Combinatorial wound healing therapy using adhesive nanofibrous membrane equipped with wearable LED patches for photobiomodulation

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Wound healing is the dynamic tissue regeneration process replacing devitalized and missing tissue layers. With the development of photomedicine techniques in wound healing, safe and noninvasive photobiomodulation therapy is receiving attention. Effective wound management in photobiomodulation is challenged, however, by limited control of the geometrical mismatches on the injured skin surface. Here, adhesive hyaluronic acid–based gelatin nanofibrous membranes integrated with multiple light-emitting diode (LED) arrays are developed as a skin-attachable patch. The nanofibrous wound dressing is expected to mimic the three-dimensional structure of the extracellular matrix, and its adhesiveness allows tight coupling between the wound sites and the flexible LED patch. Experimental results demonstrate that our medical device accelerates the initial wound healing process by the synergetic effects of the wound dressing and LED irradiation. Our proposed technology promises progress for wound healing management and other biomedical applications.

INTRODUCTION

Skin is the largest organ system of the human body and plays a key role as the first barrier protecting the internal organs from environmental hazards by restoring a physiologically compromised condition. Owing to the excellent regenerative properties of the skin, the injured skin tissue or surgical wound can be healed through the biological healing process in the four physiological events, including hemostasis, inflammation, proliferation, and mutation (remodeling) (1). Although the traditional therapies such as ointment, dressing, and derived compound are currently in practice, these strategies are insufficient for the contribution in full stages (2–5). Thus far, a major class of biomaterials such as hydrogels (6, 7), fibrous membranes (8), porous structures (9), and drug-embedded functional electrospun nanofibers have been developed for wound treatment to support pain relief and infection prevention by covering wound sites (10–12). The main goal of those strategies with engineered wound dressings is to provide a favorable environment promoting the wound healing cascade in control of the tissue formation and mediate multiple cellular fate including cell survival, proliferation, and morphogenesis. Therefore, the development of materials has focused on mimicking the microenvironment of natural extracellular matrix (ECM) for wound care purposes, including collagen, gelatin, hyaluronic acid (HA), and chitosan, and consisted of a multiscale mat or electrospun nanofibers (13–19). In particular, layered nanofibrous structures used in the treatment of wounds represent a very similar physical structure to natural ECM to facilitate the regenerative process with their high porosity and surface-to-volume ratio. Therefore, these structural advantages allow them to protect wound beds with favorable properties of their intrinsic structures as engineered scaffolds in wound healing and hemostasis (20–30).

In many biomedical applications, gelatin has been considered as one of the most promising biomaterials, as a Food and Drug Administration–approved natural polymer, due to its excellent biocompatibility, hydrophilicity, and suitable degradation profile under a physiological environment (31–34). To date, gelatin has shown excellent performance contributing to wound repair when conformally placed to the wound sites by forming a gel state to absorb the exudate with the permeability to water and oxygen in the regular healing process under an isolated wound environment (35–38). In addition to this, the easy control of the fiber diameter and pore size is advantageous in the manufacturing process when produced by electrospinning technique, resulting in favorable architectures that can mimic the biocompatible nanofibril ECM to promote wound healing process (39). However, the electrospun gelatin nanofibers generally exhibit unsatisfied mechanical strength hard to maintain the morphological integrity with a lack of stiffness when it exposed in physiological moist conditions, which has restricted the further applications of intrinsic gelatin nanofibers (40, 41). To overcome this limitation, many efforts have been made on cross-linking strategies in a simple manner. Among other material systems for the cross-linking approach, natural polysaccharide, HA, has been explored as a proper constituent of ECM in tissue engineering, mainly due to its advantageous physicochemical properties, demonstrating excellent nontoxic biocompatibility, appropriate biodegradability, nonimmunogenicity, and versatile bioactivities (42, 43). With some specific molecular configurations, the applied HA plays an important role in cell growth and drug delivery involving a series of biological processes and contributes to the structural stability that guides tissue regeneration (1, 44–46). Despite these advantages, some drawbacks still exist in the form of nanofibers, such as less-effective cell adhesion and weak mechanical stability. Thus, to improve the capability in the wound dressing

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applications, other cell adhesion–promoting molecules are additionally required in the scaffold materials system. Recently, a catechol moiety (i.e., ortho-dihydroxy phenyl group) has garnered notable attention because of its strong affinity to a wide range of substrates such as organic or inorganic compounds and even living tissues (47–49). Particularly under oxidizing or alkaline conditions, oxidative conversion of the catechol group to ortho-quinone can be derived, which is highly reactive with various organic functional groups, including thiols, amines, and other oxidized catechol via Michael-type addition or Schiff base reactions (50, 51). The ability of catechol moiety as an adhesion promoter results in beneficial network formation on biological surfaces in provided environments, indicating great potential for designing wound dressing materials targeted at skin tissues.

As an emerging technology in tissue-electronic interfaces, advanced noninvasive therapeutic devices have been recently growing, owing to their potential compatibilities in clinical applications. With some suitable choice of materials and integration strategies as transplantable electronics, the designed devices have presented unique features to enable collecting physiological data transduced through the tissue-faced devices from the human body. For example, wearable or skin-attachable devices (e.g., electronic tattoo) can be built in affordably thin substrates required to achieve conformal contact with target lesions, for on-demand treatment, in which the conformally interfaced electronic devices are to be operated on uneven and soft tissues. In particular, simple forms of flexible electronic devices in a viable format have been rapidly developed and boosted by the industrial manufacturing process in a scaled-up condition for personal health care devices. On the basis of some conceptual studies, this new type of device could be used to obtain quantitative data for monitoring the diagnostic status in time, specifically for skin diseases and the wound healing process. This progress has been extensively reported in other forms of biomedical devices, and the recent advances, to date, have witnessed many skin-fixed devices replacing or supporting traditional therapeutic protocols to evaluate the wound healing process. In this context, among other candidate platforms, flexible optoelectronic devices may be an effective option that yields the local irradiation of sufficient light energy to skin wounds. Especially in chronic ulcer wounds, photobiomodulation (PBMT) studies have suggested a viable route to treat target lesions for more rapid closure of wounds in the healing of bones, tendons, and nerves by using low-power light sources (i.e., laser); thereby, the ultimate demands would be engineering design of mature integration with the extended optical component. Thus, this low-level light therapy, known as PBMT, can be evaluated through in vitro and in vivo experiments by the demonstration of the positive effects in terms of a significant time reduction. However, due to the limited control of the geometrical mismatches on the injured skin surface during the skin therapy, it has been difficult to use continuous illumination using a conventional light source with the conformally fixed position at a desired fluence rate for the target lesions. Therefore, the challenge remains in contact with the output ends of the light source from the optoelectronic device on proximity to the tissue interface with the help of bridged biocompatible materials to facilitate the tissue regeneration for specific wound type.

Here, we report newly developed wound dressing materials integrated with a flexible substrate embedded with multiple light-emitting diode (LED) arrays that fully distribute light sources onto an entire damaged skin. This strategy exploits the high efficacy of densely produced nanofibers with sufficient porosity as a wound dressing layer that might be the promising alternative for complete skin regeneration with full-thickness level, while the light therapy based on the flexible electronic device also ensured the healing system through a direct illumination onto wound tissues. As a wound dressing material, HA–cross-linked gelatin nanofibrous fabric (HA/Gel) with catechol moiety was prepared by electrospinning for an effective acceleration of the internal wound healing process. In addition to this, the synergistic effect of light exposure [i.e., near infrared (NIR) wavelength] was studied. Key enabling characteristics of this strategy are to combine together with the unique functional wound dressing materials and mechanically compliant architectures of LED device added as an independently functional unit to support the nanofabric wound dressing. To confirm the capability of the wound dressing materials, systematic experiments were conducted: in vitro adhesion test using porcine skin and hemostatic test using the rat’s liver, respectively. In addition, the combinatorial effects of the wound dressing integrated with a flexible LED device were evaluated by in vivo rat wound model for the actual observation of the wound healing process on the wound sites. Our optoelectronic system integrated wound dressing platform (i.e., skin-adhesive medical device) has improved the regular healing process that can help repair damaged tissues with active treatment by restoring the integrity of wound skin toward clinical wound management.

**RESULTS**

**Combinatorial treatment for wound healing process: Nanofabric wound dressing with flexible LED device**

Figure 1A illustrated our main concept for an advanced wound dressing device implemented with LED arrays in a flexible format to promote regeneration of tissue by PBMT. The HA/Gel-based nanofabric wound dressing can be conformally faced to the wounded area, which may accelerate a more profound effect on tissue synthesis and initiate cellular proliferation for combinatorial treatment. In particular, an interlayered membrane made of the nanofibrous structure provides bioactive ECM to organize cells and assist in new tissue deposition as a bioactive wound dressing. The digital image (inset in Fig. 1A) shows an individual multi-LED array (total number of 30: 6 × 5 each; fig. S1), arranged on a flexible polyimide (PI) substrate, which can illuminate the coverage of the target wound area. In our experimental design, the surface light source of the multi-LED array was manufactured by standard surface-mount technology directly joining the individual LED components on a flexible printed circuit board (FPCB). This is advantageous with a straightforward strategy that is compatible with a fully established scaled-up manufacturing process when appropriate electrical components are selected. A detailed description of the substrate and associated geometric elements is presented in Fig. 1B. As described in the cross-sectional view of the schematics, to increase space efficiency and mount the isolated LED components on the circuit pads, the surface mounting was performed on the upper side of the FPCB interconnected to the lower side via holes to provide the electrical path. The copper microelectrode array (t = 10 μm) was defined by photolithography and metallization process on both sides, and the microjunctions between the LED component and electric pads were firmly fixed with a solder paste and subsequent underfill process. In addition, the device fabrication was completed...
by supplementing the coverlay layer of PI film (25 µm) on the LED-equipped substrate. This is an important process for the reliable performance of the electronic device applied in bioactive environments to prevent unwanted reactions with exposed metal electrodes. A digital image in Fig. 1C demonstrates illuminating flexible and thin multi-LEDs conformally attached to the rat skin surface. In addition, the electronic LED components and interconnects based on FPCB substrate were readily packaged with a thin layer of transparent polydimethylsiloxane (PDMS) to prevent infiltration of bioliquid before use in the following experiments, as presented in Fig. 1D. Last, a newly developed wound healing patch was created by combining a multi-LED patch and an HA/Gel-based nanofabric wound dressing as an all-in-one device (Fig. 1E); this captured digital image of the device configuration illustrates a set of LEDs built in a thin flexible substrate integrated with nanofabric dressing ($t \approx 230$ µm). In this demonstration, the example porcine skin was uniformly exposed to the red light source of multi-LED right through the wound dressing. For more accurate information, the power density was carefully measured to ensure the intensity of the light source reaching the wound region. In this work, we selected LEDs with a wavelength of 630 nm for wound healing to support dopamine-modified HA (D-HA)/Gel efficacy. This optical window

Fig. 1. Preparation and characterization of flexible LED devices. (A) Schematic illustration of the D-HA/Gel nanofibrous mesh prepared by electrospinning process and subsequent chemical cross-linking. (B) Detailed configuration of the FPCB with the LED arrays mounted on a PI substrate through via holes. (C) A digital image of the electrically powered flexible LED patch placed on rat skin. (D) A digital image of the multi-LED device after the application of transparent PDMS encapsulant. (E) A digital image of conformally attachable device demonstration on porcine skin as an all-in-one device composed of medical tape, multi-LED array, and nanofibrous membrane. (F) The measured power density of the encapsulated multi-LED array through the nanofibrous membrane and rat skin sample/nanofibrous membrane. (G) Repetitive folding test ensuring mechanical stability under the various bending radius. (H) Current/voltage characteristics of the multi-LED array corresponding to the bending radius.
The choice of this spectral range is primarily due to the fact that the NIR light source can penetrate deeply into biological tissues and reach hypodermis by a weak absorption of water, hemoglobin, collagen, and proteins. As presented in Fig. 1F, the gradual reduction of the power density of the light source was detected in each virtually situated condition. The encapsulated and the nanofibrous membrane-covered multi-LED array showed a surface power density of ~15.05 and ~13.37 mW cm\(^{-2}\), respectively. When applied to the final rat skin, a gradual attenuation of light transmission was observed at ~4.59 mW cm\(^{-2}\). In this measurement, we confirmed that the light source can penetrate through the wound dressing membrane with a minimum loss of ~11%, but the power density was decreased by ~30% through the skin compared to the originally provided power density. However, the fluence rate exposed to the multi-LED array sufficiently penetrated the skin for the wound healing experiments, synergistically combined with a wound dressing material. Because the ranges of the power density mentioned in the previous reports have been known to be ~5 to 8 mW cm\(^{-2}\) for in vivo studies (55), we postulated that the optical window range and the power density values may derive positive efficacy to help skin regeneration. Moreover, the physical reliability in the illuminating duration is one of the important parameters in the designed scheme of PBM therapy. The repetitive folding test under the various bending radius represented excellent mechanical stability of the freestanding multi-LED array, in which the power density and corresponding I-V characteristics were measured in the operating system with a certain radius curvature from 2.4 to 5.8 mm, as presented in Fig. 1 (G and H).

**Design and characterization of HA/Gel-based nanofabric wound dressing**

As a wound healing matrix, nanofiber-based membranes are well suited for severe wounds with high levels of exudate. The materials grade for maintaining homeostasis should be improved, as excessive exudate absorption may cause a separation of the wound dressing from the wound bed. To overcome this limitation, our main idea of the HA/Gel-based nanofabric wound dressing was designed as presented in Fig. 2A. The catechol-functionalized gelatin nanofibers were produced by an optimized electrospinning process and assisted with dopamine hydrochloride during the cross-linking of HA. In this synthetic scheme, the cross-linking of dopamine-containing gelatin nanofibers were proceeded by using 1-ethyl3-(3-dimethylaminopropyl) carbodiimide (EDC)\(/{N}\)-hydroxysuccinimide (NHS) to activate the carboxylic group from HA and gelatin, thereby enabling the coupling with the amine group of gelatin at a range of the acidic pH condition (Fig. 2B). The proton nuclear magnetic resonance (\(^1\)H-NMR) and Fourier transform infrared (FTIR) spectroscopy were used to confirm the cross-linking of the gelatin nanofiber with HA and the functionalized catechol group. The \(^1\)H-NMR spectrum indicated the successful grafting of HA with dopamine moieties on the gelatin backbone. For example, the new peaks at 8.6 and 7.55 parts per million (ppm) were observed in catechol-functionalized gelatin nanofibers (see fig. S2A), which are assigned to protons of the methylene group close to catechol moiety and aromatic protons in the dopamine-gelatin conjugate, respectively. In addition, the increased intensity of peak areas between 8 1.8 and 8 2.2 ppm also indicated the methyl resonance of acetamido moiety of the \(N\)-acetyl-\(d\)-glucosamine of HA. As shown in fig. S2B, the incorporation of HA in the nanofibrous structure was also confirmed by the FTIR measurement, in which the D-HA/Gel, compared to the pristine gelatin nanofibers, exhibited the new peaks at ~1000 to 1300 cm\(^{-1}\) and broad peaks at ~2900 to 3300 cm\(^{-1}\), indicating the vibration of the hydroxyl groups of HA.

As a result, the catechol-functionalized HA/gelatin nanofibers (D-HA/Gel) could be prepared in a large area (i.e., 200 mm by 300 mm; Fig. 2C), and the morphology of the samples was observed by scanning electron microscopy (SEM). As shown in Fig. 2D, the representative SEM images represent the well-ordered D-HA/Gel nanofibers. The features of the randomly organized nanofibers appeared as a hierarchical arrangement with nets interposed in the nanofiber structure, as examined in the highly magnified SEM image (inset in Fig. 2D). For more information, the diameter distributions of nanofibers were also surveyed, representing the range of ~450 to 550 nm, as presented in Fig. 2E. As the content of dopamine to gelatin increased, the morphology of samples changed significantly, which may be due to the delicate interactions of the amidation reaction between dopamine, HA, and gelatin. Therefore, the morphological changes of nanofibers were investigated, such as folded or bent structures cross-linked by HA (fig. S3), and as a result, a submesh (i.e., nets) was formed between the nanofiber networks. As previously reported (33, 56, 57), we believe that this double-layered network of HA/Gel is highly advantageous in wound dressing by mimicking the ECM of the skin structure for the regeneration of skin tissues. Besides this, the physical property is one of the important parameters in the real application of wound dressing material because appropriate mechanical strength associated with viscoelastic behavior is the main characteristic feature for maintaining homeostasis of the wound beds (58). Therefore, to analyze the correlation of dopamine in the mechanical behaviors of HA/Gel, a series of control experiments were performed on the conjugation of HA and dopamine to gelatin nanofiber by changing the dopamine content. As denoted by D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10, the amount of dopamine was finely tuned in the HA/Gel matrix. As presented in Fig. 2F, the samples containing dopamine exhibited the increased tensile strength compared to the originally produced HA/Gel (i.e., 21.9 ± 2.2 kPa). As a result of measuring the ultimate tensile strength of the samples, the values gradually improved to 30.6 ± 7.9 kPa, 37.7 ± 6.6 kPa, and 46.1 ± 16.3 kPa in the D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10 samples, respectively. Notably, these values were compatible with other strength levels or much higher mechanical stability reported for hydrgel-based wound dressing materials (59, 60).

In addition to this, the appropriate absorption capability of the exudate from the wound bed is necessary for ideal wound treatment, which can be an effective absorbent for heavy exudates to trap the biofluids. On this, the swelling capacity on the different nanofibrous membranes was thoroughly investigated by measuring changes in wet weight for 24 hours in phosphate-buffered saline (PBS) at 37°C as summarized in Fig. 2G; all the samples reached an equilibrium state after 12 hours of incubation. Notably, compared to HA/Gel membrane (516.4 ± 38.3%), the wound dressing membrane that contains increased amounts of dopamine exhibited a higher swelling capacity of 809.9 ± 79.4%, 967.2 ± 209.4%, and 1070.9 ± 46.6% for the D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10 samples, respectively. In addition, the water absorption rate of each wound dressing was evaluated by measuring the changes in water contact angle. All samples could absorb PBS quickly, and the instant contact angle on
the surface against a drop of PBS was rapidly decreased within 1 s from 61.2° ± 8.7° to 0° (fig. S4). Tissue scaffold for wound healing should have suitable degradability in recovered physiological conditions to match new tissue regeneration. To verify the degradability, the weight loss for each sample was measured in collagenase type II solution at 37°C as presented in Fig. 2H. Notably in this experiment, a faster degradation rate was observed from the samples that contain increased dopamine amounts. HA/Gel with the absence of dopamine was degraded for 6 days, but the other samples were completely degraded after 5, 4, and 3 days for D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10, respectively.

The adhesive strength of the nanofibrous wound dressing was also investigated by in vitro lap shear test using a porcine skin model as shown in Fig. 2I. The adhesiveness is a favorable property that contains increased dopamine amounts. HA/Gel with the absence of dopamine was degraded for 6 days, but the other samples were completely degraded after 5, 4, and 3 days for D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10, respectively.

Fig. 2. Preparation and characterization of nanofiber membrane. (A) Schematic illustration of the preparation D-HA/Gel nanofibrous mesh by electrospinning and chemical cross-linking. (B) Structural representation of synthesized D-HA/Gel nanofibrous mesh. (C) Photograph of a large area of D-HA/Gel nanofiber membrane. (D) Representative SEM image and (E) diameter of catechol-functionalized HA/gelatin nanofibers (D-HA/Gel). (F) Ultimate tensile strength, (G) swelling ratio (%), and (H) remaining weight (%) of each catechol-functionalized HA/gelatin nanofibers (D-HA/Gel; means ± SD, n = 5). (I) Photograph and schematic illustration of lap shear test. (J) Stress-strain curves, and (K) ultimate strength after lap shear test using nanofibers (means ± SD, n = 5). Statistical significance indicates *P < 0.05, **P < 0.01, and ***P < 0.001.
because it helps to prevent bacterial cloning and exudation of fluids in the wound site, which has been reported in the application of biomedical fields (60, 61). Since the catechol group in dopamine hydrochloride has been studied as a function of tissue adhesive material, we evaluate the adhesive capability using the inside of porcine skin (i.e., fat layer), which is closely related to the significantly improved hemostatic capacity of the nanofibrous wound dressing. For the sample preparation, the rectangularly cut porcine skin was brought into contact and uniformly pressed for 1 hour. As measured in Fig. 2 (J and K), the maximum adhesive strength of the primary HA/Gel resulted in 0.97 ± 0.29 kPa, which is a lower value compared to the control samples that only used porcine skin (i.e., 1.32 ± 0.30 kPa). On the other hand, more energy was consumed to separate the skin by increasing the adhesive strength using the D-HA/Gel5 and D-HA/Gel10 samples to 1.79 ± 0.57 kPa and 1.94 ± 0.37 kPa, respectively. This set of results suggests that the noncovalent bonding of catechol groups contributes to increasing the adhesive strength through interaction with skin proteins. To scrutinize the correlation between the physically arranged nanofibrous membrane and chemical structure of the D-HA/Gel, we tested the peeling force in a situated condition as presented in fig. S6. Obviously, the nanofibrous membrane in the dried and the wetting states showed a different behavior when we measured the shear stress of the nanofibrous membrane on porcine skin. In the dried condition, the membrane did not show sufficient adhesive properties, but enough adhesive forces were exerted in the wetting state. As shown in the SEM results in the dried state (Fig. S3), as dopamine content increases, the bending of the gelatin fiber occurs, and the cross-linking density by intermolecular interactions increases as well, where the ultimate strength reached a higher level. However, in the wet state, since dopamine and HA were conjugated to gelatin nanofiber, the membranes were highly hydrophilic, and the hydrophilicity could be increased by the dopamine catechol group, which leads to better water penetration and improved swelling behavior. In addition, when the content of the catechol group is increased, the dopamine quickly arouses intramolecular hydrogen bonds with each other, which leads the self-aggregation (62–63). Thus, D-HA/Gel10 showed the highest swelling properties. Accordingly, under this specific condition, the chance of the hydrophilic nanofibers being exposed to catalysis could be increased, the decomposition may be accelerated in the catalytic solution, leading to the fastest degradation.

**In vivo excision wound healing test and hemostatic ability of HA/Gel-based nanofabric wound dressing**

To evaluate the healing of full-thickness skin wounds with minimal intervention by HA/Gel-based nanofabric wound dressing, the dorsal skin of Sprague Dawley (SD) rats was selected as an excision wound healing model. Wounds were observed for 14 days, and the rats survived in healthy conditions during the experimental period. As shown in Fig. 3A, the biopsy punch was used to make a wound, and the HA/Gel nanofibrous wound dressing was placed in the wound sites. The prepared wound dressing was conformally stuck to the wound beds with high adhesion due to the catechol groups on the surface of the nanofibers [inset digital image (right panel) in Fig. 3A]. The structural similarity of the dense nanofibrous membrane was perfectly fit to the wound sites as illustrated in Fig. 3B, which is expected to accelerate the wound healing process with a hierarchically ordered configuration by mimicking the skin ECM (Fig. 3C). As a result, Fig. 3D represents the apparent wound healing processes during the periodic treatment for control groups. From day 4, the D-HA/Gel10–treated wounds represent slightly faster healing effectiveness than other groups, which is more obvious on day 11. The wound area was quantitatively estimated at different periodic ranges as shown in Fig. 3E. The wound area of D-HA/Gel10 was significantly decreased by ~35% on day 7 compared to the untreated groups (i.e., ~25%). After then, the wound was rapidly closed within 2 weeks.

Generally known, at a proliferative phase in the wound healing process, the granulation tissue fills the wound bed with connective tissue, and then formation of new blood vessels. At this time, the wound margins contract and pull toward the center of the wound. In addition, epithelial cells arise from the wound bed or margins and begin to migrate across the wound until the wound is covered with epithelium. This proliferative phase lasts from 4 to 24 days. To understand and evaluate the transitions of the proliferative phase, a histological examination was carried out for each case. As presented in Fig. 3F, continuous epidermis formation and vascularization appeared on the wounds treated with D-HA/Gel5 and D-HA/Gel10 compared to the control group. In hematoxylin and eosin (H&E) staining results, dopamine-containing wound dressing helped lessen the inflammation and the formation of new blood vessels (see marked yellow arrows in optical micrographs). Additional Masson’s trichrome staining was able to assess collagen deposition, which is an important factor in the remodeling of newly formed tissues. As observed, only the dopamine-containing nanofibrous membrane treatment groups showed a much higher portion of collagen in a linear form (i.e., blue-stained area). In addition, the collagen density in the D-HA/Gel10–treated group manifested the highest value. The above set of results suggested that the dopamine-containing nanofibrous meshes can accelerate the wound healing process. As previously reported (64, 65), catechol-functionalized HA patches for wound healing assessment has been confirmed, so we believe that the introduced dopamine moiety with high biocompatibility is of great help in the wound healing treatment.

We also evaluated the hemostatic ability of our prepared nanofibrous membrane using an in vivo hemorrhage model based on the superior adhesive properties of the matrix (66). Hemostasis, the phase of wound healing, begins at the onset of injury and leads to the cessation of bleeding. During this phase, the body activates the blood clotting system to block the drainage (67). To achieve the prevention of excessive bleeding, we need biocompatible hemostatic materials that work effectively and quickly. However, the conventional hemostatic materials such as alginates, proteins, gauze, and bandages do not meet the criteria well (68, 69). Therefore, we attempted to validate our nanofibrous membrane as an alternative for this application. To induce bleeding for in vivo experiments, a rat liver was punctured by a 19-gauge needle, and then the nanofibrous membrane was conformally placed on the punctured site. The measured blood loss was found to be $n = 5$ (Fig. 3, G and H). The hemostatic effect of the prepared nanofibrous membranes was obvious as captured by digital images (Fig. 3G). The weight of blood loss for the untreated control groups was $244.025 ± 77.1$ mg and $214.4 ± 19.5$ mg for HA/Gel groups, respectively. However, the weight of blood loss was significantly decreased, as measured to be $63.0 ± 32.9$ mg for D-HA/Gel5 ($P < 0.01$) and $49.74 ± 20.1$ mg for D-HA/Gel10 ($P < 0.05$), respectively. Notably, the hemorrhage control was accelerated as the content of the catechol moiety
Fig. 3. In vivo characterization of nanofibers. (A) Photograph, (B) schematic illustration, and (C) wound healing of nanofibers in excision animal model. (D) Sequential photographs and (E) quantitative area of each wound site treated for each group after day 0, day 4, day 7, day 11, and day 14 (means ± SD, n = 5). (F) Histological analysis results after wound healing (yellow arrows indicate blood vessels.) (G) The hemostatic ability of nanofibrous mesh on rat liver model. (H) Bleed amount after treatment of each group (means ± SD, n = 5) (*P < 0.05, **P < 0.01, and ***P < 0.001).
increased. These results suggest that nanofibrous membrane containing catechol moiety increases binding affinity and prevents fluid loss by exudation in wound healing. The excellent hemostatic ability of D-HA/Gel can be attributed to its tight adhesive interactions with the bleeding site. On the basis of the above results, we used D-HA/Gel10 for further PBM evaluation because of the best results in the excision wound healing and hemostatic ability test. For more information on this nanofibrous wound dressing (i.e., D-HA/Gel10), we measured air permeability and porosity to ensure superior oxygen permeability, which was found to be $21.94 \pm 0.58$ ml s$^{-1}$ cm$^{-2}$ and 89.7%, respectively.

**Cell proliferation, morphology, and migration under the NIR irradiation**

NIR irradiation has been widely used in medical use, especially for assisting wound healing (70). It is well known that the NIR light source can be absorbed into the mitochondria and promote mitochondrial respiration and adenosine triphosphate production (71). So far, it is expected that the use of light sources of specific wavelengths increases the growth factor production, cell proliferation, cell mobility, adhesion, and ECM deposition during the proliferation and remodeling phase of wound healing. In addition, for the wound dressing application using nanofiber-based ECM matrix, cell adhesion and proliferation are important parameters. On these, as a preliminary work, we cultured cells (i.e., NIH/3T3 murine fibroblasts) on the nanofibrous membrane to observe the combinatorial effects with NIR irradiation as shown in Fig. 4A. Slightly different contrast levels from cultured cells on each condition were observed. As measured in Fig. 4B, the immunofluorescence intensity levels from the F-actin in cells indicate that the NIR irradiation was highly effective on the cell proliferation by synergistic incorporation of catechol-containing nanofibers. When the cultured cells were observed by SEM on each nanofiber matrices (Fig. 4C), the fibroblasts were well adhered to the matrix surface along with the individual nanofibers. As noticed, the cells were uniformly spread by extending their filopodia following the nanostructured surface, which reveals that

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**Fig. 4. Cellular behaviors on nanofibrous membrane.** (A) Confocal microscopy of NIH/3T3 cells on HA/Gel and D-HA/Gel nanofibrous membrane with/without NIR illumination. (B) Fluorescence intensity of Alexa 488 in NIH/3T3 cells in confocal image (means ± SD, n = 5). (C) SEM images of NIH/3T3 cells on HA/Gel and D-HA/Gel nanofibrous membrane with/without NIR illumination. (D) Microscopic images showing the migration of NIH/3T3 cells (×40) with/without NIR illumination. (E) Cell proliferation of NIH/3T3 cells on HA/Gel and D-HA/Gel nanofibrous membrane with/without NIR illumination at 1, 3, and 5 days of incubation (means ± SD, n = 5) (**P < 0.01).
the nanoscale features of the cell culture platform readily support a cell growth assisted with the ECM components.

Moreover, we performed the wound healing assay (i.e., tip-scratch assay) to confirm the effect of NIR irradiation on cell migration and proliferation. As shown in Fig. 4D, the cultured cells migrated constantly for 24 hours of culture. Eighteen hours after scratching the cells, the NIR light–treated group exhibited an increase in cell migration compared to the control group (i.e., without illumination), which became more noticeable after 24 hours. These results are well matched with the cell proliferation assay as presented in Fig. 4E. The cell population was constantly increased until 5 days of culture. On day 1, no significant differences were found within the groups. However, on days 3 and 5, both HA/Gel and D-HA/Gel/NIR groups represented significantly increased values compared to other groups. On day 5, only the NIR irradiation induced a higher cell proliferation rate, indicating that the external field of the light source generates beneficial effects in promoting cell proliferation not only for the incorporation of the catechol group. On the basis of the above results, it was found that certain synergetic cooperation of dopamine and NIR irradiation promotes cell growth and proliferation on nanofiber matrices, in which the advantageous topographic features as an efficient scaffold greatly influence the cell distributions. Cytoskeletons in cells can be reconstructed by the surrounding microenvironments such as mechanical stimulation and biochemical cues, which lead to agglomeration and alignment of actin filaments for stress fiber formation (72). Cell cycle progression can be regulated by topological stimulation (e.g., nanofiber surface) for an integrin-mediated adhesion to the ECM via various signal pathways, such as G1 phase cyclin-dependent kinase and/or focal adhesion kinase. Because dopamine can promote cell adhesion, the incorporation of dopamine and other external stimuli effectively increased the initial cell adhesion in determining cell cycle progression, which finally enhanced the proliferation of the cells. For more information, we qualified the impact on the NIR irradiation by the careful observation of cell migration and proliferation in a certain period. Figure S6A presents a series of the captured images on the migrating cells. Here, the average speed of the cell movement could be estimated, which indicates that the cells, exposed to NIR light source, showed faster movement compared to the nontreated cells (fig. S6B). In addition, the average migration speed measured at each predetermined time also shifted at a faster rate when NIR light was irradiated, as appeared in fig. S6C. Consistent with this, when several cells were tracked, and the average speed per hour was compared, the groups exposed to NIR light migrated faster (fig. S6D), which was visually confirmed by tracking the movement of the same cells for 48 hours, as shown in fig. S6E (see also movie S1 and the Supplementary Materials).

**In vivo test for wearable LED patch–assisted wound healing**

The wound healing effects of the nanofibrous membrane with NIR irradiation were investigated by an in vivo experiment as presented in Fig. 5A. Full-thickness incisions were made on the rat’s skin, and the wound treatment was performed with the nanofibrous membrane and a flexible multi-LED array for 10 days. The lower panel in Fig. 1A describes the main concept of the wound healing process synergistically assisted by the wound dressing built with a LED patch. As captured in Fig. 5B, the light source of LED arrays mounted on the flexible PI substrate was fully irradiated by covering the wound sites. Figure 5C illustrates a sequential process of the size cut wound dressing attachment (i.e., D-HA/Gel membrane) and subsequent conformal contact of the flexible LED array right on the wound region. The relatively thin free-standing LED patch was encapsulated with a transparent elastomeric material (i.e., PDMS, <1 μm) that perfectly fits this purpose because of its surface tackiness. As a result, the obvious effectiveness was evaluated by the observation of the wound sites (Fig. 5, D to H). In particular, the treatment at the periodic time was engaged up to 10 days as displayed in Fig. 5D; compared with the other control groups, the D-HA/Gel wound dressing containing the catechol group represented a rapid wound closure. Notably, only the D-HA/Gel groups indicate efficient wound closure mainly due to the tightly bonded adhesive ability of dopamine moiety. As appeared in Fig. 5E, additional NIR treatment together with the functional wound dressing results in relatively less scarring when directly compared to the suturing group that known conventional wound closing method. This noticeable wound closing is greatly advantageous in minimized scar tissue to be clearly confirmed by this in vivo experiment, and the developed chemical conformation of the membrane material was ready to extend to functional wound healing support caused by other lesions with a help of the electronic device.

The mechanical recovery of the healed area can be one of the important considerations in the wound dressing choice. The graphs support the evidence for the above results (Fig. 5, F and G). The control group showed a low tensile strength, as measured to be 3.23 ± 1.16 N, but twofold higher tensile strength was shown on the use of HA/Gel and D-HA/Gel nanofibrous membrane, as measured to be 7.67 ± 1.05 N and 8.01 ± 0.86 N, respectively. On the other hand, when an additional multi-LED patch was applied to the wound lesions, the tensile strength for even the control group (i.e., without membrane) increased to 5.62 ± 0.75 N compared to the control group without the use of the multi-LED patch. Although the tensile strength of conventional sutting showed the highest tensile strength of 11.02 ± 2.15 N, the treatment of the D-HA/Gel with LED patch also resulted in relatively high tensile strength of 9.44 ± 0.74 N; statistically, those results were not significant (P = 0.21). However, the application of the D-HA/Gel with a multi-LED patch represented no allergic side effects or fewer scars on wound sites. Conclusively, our results demonstrate that the treatment by the simultaneous combination of D-HA/Gel–based wound dressing with the wearable LED patch accelerates the wound healing rate and induces stronger actual tissue bonding and minimized scars. On the basis of the previous reports, the wound dressing materials containing catechol groups had an improved healing effect compared to the absence of catechol. In addition to this scheme, we confirmed that the NIR light irradiation takes advantage of a more beneficial effect on the wound healing process (62, 63, 73, 74).

**DISCUSSION**

We have developed a catechol-functionalized nanofibrous wound dressing material that mimicked the native morphological features of ECM through the cross-linking of gelatin nanofibers with HA. We successfully demonstrated the feasible treatment using D-HA/Gel membrane attached to a flexible multi-LED unit for the accelerated wound healing strategy. With dopamine-containing catechol groups, the nanofibrous membrane represented improved tissue adhesion and mechanical compatibility that were beneficial in the wound healing process. In the material system, the intertwined morphology...
of the dopamine-containing nanofibrous membrane D-HA/Gel was more similar to the natural ECM and effective in wound healing compared to the HA/Gel composition. The improved mechanical strength is extended to the swelling capability and degradable conformation. In addition, as an in vitro approach, the cultured NIH/3T3 cells on a D-HA/Gel substrate with the NIR irradiation exhibited an enhanced proliferation rate by the nanoscale topographical cues. The in vitro experimental results revealed that the D-HA/Gel–based wound dressing conformally mounted with flexible multi-LED was obviously viable on the healing process and tissue bonding strategy, even having an excellent hemostatic capacity.

Previously, many studies have been reported about wound healing with light. Han et al. (75) reported the upconversion nanoparticle (UCNP)/HA–Rose Bengal (RB) conjugate complex for photochemical tissue bonding. The noninvasively penetrated RB was activated by
green light converted by UCNP and facilitated wound healing through the cross-linking between radicalized collagen matrices. Mao et al. (76) also reported the self-contracting hydrogel for non-invasive wound closure. They developed oxidized starch/gelatin-based shape memory hydrogel and induced wound healing on physiological temperature by self-contraction on the wound site. In addition, a bioadhesive using photocrosslinkable hydrogel for wound healing was presented (77). This specified hydrogel was cross-linked by blue light and could improve the wound healing process and inhibit bacterial infection. These previous studies showed successful wound healing using specific materials systems, which were closely related to the induction of collagen cross-linking by photoconversion of UCNP and the wound closure using NIR-responsive self-contracting hydrogels directly attached to the wound sites. Our systematically surveyed results imply that further improved HA/Gel-centered wound dressing materials are not limited to the additional other chemistries and functionalization with unprecedented efficacy. Therefore, to support wound healing of scar tissue, our newly developed biomedical material mounted on an electronic device is expected to contribute to the combinatorial therapeutic agent, which has a great potential for use in futuristic skin-attachable electroceuticals (78).

MATERIALS AND METHODS

LED patch fabrication

The PI substrate was used as an initial substrate that was sputtered with Cu on both sides, and then primary metallization was performed for via holes. The typical size of the PI substrate was 10 cm by 10 cm, and the thickness of the PI and Cu patterns were set as 25 and 1 μm, respectively. To pattern the sputtered Cu thin films, photolithography and etching process were used. Packaged LED chips (wavelength = 630 nm; Wurth Electronics, Niedernhall, Germany) were manually placed on the contact pads after applying solder paste (i.e., Sn3.0Ag0.5Cu; Kester, Itasca, USA), and then a reflow process was processed at a temperature of 250°C with a melt interval duration of 1 min. Next, a waterproof encapsulant (i.e., PDMS) was applied using a dip-coating process on the surface of the PI chip. The LED mount LEDs (10:1 mixing ratio of prepolymer and curing agent; SYLGARD 184, Dow, USA), which were cured in an oven at 80°C for 1 hour. The performance of the LEDs and interconnected stabilities was measured by a semiconductor parameter analyzer (Keithley 4200A, Keithley, USA), and the mechanical durability of the LED patch was repeatedly tested by loading pressure in a folded state (CT3 analyzer, Brookfield, USA).

Wound dressing materials

Gelatin (type A) from porcine skin, PBS, and NHS were purchased from Sigma-Aldrich. EDC and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were obtained from Tokyo Chemical Industry Co. HA (500 kDa) was purchased from Lifecore Corporation. The deuterated water (D₂O) was purchased from Sigma-Aldrich, and collagenase II was obtained from Worthington-Biochemical Corporation. All reagents were used without further purification. To prepare HA-cross-linked gelatin nanofibers, the gelatin (type A) from porcine skin was dissolved in HFIP with a concentration of 12.5% (w/v) and stirred for 6 hours at room temperature. The solution was transferred to a plastic syringe with 22-gauge stainless steel needle and extruded at an applied voltage of 11 kV from a high voltage power supplier (HV30, NanoNC, Korea). The distance between the needle tip and collector was set at ~10 cm. The electrospun gelatin nanofibers were immersed in the cross-linking solution for 24 hours at room temperature. The cross-linking solutions were prepared by mixing the ethanol solutions including EDC/NHS/dopamine and aqueous HA solutions. The EDC and NHS were dissolved at 50 and 25 mM concentration into 95% ethanol solutions, respectively, and different amounts of dopamine hydrochloride were added, with the final molar ratio to gelatin being 0, 1, 5, and 10 to enable the synthesis of D-HA/Gel with different amount of dopamine hydrochloride content. The HA was dissolved in deionized (DI) water and then poured into the 95% ethanol solutions to form the final concentration of 0.05% (w/v) and stirred to obtain a homogeneous solution. After 24 hours, the samples were rinsed with distilled water three times and vacuum-dried for further use. The fabrication process was carried out under nitrogen flushing and dark condition to avoid oxidation of dopamine. ¹H-NMR analyses were performed by sufficiently dissolving the sample in D₂O at a concentration of 1 mg ml⁻¹. ¹H-NMR spectra were recorded at 298 K with Bruker AVANCE NEO 500 (500 MHz for ¹H).

Surface characterization

To characterize the chemical structure of the samples composed of gelatin, HA, and dopamine hydrochloride, FTIR spectra were acquired by using IR Affinity-1 spectrophotometer (SHIMADZU Corp., Japan). All spectra were recorded in transmittance mode and in the wave number range of 600 to 4000 cm⁻¹. The surface morphology of the samples was characterized by field emission SEM (FE-SEM; SUPRA40VP, Carl Zeiss, Germany) at an accelerating voltage of 15 kV. Before the measurements, all samples were coated with sputtered Au. The average diameter and distribution of nanofibers were calculated by selecting at least 100 different fibers from SEM images using ImageJ (National Institutes of Health, USA). The tensile strength was measured by universal testing machine (texture analyzer, YEONJIN Corp., Korea) in tension mode under constant conditions (21°C, 60% Relative humidity, RH). The samples were cut into rectangular strips (10 mm by 40 mm) and mounted vertically by gripping the ends with metallic clamps with a gauge length of 20 mm before the start. The samples were stretched at a rate of 10 mm min⁻¹, and the test ended when the samples were fragmented. All experiments were carried out with five replicates for each sample to obtain the average data. The water contact angles of the samples were measured by deionized water (DI) and the surface was continuously visualized within 5 s by digital microscopy (Dino-Lite, AM 413TL, Taiwan). Each sample was tested three times to get the average contact angles.

Swelling test

The water absorbptivity of the nanofiber samples was evaluated by a weighing method. The specimens that were previously weighed at dry state were immersed in PBS (Chembio) solution and incubated at room temperature. At predetermined time points, the wet weight (W₃₆) of samples that was taken out and the wiped-off excess solution using filter paper was measured. The swelling ratio of the samples was calculated using Eq. 1 (4)

\[
\text{Swelling ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100\% \quad (1)
\]

where Wₙ is the hydrated mass of the sample and W_d is the dry mass of the sample.
Biodegradability test in vitro
To investigate the degradation rate of the samples, each sample was weighed at dry state \( (W_0) \) and immersed in collagenase type II solution \( (2 \text{ U ml}^{-1}) \) at 37°C. The solution was replaced every other day. At a specific time point, the samples were taken out and rinsed with DI water three times, followed by freeze drying and measurement of the remaining sample weight \( (W) \). The percentage of remaining weight \( (W\%) \) was calculated using Eq. 2

\[
W\% = \frac{W}{W_0} \times 100
\]  

Porosity and air permeability of nanofibrous membrane
From swelling test of nanofibrous membrane, we could calculate the porosity of D-Gel/HA10. Therefore, the porosity was measured according to Eq. 3

\[
\text{Porosity} = \frac{V_m - V_p}{V_m} \times 100 = \left(1 - \frac{W_m}{\rho V_m}\right) \times 100
\]  

\( V_m \) is the total volume of the fabric \( (\text{cm}^3) \), \( V_p \) is the actual volume of the fibers \( (\text{cm}^3) \), \( \rho \) is the density of the fibers \( (\text{g cm}^{-3}) \), and \( W_m \) is the mass of the fabric \( (\text{g}) \).

In addition, the air permeability test was carried out according to ASTM D737 on Gurley 4190N Densometer (Thwing-Albert, West Berlin, NJ). Each tested specimen was first placed onto the test head of the test instrument. Then, the densometer test measures the time required for a given volume of air \( (300 \text{ ml}) \) to flow through a standard area of nanofibrous membrane \( (\text{D-Gel/HA10}) \) tested under light uniform pressure. The air pressure is supplied by an inner cylinder of specific diameter \( (1.0 \text{ inch}) \) and standardized weight, floating freely within an outer cylinder partly filled with oil to act as an air seal. The air permeability result was calculated using Eq. 4 and denoted as unit of \( \text{ml s}^{-1} \text{cm}^{-2} \)

\[
\text{Air permeability} = \frac{\text{Volume}}{\text{Area} \times \text{Time}} = \frac{300 \text{ ml}}{\pi \times \left(\frac{2.54}{2}\text{ cm}\right)^2 \times \text{Time (measured, s)}}
\]

In vivo hemostatic ability test
A rat hemorrhaging liver model was used to investigate the in vivo hemostatic ability of the nanofiber samples. The rats were anesthetized as described above, and the abdominal surgery was carried out to expose its liver. The serous fluid around the liver was cleared, and preweighed filter paper was placed beneath the single lobe of the liver. Bleeding from the liver was initiated by punching the liver with a 19-gauge needle, and the bleeding conditions were observed. The experiment was performed by applying the samples on the bleeding site of the liver, and filter paper that absorbed blood was weighed and compared to the control group without any treatment.

Cell culture
NIH/3T3 murine fibroblasts were routinely cultured in high glucose containing Dulbecco’s modified Eagle’s medium (Welgene, Daegu, Korea) with 10% fetal bovine serum (Welgene) and 1% antibiotic-antimycotic solution \( (10,000 \text{ U of penicillin, amphotericin B (25 \mu g ml}^{-1}) \), and 10 mg of streptomycin; Sigma-Aldrich) in incubator under humidified 5% CO\(_2\) atmosphere at 37°C. Cells were detached from the flask at 70% confluency using trypsin-EDTA and seeded at a density of \( 1.5 \times 10^4 \) cells per well on tissue culture plate, TCP and prepared HA/Gel, D-HA/Gel1, D-HA/Gel5, and D-HA/Gel10 nanofibrous matrices. Fresh media were added every 2 to 3 days, and all samples were incubated in the same cell culture condition for in vitro assay. For NIR radiation, LED patch (light wavelength = 630 nm, luminous intensity, \( (IV); \) = 90 mcd) was radiated on the cells in well plates for 10 min at the room temperature.

Immunocytochemical analysis
The nuclei and F-actins of NIH/3T3 murine fibroblasts were confirmed using the immunofluorescence staining. After incubation of 5 days, cells were fixed with a 3.7% formaldehyde solution (Sigma-Aldrich) for 10 min and permeabilized in 0.1% Triton X-100 (Sigma-Aldrich) for 5 min, followed by blocking with a 2% bovine serum albumin (GenDEPOT, Barker, TX, USA) solution for 30 min. Subsequently, the cells were treated with Alexa Fluor
Cell migration assay
To determine the effect of NIR illumination on cell migration and proliferation, the cell scratch healing assay was conducted. The cells were seeded into well plate and cultured until confluent. Scratches were made by scraping the cell monolayers using a pipette tip. Then, for NIR radiation groups, LED light was radiated on the cells in well plates for 10 min at the room temperature. At 0, 12, and 24 hours after scratching, the images were taken using optical microscope to assess the degree of cell migration into the scratched area. Quantification of the effect of NIR illumination on cell migration was analyzed by taking a video of the cell proliferation in live cell incubator using IX81 microscope equipped with Olympus DP74 camera for 48 hours.

Cell proliferation evaluation
Cell proliferation was evaluated by a cell counting kit-8 assay (CCK-8, Dojindo, Kumamoto, Japan). NIH/3T3 murine fibroblasts were seeded on each nanofiber matrices as above and incubated for predetermined periods. The CCK-8 assay was followed at each time point according to the manufacturer’s protocol. Briefly, the CCK-8 solution mixture with media (ratio of 1:9) was dispensed into each sample and incubated for 2 hours at 37°C in the dark. After the completion of the incubation period, the 100 μl of supernatant was collected and transferred into a new 96-well plate. The absorbance values were measured at 450 nm with SpectraMax 340 ELISA Reader (Molecular Devices, CO., USA).

In vivo incision wound healing test under LED irradiation
In vivo incision wound healing test was carried out to evaluate the effect of the nanofibrous membrane and NIR light illumination on wound healing process. In this study, after 7 days of acclimatizing, rats were anesthetized by inhalation of the isoflurane through an anesthesia apparatus. Then, the dorsal area of rat was shaved, and alcohol disinfection was conducted. Then, incision wounds in size affixed to a 2 cm on skin tissue of rat were made using a blade and scratches were made by scraping the cell monolayers using a pipette tip. For the LED irradiation groups, LED light was radiated on the cells in well plates for 10 min at the room temperature. At 0, 12, and 24 hours after scratching, the images were taken using optical microscope to assess the degree of cell migration into the scratched area. Quantification of the effect of NIR illumination on cell migration was analyzed by taking a video of the cell proliferation in live cell incubator using IX81 microscope equipped with Olympus DP74 camera for 48 hours.

Statistical analysis
Data are expressed as means ± SD from several animals in a group in a few separate experiments. Statistical analysis was carried out with analysis of variance to compare the differences using GraphPad Prism (GraphPad Software, San Diego, CA). P values less than 0.05 were considered statistically significant.

Supplementary Materials
Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abn1646

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