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ORIGINAL ARTICLE

Effect of gelatin methacryloyl hydrogel on healing of the guinea pig vaginal wall with or without mesh augmentation

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Abstract

Introduction and hypothesis The aims of this study were to evaluate the effectiveness of gelatin methacryloyl as an adjunct to anterior vaginal wall injury with or without vaginal mesh compared with traditional repair with suture.

Methods Virginal cycling Hartley strain guinea pigs ($n=60$) were randomized to undergo surgical injury and repair using either polyglactin 910 suture or gelatin methacryloyl for epithelium re-approximation or anterior colporrhaphy with mesh augmentation using either polyglactin 910 suture or gelatin methacryloyl for mesh fixation and epithelium re-approximation. Noninjured controls ($n=5$) were also evaluated. After 4 days, 4 weeks, or 3 months, tissues were analyzed by hematoxylin & eosin in addition to immunolabeling for macrophages, leukocytes, smooth muscle, and fibroblasts.

Results Surgical injury repaired with suture was associated with increased inflammation and vessel density compared with gelatin methacryloyl. Vimentin and α-smooth muscle actin expression were increased with gelatin methacryloyl at 4 days ($p=0.0026$, $p=0.0272$). There were no differences in changes in smooth muscle or overall histomorphology after 3 months between the two closure techniques. Mesh repair with suture was also associated with increased inflammation and vessel density relative to gelatin methacryloyl. Quantification of collagen content by picrosirius red staining revealed increased thick collagen fibers throughout the implanted mesh with gelatin methacryloyl compared with suture at 4 weeks (0.62 ± 0.01 μm^2 vs 0.55 ± 0.01, $p=0.018$). Even at the long-term time point of 3 months, mesh repair with suture resulted in a profibrotic encapsulation of the mesh fibers, which was minimal with gelatin methacryloyl. Smooth muscle density was suppressed after mesh implantation returning to baseline levels at 3 months regardless of fixation with suture or gelatin methacryloyl.

Conclusions These results suggest that gelatin methacryloyl might be a safe alternative to suture for epithelium re-approximation and anchoring of prolapse meshes to the vagina and may improve chronic inflammation in the vaginal wall associated with mesh complications.

Keywords Mesh anchoring · GelMA · Gelatin methacryloyl · Vaginal surgery · Pelvic organ prolapse · Reconstruction surgery

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Introduction

Approximately 11% of women will undergo surgery for pelvic floor disorders in their lifetime [1] and this number is believed to increase 46% by 2050 [2]. Polypropylene meshes are frequently used to aid in the surgical repair of pelvic organ prolapse (POP) and urinary incontinence. However, vaginal mesh has been under much scrutiny over the past few years and as of April 2019, the FDA mandated that all companies stop producing and selling transvaginal mesh kits for the treatment of POP owing to the high rate of mesh complications, including mesh exposure and pain [3]. Although lower for sacrocolpopexies than for transvaginal mesh, mesh

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complication rates remain problematic for abdominal mesh placement occurring at a rate of 10.5% within 7 years after placement [4].

Current evidence supports the phenomenon of mesh wrinkling as a contributing factor in mesh exposure [5]. Mesh wrinkling cause changes in the mesh pore geometry, which may cause mesh deformation, making it more susceptible to bacterial infiltration, to infection, and to impairment of the ability of the host's immune system to infiltrate the mesh to prevent such infections. It can also lead to an increased amount of mesh material in a given area, which can cause an enhanced foreign body response, further placing the patient at risk of mesh exposure [6]. Sutures used to attach mesh to the vagina can act as point loads and cause mesh wrinkling; increasing the number of point loads increases the degree of mesh wrinkling [5, 6].

Gelatin methacryloyl (GelMA) hydrogels have a wide variety of biomedical applications in regenerative medicine, drug delivery, and tissue adhesives [7, 8]. GelMA is made by crosslinking gelatin and methacrylic anhydride; it has been thoroughly studied as an adhesive compound with hemostatic properties in the lung, kidneys, and vascular systems [7]. Furthermore, in vivo experiments using GelMA in large animals demonstrated a low inflammatory host response, as well as fast in vivo degradation, while allowing for adequate time for wound healing. It can also function under wet conditions, such as on vaginal fibromuscular tissue [9]. GelMA has yet to be tested on the reproductive tract, specifically vaginal tissue, or tested as an adhesive material that allows for the adherence of foreign material, such as vaginal mesh.

The aim of this study was to determine if GelMA can be used as a safe alternative to suture for mesh anchoring to vaginal tissues (e.g., sacrocolpopexies and vaginal mesh placement). We hypothesized that GelMA would result in a safe alternative to suture for both repair of vaginal wall epithelium and implantation of vaginal mesh, without detrimental effects in inflammatory response and similar wound healing. To test our hypothesis, we compared the effect of suture or GelMA on healing of the vaginal wall after surgical injury with or without mesh augmentation using a preclinical animal model.

Materials and methods

Guinea pigs

All guinea pigs were studied and euthanized in accordance with the standards of humane animal care described by the National Institutes of Health Guide for the Care and Use of Laboratory Animals, using protocols approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center. A total of 65 virginal female adult Hartley strain guinea pigs at 12 weeks of age from Elm Hill Labs (Chelmsford, MA, USA) were housed in Institutional Animal Care and Use Committee-approved facilities under a 12-L:12-D cycle at 22 °C. After facility acclimation, the animals were randomized into one of four surgical groups. Animal weights were similar in all groups (794 ± 110 g, mean ± SD).

Treatment groups

Using a list randomizer, animals were randomized to one of four surgical groups (surgical injury plus (1) suture, (2) GelMA, or anterior colporrhaphy with (3) mesh + suture, and (4) mesh + GelMA). Five noninjured controls underwent anesthesia without surgery. Briefly, the vaginal membrane was incised allowing access to the vagina. The anterior vaginal wall was then grasped with two hemostats and a 7-mm incision was made along the anterior vaginal wall. The underlying muscularis was then dissected from the vaginal epithelium. If mesh was used, lightweight polypropylene monofilament mesh (Uphold Lite, Boston Scientific, Marlborough, MA, USA, measuring 4×6 mm) was then fixed to the underlying muscularis and secured. The vaginal wall epithelium was re-approximated with either suture or 10% GelMA. To prepare the GelMA solution, 10% (*w*/*v*) porcine GelMA was dissolved in phosphate-buffered saline (PBS) with 0.5% (*w*/*v*) lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as a photoinitiator at 37 °C. Group 1 underwent anterior vaginal surgical injury and vaginal epithelium was re-approximated with 4–0 polyglactin 910 suture in a running nonlocking fashion. Group 2 underwent a surgical injury closed with 30 µl of GelMA, which was polymerized using LED light as described by Noshadi et al. [8]. Group 3 underwent anterior vaginal wall colporrhaphy with mesh placement using four interrupted stitches of 4–0 polyglactin 910 suture to anchor the mesh. The anterior vaginal wall epithelium was then re-approximated with 4–0 polyglactin 910 suture in a running fashion. Group 4 underwent anterior colporrhaphy with mesh placement using 30 µl of GelMA polymerized using LED light (10 mW/cm^2) for 3 min to anchor the mesh followed by re-approximation of the vaginal epithelium with an additional 30 µl of GelMA and 3 min of polymerization with LED light. Sample size was determined by the resource equation method [10]. There was a total of four surgical categories, each with three time points for tissue collection: 4 days postoperatively, 1 month postoperatively (28 days), and 3 months postoperatively (84 days). An additional 5 guinea pigs were used as noninjured controls. Therefore, the guinea pigs were randomized into a total of 13 groups, each of which contained 5 guinea pigs.

Tissue processing and histomorphology

After euthanasia with 0.3 ml pentobarbital (390 mg/ml; Virbac, Fort Worth, TX, USA), the abdominal wall was opened and the animal perfused with 60 ml of PBS, followed by 180 ml of neutral buffer formalin (10%). After perfusion, the pubic symphysis was disarticulated and the uterine horns, bladder, cervix, and vagina were dissected down to the perineal skin and excised en bloc. After excision, each specimen was grossly examined to evaluate for mesh erosion. The bladder was then dissected off the anterior vaginal wall and lower uterine segment taking care not to damage the urethra and then discarded. The remaining specimen was then fixed in neutral buffer formalin (10%). Tissues were subsequently processed and embedded in paraffin blocks. Cross-sections (4 µm) of the vagina and urethra were taken every 100 µm to obtain four separate cross-sections at different levels of the vagina for analysis. Four cross-sections were placed on a single slide and underwent immunolabeling with a single or double antibody (Supplementary Table 1). Additional slides (4 cross-sections at 100-µm intervals/slide) were stained with hematoxylin and eosin (H&E) using standard techniques. Images of each cross-section were captured and analyzed using a Nikon E1600 microscope and Nikon NIS Elements AR software (Melville, NY, USA).

Immunohistochemistry

Antigen retrieval was performed on formalin-fixed and paraffin-embedded vaginal cross-sections by steaming slides in 10 mM sodium citrate buffer (pH 6.0) for 30 min. Tissues were then immunolabeled with antibodies (Supplementary Table 1). Slides were scanned using Confocal Zeiss LSM880 Airyscan (Jena, Germany). Briefly, sections were divided into five groups to evaluate a total of eight antibodies. Group 1 included primary rabbit antiTNFα; group 2 was double labeled and included primary mouse anti smooth muscle α-actin (anti-α-SMA) conjugated with EF-570 and primary anti-rabbit vimentin; group 3 was double labeled with primary rabbit anti-collagen I and mouse anti-collagen III; group 4 included rabbit anti-arginase I and mouse anti-CD45 conjugated with AF-488; group 5 was labeled with rabbit anti-CD68. All sections were incubated overnight (21 h) at 4 °C with their corresponding primary antibodies after blocking for 2 h with 10% normal goat serum (Thermo Fisher Scientific, Waltham, MA, USA). The slides were incubated for 1 h with the corresponding anti-mouse and anti-rabbit secondary antibodies, as listed in Supplementary Table 1. ProLong Gold antifade reagent with 4′,6-diamidino-2-phenylindole (DAPI; Invitrogen, Eugene, OR, USA) was placed with a coverslip and dried in the dark for 24 h at room temperature. Slides were scanned using Confocal Zeiss LSM880 Airyscan (Jena, Germany) with a ×20 objective. A single fluorescent image of the vaginal muscularis from each slide (at the site of injury if present) was analyzed by two authors (LJ and HS).

Image analysis

For all antibodies, image analysis was performed using ImageJ 1.52 software (NIH, Bethesda, MD, USA) using a basal threshold of fluorescence for quantification of signal area. Results were analyzed as area fraction (percentage of pixels) of each above antibody, excluding area of mesh fiber, blood vessels, and epithelium. For CD45 and CD68, a proportion of positively labeled cells per image was calculated by divining the number of antibody-positive labeled cells by the total number of DAPI-labeled cells per field.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 7.0 (La Jolla, CA, USA). Data were tested for distribution before statistical analysis. For discrete variables (vessel counts, cell counts), the medians of each specimen per group were averaged. Then, the mean ± SEM of the averages was used to determine significant differences between groups using a two-way ANOVA. For continuous variables and normally distributed data involving time as an additional variable, a two-way analysis of variance was used followed by the appropriate post-hoc tests, including Dunnett for comparison with noninjured controls and a pairwise test using the Bonferroni multiple comparisons procedure between all groups. For nonparametric data, Kruskal–Wallis was used to compare overall significance and Mann–Whitney was used to compare between groups. A p value of less than 0.05 was used to determine significance.

Results

Effect of GelMA on surgical injury of the vaginal wall without mesh: histomorphology

To determine if GelMA was tolerated and effective in vaginal wound healing, we initiated studies of healing of the vaginal wall after surgical injury of the anterior vaginal wall in nonpregnant guinea pigs. Animals underwent anterior vaginal wall injury and repair with either GelMA or suture for epithelial re-approximation. Thereafter, vaginal tissues were harvested at different time points representing early (4 days), intermediate (4 weeks), and long-term (3 months) phases of wound healing ($n = 5$ in each group at each time point). Noninjured controls underwent anesthesia without

injury ($n = 5$) and tissue was harvested 1 week after anesthesia. There were no surgical complications or episodes of wound dehiscence.

Hematoxylin & eosin staining of vaginal tissue obtained from the mid-vagina after anterior vaginal wall surgical injury revealed a dramatic inflammatory reaction 4 days after vaginal injury and suture repair (Fig. 1b). Specifically, hemorrhage and inflammatory infiltrates extended 300–500 µm from the suture (Fig. 1b). Further, the epithelial layer was not approximated at sites of needle entry (5 out of 5 animals at 4 days, Fig. 1b). Although re-approximation with GelMA also resulted in localized inflammation, hemorrhage and size of the inflammatory reaction were decreased compared with suture (Fig. 1e). Importantly, with GelMA, the vaginal epithelium was closed even at the early time point of 4 days (Fig. 1e). Vessel counts conducted on H&E-stained slides demonstrated increased vessel number with injury regardless of repair. Suture repair, however, was associated with increased vascularity at 4 days ($p < 0.0001$), 4 weeks ($p < 0.0001$), and 3 months ($p < 0.001$) compared with GelMA (Fig. 2a).

Both suture and GelMA remained visible in all tissues obtained at 4 weeks (Fig. 1b, e) whereas both were dissolved (at the microscopic level) by 3 months (Fig. 1d, g). By 3 months, healing of the vaginal wall was complete. Repair with suture or GelMA was virtually indistinguishable with resolution of inflammation, closure of the injury site, and restoration of vaginal wall integrity (Fig. 1d, g).

Immunolabeling with SMA was conducted to determine the effect of surgical injury with repair on smooth muscle in the vaginal wall (Fig. 3). Quantification of the immunolabeling area revealed that smooth muscle content had decreased significantly by 4 days ($p = 0.04$) and 4 weeks ($p = 0.03$) compared with controls, after colporrhaphy, regardless of

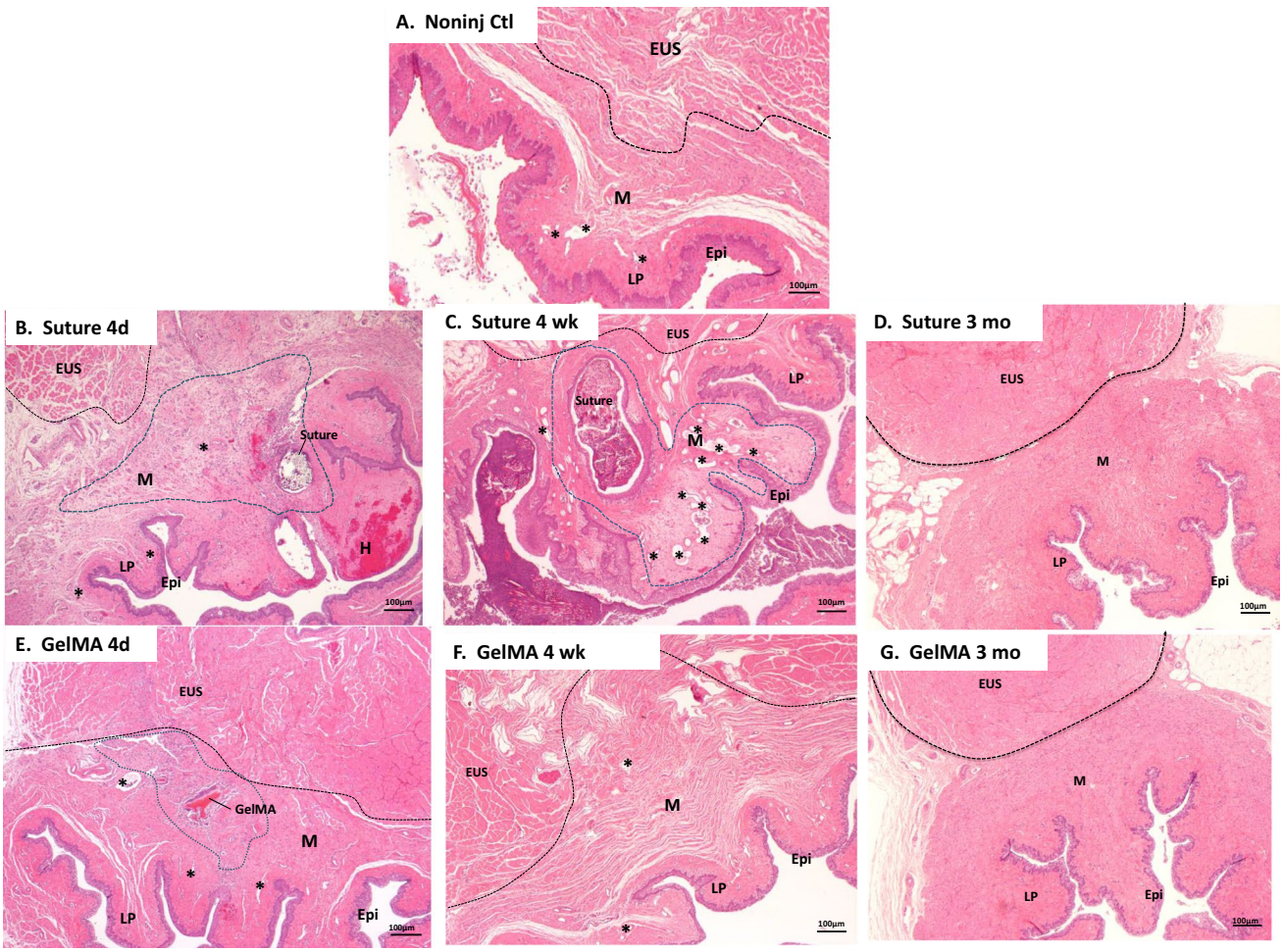

Fig. 1 Effect of GelMA or suture on healing of the vagina after anterior vaginal wall surgical injury without mesh. Representative sections of anterior vaginal wall from cycling guinea pigs stained with H&E. **a** Histology of noninjured control. **b** 4 days after suture repair. **c** 4 weeks after suture repair. **d** 3 months after repair with suture. **e** 4 days after repair with GelMA. **f** 4 weeks after repair with GelMA. **g** 3 months after repair with GelMA. *Epi* vaginal epithelium, *LP* lamina propria, *M* muscularis, *EUS* external urethral sphincter, *H* hemorrhage. *Dashed lines* indicate area of inflammatory infiltrate. *Asterisk* indicates blood vessels

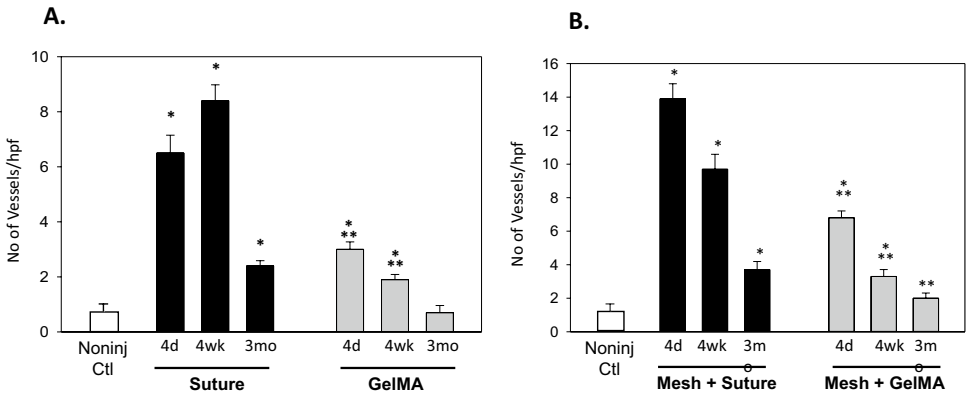

Fig. 2 Effect of suture or GelMA on blood vessel density in the vaginal wall. **a** After surgical injury. **b** After anterior colporrhaphy implanted with mesh. Number of blood vessels per high power field (*hpf*) were quantified as described in Materials and methods. Data represent mean ± SEM of 5 animals in each group (median of 5 non-overlapping fields in each animal). *$p < 0.05$ compared with noninjured control (*Noninj Ctl*). **$p < 0.05$ compared with suture, two-way ANOVA

closure method in both the muscularis (Fig. 3e) and the lamina propria (Fig. 3f). The content of SMA recovered at 3 months to suprabasal levels ($p = 0.01$).

Effect of GelMA on surgical injury of the vaginal wall with mesh augmentation

Having established that GelMA was well-tolerated in our animal model and resulted in closure of the vaginal wall after anterior vaginal wall injury, we sought to determine if augmentation of surgical injury with vaginal mesh anchored with GelMA was similar to that of suture. To facilitate comparisons with experiments conducted without mesh, vaginal tissues were harvested at early (4 days), intermediate (4 weeks), and long term (3 month) phases of wound healing after injury and mesh with suture or GelMA. There was no evidence of mesh erosions into the vagina, bladder, or urethra in any specimen. In addition, there were no cases of mesh migration; all mesh was found to be correctly placed after tissue collection.

After mesh implantation (Fig. 4), mesh pores were distributed appropriately within the vaginal muscularis and accompanied by an inflammatory reaction in both suture and GelMA groups (Fig. 4). Similar to vaginal injury without mesh, suture fixation of mesh resulted in a pronounced inflammatory reaction, which was more severe than without mesh (Fig. 4a, b vs Fig. 1b, c). At 4 days, mesh fixation with either suture or GelMA resulted in an inflammatory response encircling the mesh (Fig. 4a, d). However, in contrast to mesh fixation with suture, the inflammatory response was blunted with GelMA at 4 weeks (Fig. 4b vs e). Inflammation persisted for 3 months if mesh was fixed with suture (Fig. 4c). Furthermore, with suture, the mesh was encapsulated by a dense foreign body reaction, which was minimal with GelMA (Fig. 4c). With GelMA, inflammation was minimal at 3 months with mesh (Fig. 4f).

Immunolabeling for macrophages with CD68 antibodies revealed that macrophages were distributed throughout the vaginal wall in control noninjured animals (not shown). With mesh, however, CD68+ macrophages accumulated near mesh, suture, or GelMA with no significant differences among groups (not shown). To determine if macrophage subtypes may differ among the two groups of mesh fixation, sections were labeled and quantified for TNF (a marker of pro-inflammatory type 1 macrophages, M1) or arginase (a marker of type 2 wound healing type macrophages, M2; Fig. 5a, b). The area of M1 macrophages was decreased in the intermediate phase of healing with GelMA compared with suture ($2{,}717 \pm 524.8$ μm^2 vs $5{,}529 \pm 986.7$ μm^2, $p = 0.04$; Fig. 5d). Further, M1 macrophages were decreased with GelMA compared with noninjured controls at 3 months ($1{,}603 \pm 373.4$ μm^2 vs $3{,}257 \pm 301.7$ μm^2, $p = 0.0062$; Fig. 5a). In contrast, although not statistically significant, M2 macrophages transiently increased with GelMA (Fig. 5b).

Similar to our findings with colporrhaphy alone (Fig. 2a), vessel density increased with placement of mesh ± suture or GelMA (Fig. 2b). The number of vessels increased significantly with suture during the acute phase at 4 days ($p < 0.0001$), and was even greater than without mesh. Vessel density decreased with time but remained significantly increased at 3 months relative to noninjured controls (Fig. 2b). Across all time points, vessel density was decreased with mesh + GelMA compared with mesh + suture with normalization of vessel counts by 3 months (Fig. 2b).

To determine if mesh fixed with either suture or GelMA resulted in changes in the two predominant types of fibrillar collagen in the vaginal wall (Col I and Col III), sections

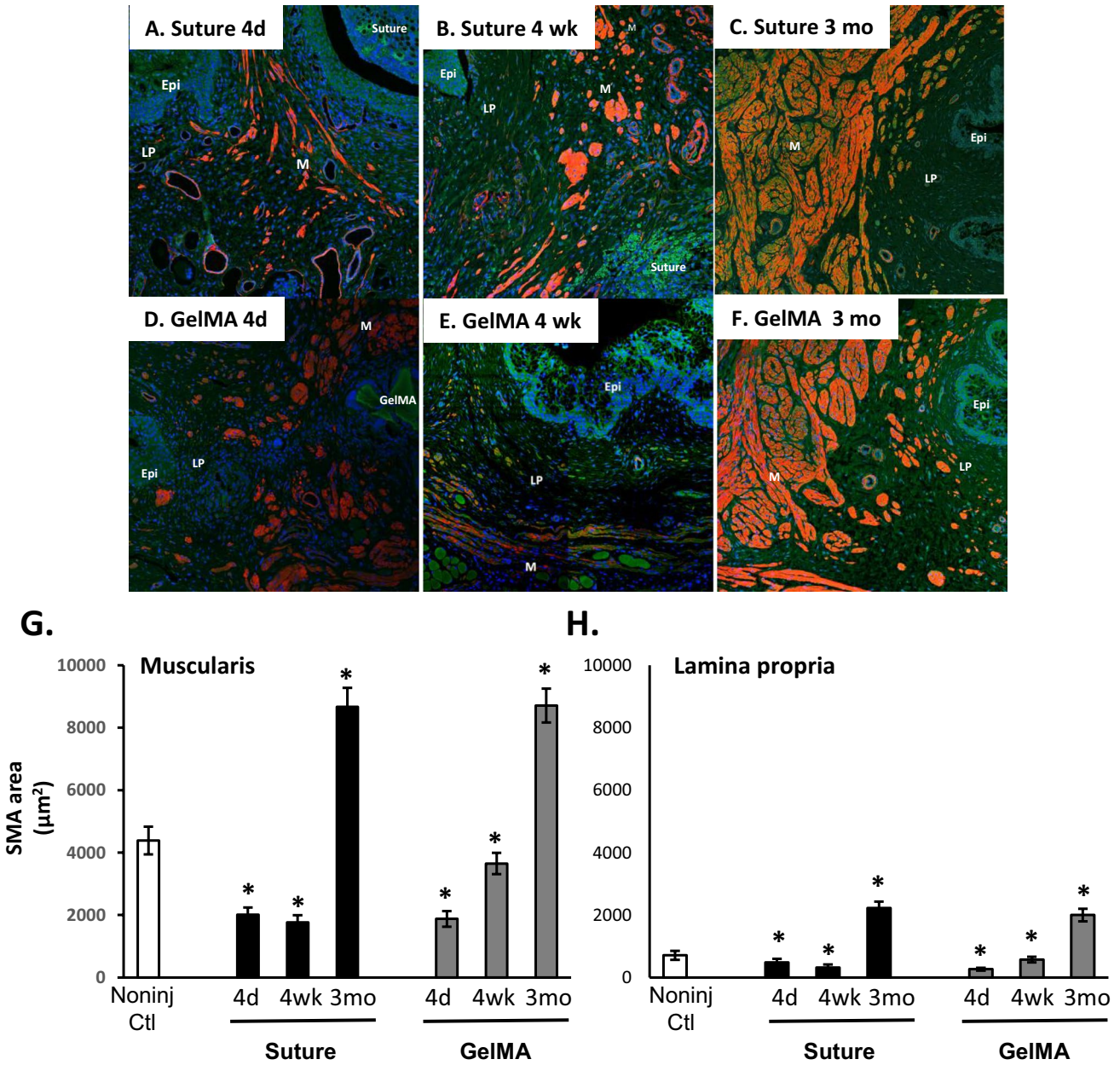

Fig. 3 Effect of suture or GelMA on smooth muscle and fibroblasts in the vaginal wall after surgical injury without mesh. Sections from tissues repaired with suture at **a** 4 days, **b** 4 weeks, or **c** 3 months, or with GelMA at **d** 4 days, **e** 4 weeks, or **f** 3 months were double labeled with anti-SMA (*red*) and anti-vimentin (*green*) antibodies as described in Materials and Methods. Quantification of the smooth muscle area is presented in panels **g** and **h**. *$p < 0.05$ compared with noninjured controls. *Epi* epithelium, *M* muscularis, *LP* lamina propria, *EUS* external urethral sphincter

were stained with picrosirius red (Supplementary Fig. 3) or double immunolabeled with Col I and Col III antibodies (Fig. 6). Picrosirius red staining revealed less collagen surrounding the mesh (Supplementary Fig. 3). If mesh was fixed with suture, the halo of decreased collagen staining was more pronounced, consistent with fibrosis. By 4 weeks with suture, the area of total collagen staining was decreased significantly compared with nonsurgical controls at 4 weeks ($0.55 \pm .01$ suture vs 0.66 ± 0.01 µm^2 control, $p < 0.05$). With GelMA, however, collagen staining was similar to controls (0.62 ± 0.01 µm^2) and collagen fibers appeared more organized surrounding GelMA (Supplementary Fig. 2b) relative to disorganized fibers surrounding suture (Supplementary Fig. 2a). Immunolabeling with collagen antibodies revealed that the temporal pattern of Col I content was similar in mesh + suture and mesh + GelMA (Fig. 6). Specifically, Col

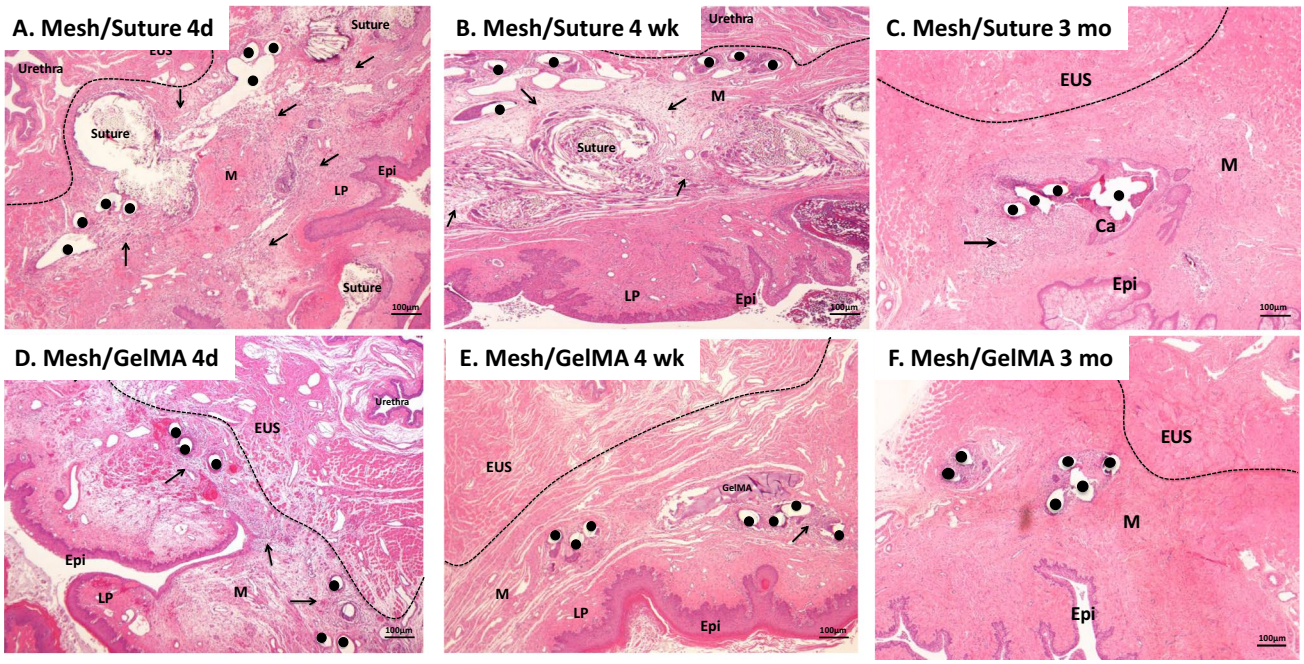

Fig. 4 GelMA or suture affects healing of the vagina after anterior colporrhaphy with mesh. Representative sections of anterior vaginal wall from cycling guinea pigs stained with H&E. **a** 4 days after suture repair. **b** 4 weeks after suture repair. **c** 3 months after suture repair. **d** 4 days after repair with GelMA. **e** 4 weeks after repair with GelMA. **f** 3 months after repair with GelMA. Arrows indicate inflammatory reaction. *Black circles* indicate mesh pores. *Epi* epithelium, *LP* lamina propria, *M* muscularis, *EUS* external urethral sphincter, *Ca* fibrotic reaction encapsulating mesh fibers

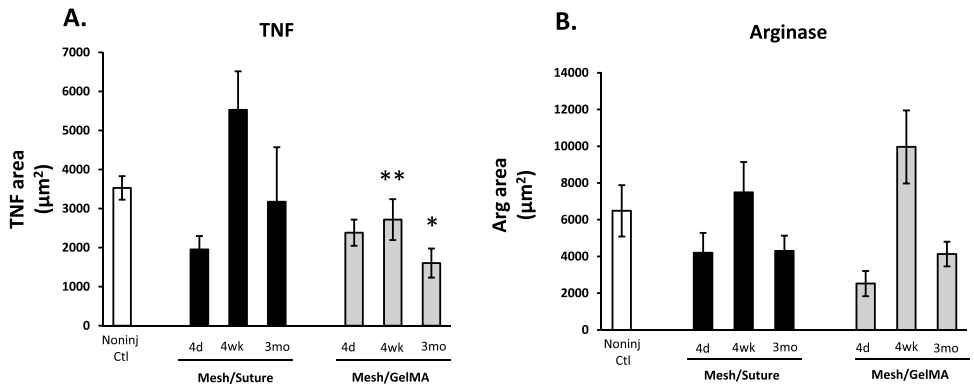

Fig. 5 Effect of GelMA or suture on macrophage populations in the vagina after anterior colporrhaphy with mesh. **a** Immunolabeled area of TNF in the anterior vaginal wall of noninjured controls, 4 days, 4 weeks, and 3 months after mesh + suture, and 4 days, 4 weeks, and 3 months after mesh + GelMA. $*p < 0.05$ compared with noninjured controls, $**p < 0.05$ compared with 4 weeks with suture. **b** Immunolabeled area of Arg in the anterior vaginal wall of noninjured controls, 4 days, 4 weeks, and 3 months after mesh + suture, and 4 days, 4 weeks, and 3 months after mesh + GelMA

I was increased by 4 weeks in both groups compared with noninjured controls and this increase was maintained at 3 months (Fig. 6a–e). The pattern of Col III immunolabeling differed in the two groups (Fig. 6f). With mesh + suture, Col III was decreased at all time points. Findings of increased Col I and suppression of Col III persisting to 3 months can signify a "pro-fibrotic" matrix. With mesh + GelMA, however, Col III was not suppressed (Fig. 6f).

The impact of mesh fixed with suture or GelMA on smooth muscle cells was studied by immunolabeling with SMA (Supplementary Fig. 1). Similar to colporrhaphy repaired without mesh (Fig. 3), the area of SMA decreased in both the muscularis and the lamina propria after mesh implantation at 4 days and 4 weeks (Supplementary Fig. 1). The magnitude of an early decrease in SMA (~60%) was similar with or without mesh. In contrast to simple repair of

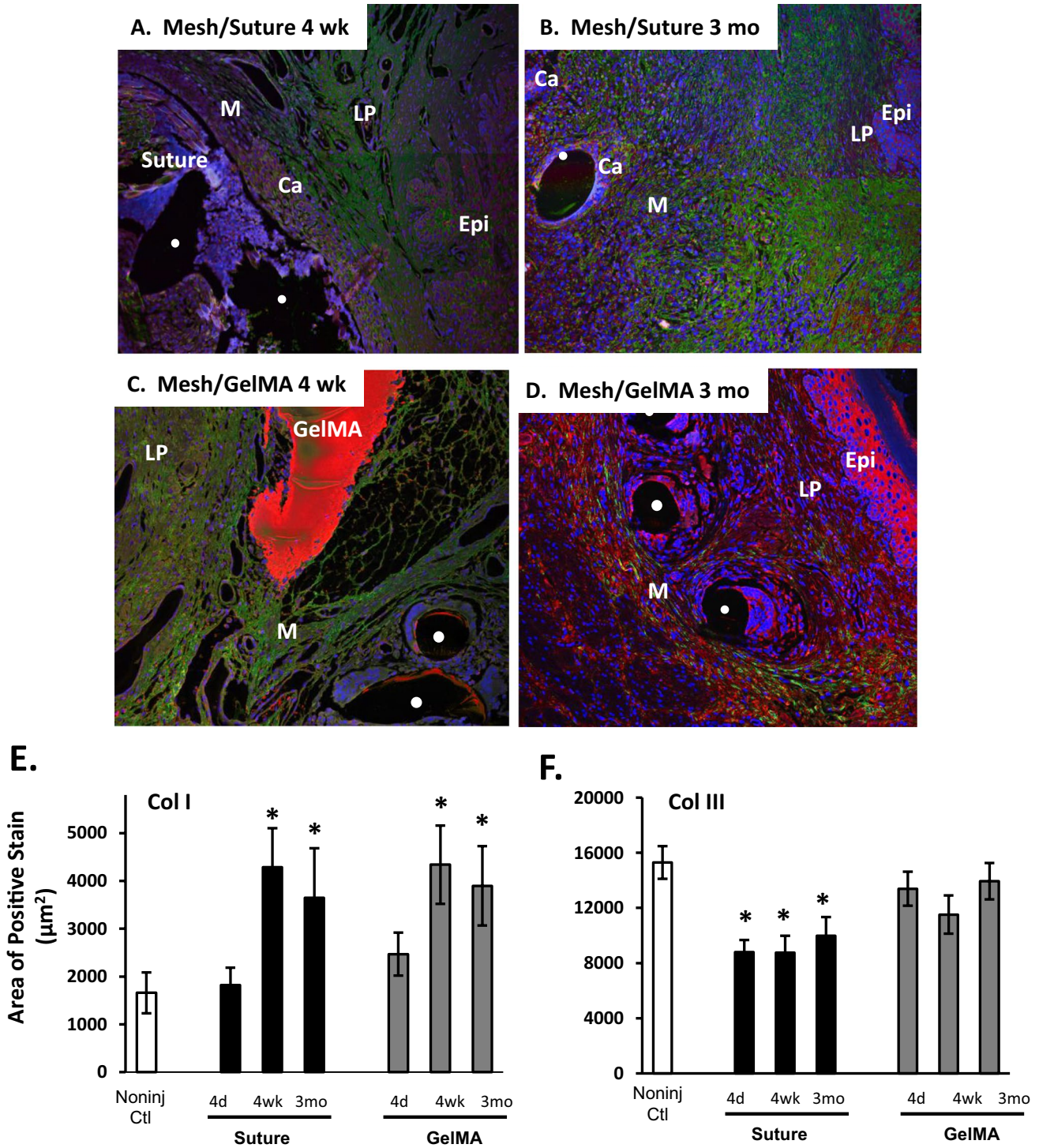

Fig. 6 Impact of suture or GelMA on collagen expression in the vaginal wall after anterior colporrhaphy implanted with mesh. Tissue sections were immunolabeled for Col I (*green*) and Col III (*red*) in anterior vaginal wall sections from animals implanted with mesh/suture for **a** 4 weeks and **b** 3 months, or GelMA for **c** 4 weeks or **d** 3 months. Quantification of **e** Col I or **f** Col III area. *Black circles* indicate mesh pores. $*p < 0.05$ compared with noninjured controls. *Epi* epithelium, *LP* lamina propria, *M* muscularis, *EUS* external urethral sphincter, *Ca* profibrotic capsule

colporrhaphy in which SMA increased to suprabasal levels by 3 months (Fig. 3g, h), with mesh, SMA returned only to baseline levels (Supplementary Fig. 1e, f).

Quantification of vimentin (expressed in both smooth muscle cells and fibroblasts), revealed that vimentin was distributed evenly throughout the lamina propria and muscularis. Unlike SMA, vimentin expression was increased after mesh placement at all time points and was similar with both suture and GelMA (Supplementary Fig. 2).

Discussion

Our results using lightweight polypropylene monofilament implanted vaginally agree with others demonstrating an acute and chronic inflammatory reaction to implanted mesh [11]. The immune cell profile of excised mesh in the chronic phase of wound healing also demonstrated macrophages surrounding mesh fibers with a predominance of the M1 subtype [12]. In agreement with results from other surgical fields [13–15], suture was also accompanied by a pronounced pro-inflammatory reaction, which was amplified by the presence of mesh. This finding has potential clinical relevance, as vaginal mesh excised for pain or erosion is associated with acute and chronic inflammation [12, 16]. We also observed tissue hemorrhage at the sites of needle insertion for vaginal repair with suture. Without a doubt, bleeding at the time of surgical incision should be controlled, but suture placement is accompanied by needle injury and risk of additional hemorrhage, often demanding additional suture, thereby potentiating the risk of additional bleeding. Here, we also found that fixation with GelMA resulted in less inflammation, less tissue hemorrhage during the acute phase of wound healing, and a positive matrix profile relative to suture without complications. In this regard, GelMA is superior to suture.

Here, we found that after simple colporrhaphy smooth muscle recovery was robust, possibly because of stem cell recruitment and fibroblast differentiation. Interestingly, with mesh, smooth muscle regenerated, but not to the extent that it did in the absence of mesh. Our findings of decreased vaginal smooth muscle with injury are consistent with those of Shaffer et al. [17] and Gualtieri [18, 19], in which vaginal mesh resulted in decreased smooth muscle. It is possible, therefore, that loss of smooth muscle was similar with and without mesh, but the presence of mesh and the associated increased inflammation impaired its full smooth muscle regenerative capacity.

Angiogenesis is predominantly driven by ischemia and inflammation. Hypoxia plays a critical role in this process, as cells sense and respond to hypoxic conditions by changing gene expression [20], In monocytes, the predominant genes modulated by hypoxia encode proteins involved in angiogenesis or belonging to cytokines and growth factors [20]. Hypoxia-mediated angiogenesis is crucial for matrix remodeling in connective tissues [21] and is important for fibroblast proliferation. This process may serve as a double-edged sword, however, if hypoxia results in tissue necrosis or excessive proliferation and fibrosis. Here, we found that blood vessel density increased with wound healing but was augmented significantly with suture. Barbed suture has been shown to be as efficacious as conventional suture with knots but has the added benefit of improved wound healing owing to the reduction of tissue ischemia [22]. Theoretically, GelMA may further improve wound healing by not only decreasing tissue ischemia but also avoiding repeated tissue trauma with needle penetration. Angiogenesis is also mediated by pro-angiogenic chemokines and perturbation of vascular endothelial growth factor (VEGF) function [23]. As suture was also accompanied by increased inflammation, the combination of ischemia and inflammation may also induce angiogenesis.

One strength of this study is the animal model. Whereas other commonly used laboratory animals such as rats and mice have limited smooth muscle in the vaginal wall, smooth muscle of the guinea pig vagina is robust and similar to that of humans [24]. Limitations of the animal model, however, include the lack of gravitational forces on the vaginal wall in quadrupeds and the use of young premenopausal animals without POP. This allowed us to analyze the effects of vaginal mesh and different fixation techniques under optimal conditions of the vaginal matrix. In women, application of vaginal estrogen is common practice prior to mesh implantation. Thus, use of normal cycling estrogenized guinea pigs is relevant. It should be emphasized that our positive findings with GelMA in guinea pigs may differ if implanted into the abnormal matrix of the vaginal wall of women with POP or other comorbidities (e.g., diabetes, aging, and obesity). Further, erosion of mesh was evaluated up to 3 months, which may change with longer implantation times and aging. Nevertheless, these results suggest that GelMA might be a safe alternative to the placement of sutures, which can be a difficult and time-consuming procedure.

Immunohistochemistry and analysis facilitate localization of matrix and cell components, which offers an added layer of interpretation compared with total protein in tissue homogenates. Nonetheless, quantification is limited by cell counts or area density, which may not reflect cellular protein content in every situation. Our quantification criteria were strictly controlled, facilitating not only protein localization but also quantification, at least at the tissue level. More in-depth studies are needed to understand the molecular mechanisms by which suture or GelMA alter wound healing responses to vaginal mesh and, in particular, the excessive profibrotic capsulation of mesh fibers with suture.

In summary, our studies in young estrogenized guinea pigs reveal that GelMA is a safe alternative to suture for vaginal epithelium closure and the anchoring of POP meshes to the vagina in the short term. Studies including longer time points and biomechanics are needed to evaluate the long-term durability of repairs using GelMA. Further studies including large animal preclinical models are necessary to assess feasibility of use in humans.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00192-021-05031-2.

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Contributions L. Jackson: data collection or management, data analysis, manuscript writing/editing; H. Shi: data collection or management, data analysis, manuscript writing/editing; J. Acevedo: data collection or management, data analysis, manuscript writing/editing; S. Lee: data analysis, manuscript writing/editing; N. Annabi: protocol/project development, data analysis, manuscript writing/editing; A. Word: protocol/project development, data analysis, manuscript writing/editing; M. Florian-Rodriguez: protocol/project development, data collection or management, data analysis, manuscript writing/editing.

Declarations

Conflicts of interest The authors report no conflicts of interest.

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