheets (concentration of 1×10^5 sheets mL^{-1}) were first cast onto a PTFE plate, which was then dried in a desiccator overnight. The macroscopic morphologies of the fragmented PLLA nanosheets adhered on the SiO_2 substrate (Figure 2Ai) and the resulting film detached from a PTFE plate (Figure 2B) were photographed using a digital camera Olympus C-5050 Zoom (Olympus Co.). Microscopic morphologies (Figures 2Aii and iii) were recorded using a digital microscope (Keyence Co., Osaka, Japan) and a Hitachi S-4500 field emission scanning electron microscope at an accelerating voltage of 5 kV. For SEM images, the substrates were coated with tetroxide (thickness: ca. 10 nm) using an osmium plasma coater (NL-OPC80, Nippon Laser & Electronics Lab., Nagoya, Japan) prior to observation.

To visualize the ubiquitous patchwork of the nanosheets on several irregularly shaped interfaces, the fragmented nanosheets labeled with rhodamine at a concentration of 1×10^4 sheets mL^{-1} (30 mL) were suspended in a glass beaker. A 18G needle (Termo Co., Tokyo), artificial skin derived from polyurethane resin, and the lower half of a mouse body including the perineum (C57BL/6, 7 weeks old, weight 20 g, Japan SLC, Tokyo) were vertically dipped and lifted into/from the beaker once each cycle and dried at RT for ca. 30 min. Before this procedure, the mice were deeply anesthetized with sodium pentobarbital at a dose of 2.5 mg per body and the hair of the lower half of the mouse body was clipped and depilated with hair removal cream (Shiseido Co. Ltd.). The macroscopic morphologies of the fragmented PLLA nanosheets adhering to the interfaces were observed by a fluorescence stereomicroscope (MVX10, Olympus Co.) equipped with a digital color camera (DP72, Olympus Co.) for Figures 2Ci,ii or a digital camera (C-5050 ZOOM, Olympus Co.) for Figures 2Ciii,iv or by an in vivo imaging system (OV100, Olympus Co.) for Figures 2Cv,vi.

The surfaces of the substrates were observed using a stereomicroscope (SZX7; see Figure 2D) and the covering effect of fragmented PLLA nanosheets (surface coverage) was calculated from data obtained using an image analyzer (DP2-BSW, Olympus Co., Tokyo). The average thickness of fragmented PLLA nanosheets adhering to the SiO_2 substrates was also analyzed in parallel using a surface profiler α-step (KLA-Tencor Co., San Jose, CA). Each experiment was performed at least three times. Values are given as the mean ± SD.

In vitro bacterial permeability assay: A suspension of fragmented PLLA nanosheets (50 μL) at a concentration of 8.3×10^3 sheets mL^{-1} was dropped onto the outside of a Transwell Membrane (TM, diameter: 6.5 mm) kit with a pore size of 8 μm (Corning Co., Inc., NY) (Figure 3A). Accelerated permeability measurements of *P. aeruginosa* were made using three additional large pores (diameter: 1.2 mm each), corresponding to a total area of 3.4 mm^2. The TM coated with fragmented nanosheets was positioned across a 24-well plate, which was filled with 100 and 600 μL of RPMI-1640 medium containing a 10% (v/v) fetal bovine serum (FBS; without antibiotic) in the inner and outer compartments, respectively. A 10 μL suspension of *P. aeruginosa* (1×10^8 CFU mL^{-1}) was carefully added to the inner compartment and then cultured for 6 h. The number of bacteria in the outer well that had passed through the nanosheet-coated TM was subsequently determined (Figure 3C). A control TM was also made using three additional pores without any coating. Each experiment was performed at least three times. Values are given as the mean ± standard error of mean (SE). For Figure 3B, microscopic morphology of the TM surface after coating with the fragmented nanosheets was recorded using a scanning electron microscope (VE-9800, Keyence Co., Osaka, Japan) at an accelerating voltage of 1 kV.

In vivo therapeutic barrier effect of the fragmented PLLA nanosheets: All animal studies were approved by the Animal Research Committee of the National Defense Medical College. Male C57BL/6 mice (7 weeks old, weight 20 g, Japan SLC) were studied. The mice were deeply anesthetized with sodium pentobarbital at a dose of 2.5 mg per body and their dorsal hair was clipped and depilated with a hair removal cream (Shiseido Co. Ltd.). After this procedure, dorsal skins of mice were exposed for 4 s to water heated to 70 °C through a Walker–Mason template to induce SDB injury.[6b] The burn area was controlled to be approximately 20% of the total body surface area. The suspension of fragmented nanosheets (1.4×10^4 sheets mL^{-1}, 30 μL) was dropped on the site of the dermal burn wounds (1 cm circle) and then dried for approximately 5 min. Next, a suspension of *P. aeruginosa* (1×10^7 CFU μL^{-1}, 10 μL) was carefully dropped on the nanosheets and the mice were wrapped with an OpSite Flexifix transparent film roll (Smith & Nephew Wound Management KK, Tokyo). As a negative control, we induced SDB injury in mice but did not treat them with a fragmented-nanosheet coating. Skin was sampled from the mice 3 or 6 days after treatment with fragmented nanosheets, fixed with 10% formaldehyde, and then stained with H&E (Figure 3D and Figure S6 in the Supporting Information). To examine the barrier effects of the repeated nanosheet-pachwork, skin with SDB-induced injury was coated on day 1 with the fragmented nanosheets (1st patchwork: 1.4×10^4 sheets mL^{-1}, 30 μL) and were wrapped with an OpSite film roll. On day 3 after medical intervention, the region of the nanosheet-patchwork was coated again with the nanosheets (2nd patchwork) or not, and then *P. aeruginosa* (1×10^7 CFU μL^{-1}, 10 μL) was dropped onto the area and the skin was wrapped with an OpSite film roll again. Skin samples were then taken from the mice on day 6 after treatment with fragmented nanosheets, fixed with 10% formaldehyde, and then stained with H&E (Figure S8, Supporting Information). Each experiment was performed at least three times.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the authors.

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