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ORIGINAL ARTICLE

Matriderm® 1 mm versus Integra® Single Layer 1.3 mm for one-step closure of full thickness skin defects: a comparative experimental study in rats

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Abstract
Purpose Dermal templates, such as Matriderm® and Integra®, are widely used in plastic and reconstructive surgery, often as two-step procedures. A recent development is the application of thin dermal templates covered with split thickness skin grafts in one-step procedures. In this experimental study, we compare the two thin matrices Matriderm® 1 mm and Integra® Single Layer in a one-step procedure with particular focus on neodermis formation.
Methods Matriderm® 1 mm and Integra® Dermal Regeneration Template—Single Layer (1.3 mm) were compared in a rat model. In three groups of five animals each, a full thickness wound was covered with (a) Matriderm® 1 mm and neonatal rat epidermis, (b) Integra® Single Layer and neonatal rat epidermis, or, (c) neonatal rat epidermis only (control). Histological sections 2 weeks post transplantation were analyzed with regard to take of template and epidermis, neodermal thickness, collagen deposition, vascularization, and inflammatory response.
Results Take of both templates was complete in all animals. The Matriderm®-based neodermis was thinner but showed a higher cell density than the Integra®-based neodermis. The other parameters were similar in both matrices.
Conclusion The two templates demonstrate a comparable biological behavior early after transplantation. The only difference was found regarding neodermal thickness, probably resulting from faster degradation of Matriderm®. These preliminary data suggest that both dermal templates appear similarly suitable for transplantation in a one-step procedure.

Keywords Dermal substitute · Matriderm® 1 mm · Integra® Single Layer · One-step procedure · Rat model

Introduction

Dermal substitutes are widely used in plastic, reconstructive, and burn surgery since many years [1–8]. Most templates are relatively thick and thus involve a two-step procedure with an interval of several weeks between dermal matrix implantation and skin grafting. Consequently, valuable time is lost and cost increases. Several years ago, a company producing Matriderm® (Matriderm) (Dr. Suwelack Skin & Health Care AG), which is an acellular lyophilized collagen and elastin based biomatrix, introduced a thin (1 mm) dermal template. It has been successfully used in one-step procedures in combination with split thickness skin grafts (STSG) [9–19]. More recently, Integra Life Sciences also developed a thin version, namely Integra® Dermal Regeneration Template Single Layer (Integra Single Layer), an acellular, permanently cross-linked collagen and glycosaminoglycan based biomatrix

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(1.3 mm). In contrast to the well-established bilayered Integra® Dermal Regeneration Template [3–6, 20, 21], it only consists of a thin dermal regeneration template. Obviously, Integra Single Layer lends itself ideally to also be used in one-stage procedures in combination with STSG, just like Matriderm.

The aim of this experimental study (rat model) was to compare these two thin matrices used in a one-step procedure with a particular focus on neodermis formation.

Methods

Study design

Matriderm and Integra Single Layer were compared in a one-step procedure on immuno-incompetent rats. In three groups of five animals each, a full thickness skin wound was created and covered with either (a) Matriderm 1 mm and neonatal rat epidermis, (b) Integra Single Layer 1.3 mm and neonatal rat epidermis, or (c) coverage with neonatal rat epidermis only (control). Two weeks later, the transplants were excised and processed for histology.

Materials and methods

Matriderm (thickness 1 mm) (Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany) and Integra Single Layer (thickness 1.3 mm, [7]) (Integra Life Sciences Corporation, Plainsboro, NJ, USA) were cut into round pieces with a diameter of 2.5 cm to exactly fit the transplantation devices (modified Fusenig chambers [22]), and soaked in DMEM (Dulbecco modified Eagle medium, Invitrogen, Carlsbad, CA, USA) for 10 min immediately before implantation.

The surgical protocol was approved by the local committee for experimental animal research (permission number 65/2009). Immuno-incompetent female nu/nu rats, 8–10 weeks old (Harlan Laboratories, The Netherlands), were prepared and anesthetized as previously described [23]. To protect the implant and to prevent wound closure from the surrounding skin, Teflon rings with a diameter of 2.6 cm were sutured into full-thickness skin defects created on the backs of the rats, using non-absorbable polyester sutures (Ethibond®, Ethicon, Piscataway, NJ, USA). In experimental animals, the matrices were inserted into the rings and then covered with neonatal rat epidermis devoid of any dermal elements, so as to avoid potential interference of such dermal remnants with neodermis formation. In control animals, neonatal epidermis was applied directly onto full-thickness defects. The transplants were then covered with a silicone foil (Silon-SES, BMS, Allentown, PA, USA), a polyurethane sponge (Ligasano, Ligamed, Innsbruck, Austria), and a tape as wound dressing. Dressing changes and photographic documentations were performed after 7 and 14 days. The above procedures have been previously described in detail [23].

Analyses of the transplants

After 14 days, the transplant areas were generously excised and processed for cryosections (12 μm thick) as well as for paraffin sections (8 μm thick). Paraffin sections were deparaffinized, stained with haematoxylin and eosin (Sigma, St. Louis, MO, USA), and thereafter mounted within Eukitt® (Fluka, Buchs, Switzerland). This allowed us to assess take of template and epidermal coverage, cell density, and neodermal thickness.

The thickness of the neodermal compartment was measured using the graphic program Image J (http://rsb.info.nih.gov). Repetitive measures from the basal layer of the epidermis to the subcutaneous tissue were taken in different sections from each sample (approximately 50 per sample). The results were analyzed using VassarStats® online software (http://faculty.vassar.edu/lowry/VassarStats.html) performing unpaired Student's t test. A $p < 0.05$ was considered significant.

To assess collagen deposition and distribution, paraffin sections were deparaffinized and Masson's trichrome staining applied. The procedure was performed according to the manufacturer's description (Kit Sigma-Aldrich HT15).

Vascularization of the neodermis and inflammatory response against the matrix were analyzed by immunostaining. Antibodies against the following cell types were used on cryosections according to the manufacturer's description: Vascular endothelial cells (anti-CD31, BD Pharmingen), macrophages (anti-Integrin αM, Santa Cruz), and granulocytes (HIS48, Santa Cruz). As a secondary antibody we used TRITC-conjugated polyclonal goat F(ab')2 fragments directed to mouse or rabbit immunoglobulins (Dako, Baar, Switzerland). Cryosections were fixed and permeabilized in acetone for 5 min at −20°C, air-dried, washed 3× in phosphate-buffered saline (PBS, Invitrogen), and blocked in PBS containing 2% bovine serum albumin (Sigma, Buchs, Switzerland) for 30 min. Incubation with the diluted antibodies was performed in blocking buffer for 1 h at room temperature. Slides were washed three times for 5 min in PBS and blocked for additional 15 min before the second antibody was added. Finally, the slides were incubated for 5 min in PBS containing 1 μg/ml Hoechst 33342 (Sigma, Buchs, Switzerland), washed twice for 5 min in PBS, and mounted with Dako mounting solution (Dako, Baar, Switzerland)

containing 25 mg/ml of DABCO anti-quenching agent (Sigma, Buchs, Switzerland).

Pictures of immunofluorescence staining were taken with a DXM1200F digital camera connected to a Nikon Eclipse TE2000-U inverted microscope. The device is equipped with Hoechst 33342, FITC, and TRITC filter sets (Nikon AG, Egg, Switzerland; Software: Nikon ACT-1 vers. 2.70). Images were processed with Photoshop 7.0 (Adobe Systems Inc, Munich, Germany).

Results

Take of templates and epidermal coverage

Two weeks after transplantation we observed a complete take of both templates, Matriderm and Integra Single Layer, in all animals. The substitutes were fully integrated into the rat tissue, as evidenced by complete colonization of the neodermis with rat cells. Cell density was higher in Matriderm-derived neodermis than in Integra Single Layer derived neodermis (Fig. 1). Epidermal take was seen macroscopically and histologically, but was inconsistent in all animals of all three groups (Fig. 1).

Neodermal thickness

In the Matriderm group, neodermal thickness showed a range from 0.47 to 0.60 mm, with a mean thickness of 0.49 mm (SD 0.03 mm). In the Integra Single Layer group, it ranged from 0.59 to 0.85 mm, with a mean thickness of 0.67 mm (SD 0.015 mm). In the control group, it was from 0.10 to 0.34 mm, with a mean thickness of 0.27 mm (SD 0.036 mm). The two template-derived neodermises were significantly thicker than the scar-like neodermis in the control group (Matriderm-derived neodermis $p = 0.0029$, Integra Single Layer-derived neodermis $p = 0.0004$). Regarding the template-derived thickness, the Matriderm-derived neodermis was less thick than the Integra Single Layer-derived neodermis, but the difference did not reach statistical significance ($p = 0.05$).

Collagen deposition

Staining with Masson's trichrome revealed a beginning, but still scant, collagen deposition in the Matriderm-derived and similarly also in the Integra Single Layer-derived neodermis. The collagen deposition in the control specimen was more pronounced and more homogeneous (Fig. 2).

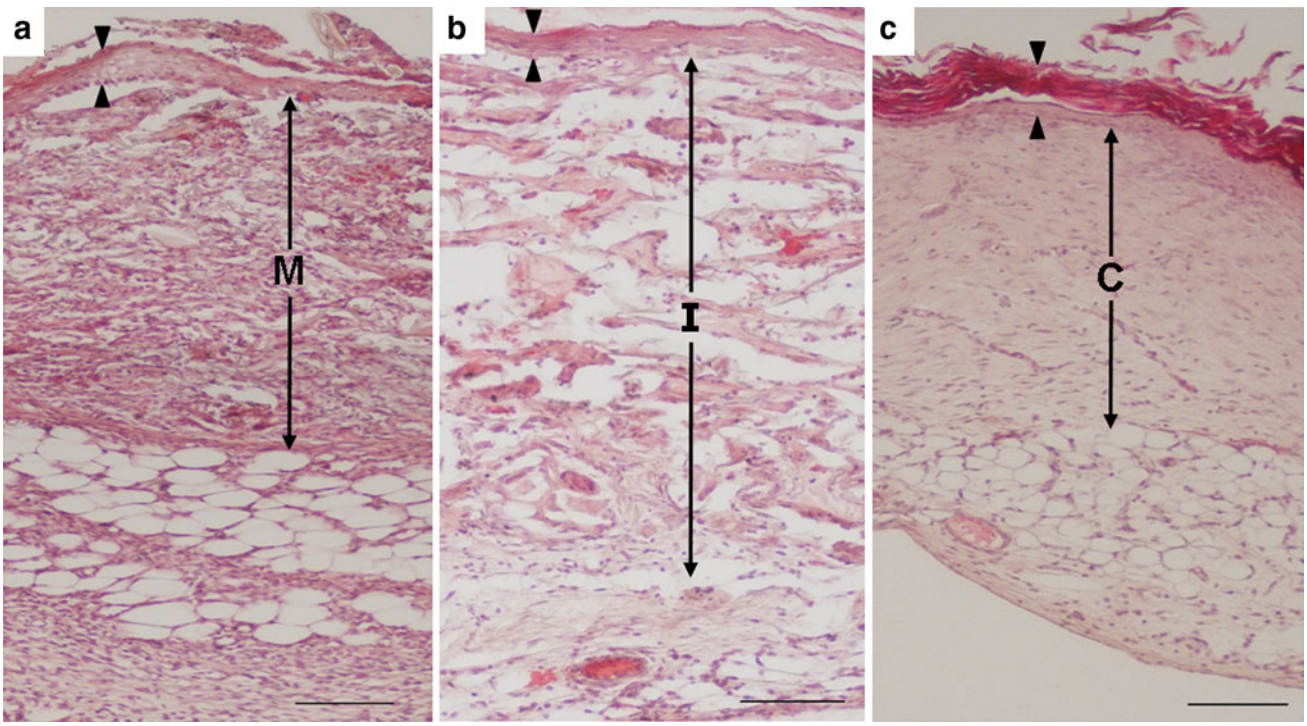

Fig. 1 Histological evaluation of the excised transplants 14 days after one-step procedure (haematoxylin and eosin staining). **a** Matriderm, **b** Integra Single Layer, **c** control. All transplants show a developed epidermis (*between markers*), a neodermis formation (*M* Matriderm-derived neodermis, *I* Integra Single Layer-derived neodermis, *C* neodermis of control group), and a populated neodermis. The remaining scaffold elements cannot be discerned. *Scale bars* 100 μm

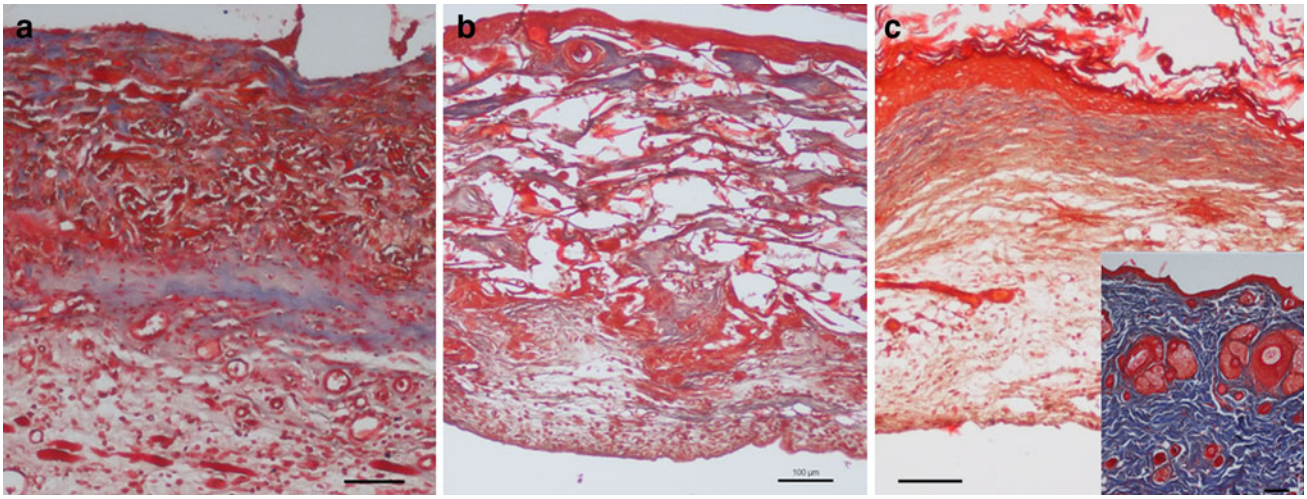

Fig. 2 Masson's trichrome staining 14 days after one-step transplantation. **a** Matriderm, **b** Integra Single Layer, **c** control. The insert depicts normal rat skin. Collagen stains blue. There is scant collagen deposition in the template-derived neodermises (**a** and **b**), while the collagen deposition in the control (**c**) is more pronounced and more homogenous, but still distinctly less than in normal rat skin (insert of **c**). *Scale bars* 100 μm

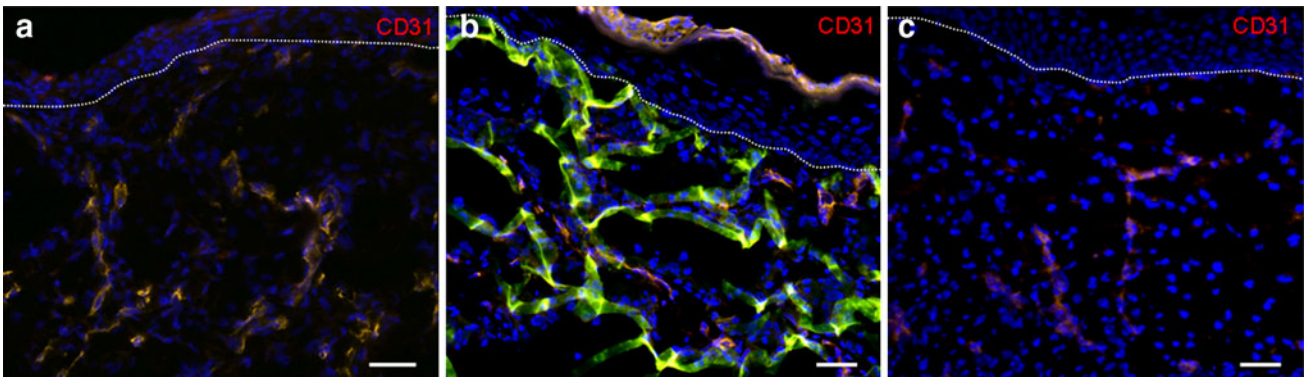

Fig. 3 Neovascularization 14 days after one-step transplantation. **a** Matriderm, **b** Integra Single Layer, **c** control. Endothelial cells are stained with CD31 (*red*), collagen in Integra Single Layer exhibits autofluorescence (*green*), cell nuclei are stained with Hoechst (*blue*). No autofluorescence is observed with Matriderm. The *dotted line* indicates the basement membrane. A neodermal capillary network can be seen from the subcutaneous structures up to the superficial neodermal areas in all three groups. *Scale bars* 50 μm

Vascularization

Staining with CD 31 demonstrated a neodermal capillary network present in a similar way in all three groups. Blood vessels could be seen from the subcutaneous structures up to the superficial neodermal areas (Fig. 3).

Inflammatory response

There were few granulocytes infiltrating the template-derived neodermises, while the number of granulocytes present in control specimens was even lower. The findings regarding macrophage infiltration were almost identical to granulocyte infiltration (Figs. 4, 5).

Discussion

This appears to be the first study comparing Matriderm 1 mm and Integra Single Layer 1.3 mm in a small animal model. In the global picture, these two templates do not demonstrate significant differences in their biological behavior early after transplantation in that template and epidermal take rate, vascularization, and inflammatory response were comparable.

Clearly, the perfect template take rate, the prompt vascularization, and the relatively low-grade inflammatory response are favorable parameters with regard to clinical applicability. There are several publications indicating that 1 mm thick Matriderm can successfully be used clinically

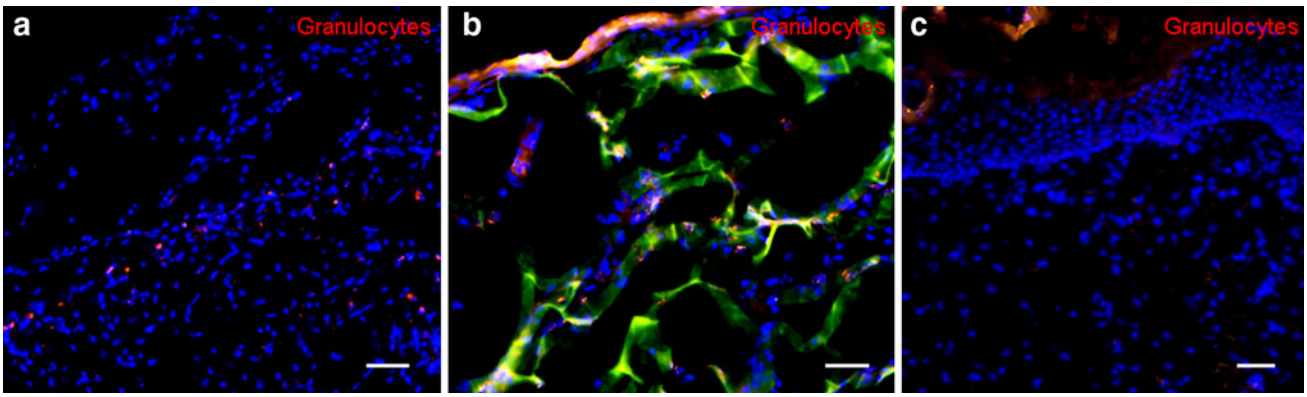

Fig. 4 Inflammatory response (granulocytes) 14 days after one-step transplantation. **a** Matriderm, **b** Integra Single Layer, **c** control. Granulocytes are stained with Granulocyte antibody HIS48 (*red*), collagen in Integra Single Layer exhibits autofluorescence (*green*), cell nuclei are stained with Hoechst (*blue*). Few granulocytes infiltrating the template-derived neodermises (**a** and **b**) can be detected, while only single granulocytes are present in the control group (**c**). *Scale bars* 50 μm

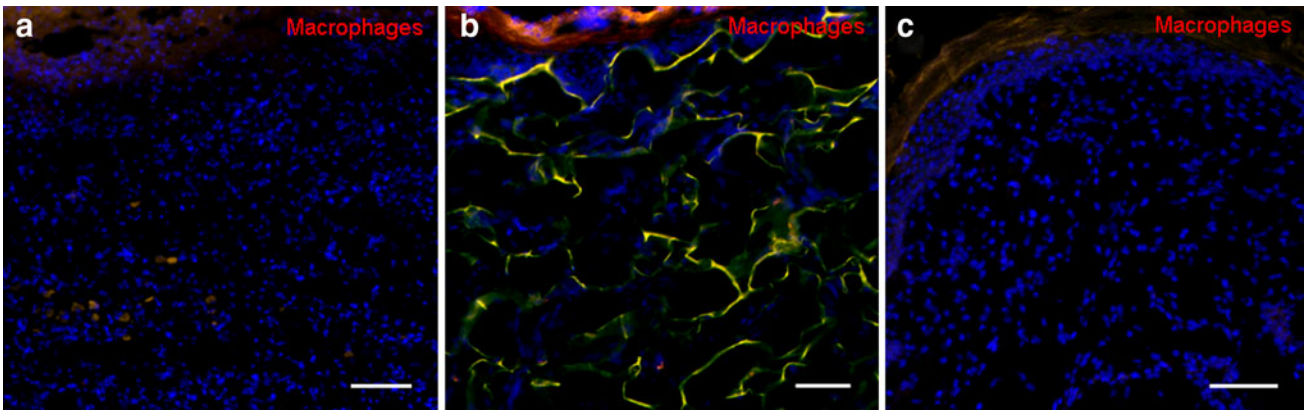

Fig. 5 Inflammatory response (macrophages) 14 days after one-step transplantation. **a** Matriderm, **b** Integra Single Layer, **c** control. Macrophages are stained with Integrin αM (*red*), collagen in Integra Single Layer exhibits autofluorescence (*green*), cell nuclei are stained with Hoechst (*blue*). There are few macrophages infiltrating the template-derived neodermises (**a** and **b**), while only single macrophages can be seen in the control group (**c**). *Scale bars* 100 μm

in a one-step procedure [13, 14, 16–18]. In contrast, there is only one publication describing the successful use of 1.3 mm thick Integra Single Layer in combination with a split thickness skin graft to reconstruct deep facial defects in a one-step procedure [24]. The existence of many more articles concerning Matriderm is probably due to the fact that it has been available since several years, whereas Integra Single Layer has only recently been put in the market.

A particularly promising finding is the fact that the template-based neodermal compartments were clearly, and significantly thicker than the one in the controls, thereby approximating the physiological dermal–epidermal ratio. It is conceivable that this positive feature remains also in the long run and provides the reconstructed skin with a near normal texture, mechanical stability, and adequate elasticity. There was also a certain difference in thickness between the two template-derived neodermises. The most tenable explanation for this phenomenon is a more rapid degradation of Matriderm because its collagen components are (as opposed to Integra Single Layer) not cross-linked. It is well known that crosslinking reinforces matrix stability and makes it less susceptible to degradation (increased resistance against enzymatic digestion) [7, 25, 26]. Yet, a potentially adverse effect of crosslinking is that degradation products can decrease cell survival, proliferation, and adhesion [7, 26], which could explain the lower cell density observed in the Integra Single Layer-derived neodermis.

Astonishingly, the take of the neonatal rat epidermis was almost identically inconsistent in all the groups, explicitly also including the control group. We therefore assume that these problems do not reflect different properties of the templates used, but rather have to do with the fact that the epidermal coverage was extremely thin (2–3 cell layers only!), fragile, and devoid of fibroblasts and collagen. Of note, multiple publications have underscored bad and

inconsistent take rates after applying cultured epithelial autografts that share many structural characteristics with the epidermal grafts used here [27–29]. Notably, in a clinical setting, STSG used for coverage by definition, contain a dermal component including fibroblasts and collagen fibres. Therefore, such take problems will most likely not occur, as also evidenced by several publications already showing successful outcomes and rapid wound coverage in one-step procedures using STSG [30, 31].

In summary, this is apparently the first experimental study to compare Matriderm 1 mm and Integra Single Layer 1.3 mm with particular focus on their suitability for a one-step application to close deep skin defects. Our data suggest that both dermal templates can be successfully implanted and that both demonstrate a similarly favorable biological behavior in terms of take, vascularization, and inflammatory response. Thus, both qualify for a one-step procedure.

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Conflict of interest The authors declare that they have no conflict of interest.

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