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Repeated penile girth enhancement with biodegradable scaffolds: microscopic ultrastructural analysis and surgical benefits

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Autologous tissue engineering using biodegradable scaffolds as a carrier is a well-known procedure for penile girth enhancement. We evaluated a group of previously treated patients with the aim to analyze histomorphometric changes after tissue remodeling and to estimate the benefits of repeated procedure. Between February 2012 and December 2016, a group of 21 patients, aged 22–37 (mean 28.0) years, underwent a repeated penile girth enhancement procedure with biodegradable scaffolds. Procedure included insertion of two poly-lactic-co-glycolic acid scaffolds seeded with laboratory-prepared fibroblasts from scrotal tissue specimens. During this procedure, biopsy specimens of tissue formed after the first surgery were taken for microscopic analysis. The mean follow-up was 38 months. Connective tissue with an abundance of connective tissue fibers, small blood vessels, and inflammatory cells were observed in all analyzed surgically removed tissue. Ultrastructural analysis of these tissue samples discovered the presence of large quantities of collagen fibrils running parallel to each other, forming bundles, with a few widely spread fibroblasts. In total, the mean values of flaccid and erect gain in girth after the second surgery were 1.1 ± 0.4 (range: 0.6–1.7) cm and 1.0 ± 0.3 (range: 0.6–1.5) cm, respectively. Microscopic evaluation of newly formed tissue, induced by autologous tissue engineering using biodegradable scaffolds, showed the presence of vascularized loose connective tissue with an abundance of collagen fibers, fibroblasts, and inflammatory cells, indicating active neovascularization and fibrinogenesis. The benefit of the repeated enhancement procedure was statistically significant.

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INTRODUCTION

Penile size has been a significant cause for concern throughout history. Men often want to enlarge their penises to improve their self-esteem and/or to impress their partners.[1] However, most of them have a normally sized and functional penis, but interpret their size as abnormal and request an enhancement surgery as a way to ease their distress and depression. Penile dysmorphophobia is presented as a special entity and is defined as a medical problem in men whose penises are normally developed but who are dissatisfied with their dimensions and who request enhancement surgery. Several papers demonstrated different methods for penile girth enhancement. It is controversial because of its unclear indications, the availability of many poorly evaluated procedures, and the risk of complications.[2–4] A novel approach using an autologous *ex vivo* tissue engineering process was described in 2006.[5] The procedure included harvesting fibroblasts from the scrotal dermal tissue and seeding them into pretreated tube-shaped biodegradable scaffolds. The preliminary clinical results were good, showing a significantly lower complication rate than reported in previously established procedures.

Regardless of these encouraging facts, only one study has analyzed histomorphometric properties of tissue remodeling following scaffold penile augmentation.[6] In this study, light microscopy and scanning electron microscopy were utilized. However, no studies have focused on microscopic ultrastructural analysis of newly formed tissue. In addition, reliability and efficacy of repeated girth enhancement procedures remain questionable. Thus, we evaluated our patients who underwent repeated penile girth enhancement procedures with biodegradable scaffolds and hereby present an ultrastructural analysis of newly formed tissue as well as outcomes in gain after repeated treatment.

PATIENTS AND METHODS

Patients

Between February 2012 and September 2016, repeated penile girth enhancement surgery with biodegradable scaffolds was performed in Belgrade Center for Genital Reconstructive Surgery (Belgrade, Serbia) in 21 men, aged 22–37 (mean 28) years. Time between the two procedures ranged from 12 to 32 (mean 19) months. Indication for secondary procedure was the patient's request for further gain in

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penile girth, due to dissatisfaction with achieved dimensions after the first surgery and desire for additional enhancement. Individuals who presented with the complaint of penile dysmorphophobia (subjective perception of small penis) were included in this study. Thus, we used this opportunity for microscopic ultrastructural analysis of changes after the first procedure. Our main goal was to get the biopsies from the patients who were already operated and who requested the repeated procedure despite good outcome in girth enhancement, due to existing penile dysmorphophobia. This study was approved by the Ethical Committee of Belgrade Center for Genital Reconstructive Surgery (Belgrade, Serbia), and all patients were thoroughly informed, once again, about the details of the procedure with possible complications; and written informed consent was obtained before surgery. Penile girth was measured in flaccid and erect state. Measurement of the erect penis was obtained by pharmacological erection induced with intracavernosal injection of 20 μg of prostaglandin E1 (Caverject, Pfizer, New York, NY, USA).

Surgical approach

Repeated surgical approach for penile girth enhancement was the same as previously described.[5] Fibroblasts were obtained from scrotal dermal tissue and expanded in culture until the total cell number reached at least 2×10^7. The cells were seeded in two poly-lactic-co-glycolic acid scaffolds (PLGA; Regen Biotech Inc., Sungnam, Korea), the pores of which enabled cellular proliferation and development of an intracellular matrix combined with vascular regeneration. The scaffolds were incubated at 37°C, 24 h before the planned surgery. Intracavernosal injection of prostaglandin E1 was used to achieve pharmacological erection allowing for the measurement of penile body girth in erection and easier dissection of penile entities during the procedure. Penis was degloved using a subcoronal incision between dartos fascia and the new layer formed after previous scaffold insertion (**Figure 1a**). Biopsy specimens from the newly formed layer were taken for histomorphometric and ultrastructural analysis. Scaffolds were opened and placed over the penile shaft excluding the urethra (**Figure 1b**). Scaffolds were fixed to the Buck's fascia close to the urethra, preventing their movement postoperatively. Penile skin was pulled back over the scaffolds and closed in circumcision manner in two layers (**Figure 1c**). The penis was dressed with an elastic bandage for 7 days postoperatively, and urinary catheter was not used. Prophylactic antibiotics (cephalosporins; 515-01-3104-12-001, Galenika, Belgrade, Serbia) were used in all patients for 5 days. Sexual intercourse was restricted for 1 month following surgery. Biopsy specimens were fixed in 4% formaldehyde (F1635; Sigma-Aldrich, St. Louis, MO, USA) and in 3% glutaraldehyde (G5882; Sigma-Aldrich) for light and electron microscopy, respectively.

Light microscopy

Formalin-fixed tissue specimens were embedded in paraffin. Tissue sections (5-μm thick) were deparaffinized in xylol (534056; Sigma-Aldrich) and rehydrated in serial alcohols and were later used for hematoxylin-eosin (HE) (hematoxylin, 104302; eosin, 115935; Merck KGaA, Darmstadt, Germany) staining and double immunostaining (DakoCytomation EnVision®, Doublestain System [HRP-AP], Dako Inc., Carpinteria, CA, USA). Digital images of HE and immunostained sections were made on a Photomicroscope Olympus (Olympus BX41TF; Olympus, Tokyo, Japan) with a digital camera (Olympus C5060; Olympus) for the acquisition and analysis of the images.

Immunostaining

Formalin-fixed biopsy specimens were pretreated using heat-induced epitope retrieval procedure and blocked with peroxidase blocking solution (Dako Inc.). Sections were incubated with primary antibody Ki-67 (dilution 1:100; Novocastra, Leica Biosystems, Nussloch GmbH, Germany) followed by the incubation with the HRP-labeled Polymer (Dako Inc.). The initial reaction was completed with incubation of the Liquid DAB+ substrate-chromogen. On completion of the first reaction, incubation of the Doublestain Block (Dako Inc.) was performed. Specimens were then incubated with the second primary antibody vimentin (dilution 1:100; Dako Inc.) and subsequently with Rabbit/Mouse (LINK; Dako Inc.). After rinsing, sections were incubated with AP-labeled Polymer (Dako Inc.). The second antigen stain was completed with incubation of the Fast Red substrate-chromogen (Dako Inc.). Specimens were counterstained with hematoxylin (104302; Merck KGaA).

Electron microscopy

Glutaraldehyde-fixed tissue specimens were rinsed in cold cacodylate buffer (20840; Sigma-Aldrich), postfixed in 1% osmium tetroxide (O5500, Sigma-Aldrich), dehydrated in graded ethanol and propylene oxide (82320, Sigma-Aldrich), and processed for embedding in EPON (45345; Sigma-Aldrich). Thin sections were mounted on copper grids and stained with uranyl acetate (AGR1260A; Agar Scientific Ltd., Essex, UK) and lead citrate (AGR1210; Agar Scientific Ltd.) and further examined by electron microscope (Morgagni 268D; FEI, Hillsboro, OR, USA).

Instruments and statistical analyses

A retrospective evaluation of penile girth in flaccid and erect penis before surgery, then after the first and second surgery was performed. Satisfaction was estimated using a short questionnaire modified from a validated study for long-term outcome evaluation in hypospadias.[7] Surgery was assessed on a scale from 1 to 5 with 5 being the best ([1] very dissatisfactory; [2] dissatisfactory; [3] good; [4] very good; and [5] excellent). The results were analyzed with Statistica 6.0 software (StatSoft, Dell Software, Palo Alto, CA, USA). Obtained results were compared between groups using the two-tailed paired student's *t*-test, at 95% level of significance ($P < 0.05$).

RESULTS

Penile girth measurement

The mean follow-up was 38 months and ranged from 13 to 66 months. Girth measurements were taken in the flaccid and erect conditions. The average flaccid and erect girths (mean ± standard deviation [s.d.]) before the second procedure were 11.6 ± 0.8 cm (range: 9.8–12.8 cm) and 13.1 ± 0.5 cm (range: 12.4–14.1 cm), respectively. Girth after the second enhancement surgery was recorded at midshaft, in the flaccid and erect conditions, at 12.7 ± 0.6 cm (range: 11.5–14.0 cm) and 14.0 ± 0.4 cm (range: 13.3–14.7 cm), respectively. In total, the mean values of flaccid and erect gain in girth after the second surgery were 1.1 ± 0.4 cm (range: 0.6–1.7 cm) and 1.0 ± 0.3 cm (range: 0.6–1.5 cm), respectively (**Table 1**). There was a significant improvement for men with repeated surgery compared to the gain in girth after the first procedure ($P < 0.001$).

There were no major complications after surgery. In two cases, partial superficial necrosis of the penile skin was successfully treated conservatively. In long-term follow-up, all men reported good quality of totally preserved erection without changes of penile sensitivity. Furthermore, there were no complications related to prostaglandin E1 test such as priapism or prolonged erection. There were no unsatisfied patients. In total, the patients appraised the outcomes

after the second surgery as follows: the result was graded as excellent (mark 5) by 52.38%, very good (4) by 28.57%, and good by 19.05% (3) of patients (**Table 1**).

Light and electron microscopic findings

Connective tissue with an abundance of connective tissue fibers, frequent small blood vessels, and small number of inflammatory cells were observed in all analyzed tissue samples collected during surgery. White adipocytes, singular or in small groups, were also seen in all of the samples (**Figure 2a**). Double staining for Ki-67 and vimentin shows that the majority of proliferative cells are fibroblasts (**Figure 2b**).

Ultrastructural analysis of these tissue samples discovered an abundance of collagen fibrils that were regularly aligned, forming bundles, with a few widely spread fibroblasts (**Figure 3a**). Observation under the transmission electron microscope showed adjacent collagen bundles orientated orthogonally to one another. In some biopsy specimens, the presence of lipid droplets was found in fibroblasts (**Figure 3b**). Lipid droplets were small and variable in size and number. Mast cells were seen in all tissue samples that were analyzed (**Figure 3c**). Some of the mast cells were partly degranulated.

DISCUSSION

Several studies on penile length and girth and on what should be considered to be a "normal" penile size are published. The authors of these studies measured various aspects of penis size: length in flaccid, flaccid stretched and erect state, and girth in flaccid and erect state. The variability of the recorded values depended on the population included in the study as well as on the measuring technique. The average length in these studies, measured between the top of the glans and the base of the penis, in flaccid, flaccid stretched, and erect penis was 9 cm, between 12 cm and 13 cm, and between 14 cm and 16 cm, respectively. Regarding girth, the average circumference in the middle part of the penile shaft in flaccid and erect penis was between 9 cm and 10 cm, and 12 cm and 13 cm, respectively.[2,8–10]

Penis girth enhancement procedures are even more controversial than penis lengthening procedures. There is no recommended indication for penile girth enhancement in medical literature, and operative techniques are still not standardized.[1–4,11] The goal of such a procedure would be a symmetrical increase in the girth of the penis. These procedures have been described and used either by patients themselves or by doctors such as plastic surgeons, urologists, or even dermatologists.[11] However, severe complications such as penile disproportion with serious disfigurement and dysfunction are not an uncommon outcome, requiring difficult corrections. According to literature, no satisfactory method has been reported for penile girth enhancement to date. Several reports advocated different methods for girth enhancement such as biosynthetic materials, natural biological tissue, and tissue-engineered materials, with varying degrees of success.[12] For instance, natural biological tissues have good histocompatibility and are very easy to obtain, but the materials are absorbed. Biosynthetic materials are very stable, but reportedly associated with prosthesis exposure and poor penile morphology.

We published the use of PLGA scaffolds pretreated with autologous fibroblasts for penis girth enhancement.[5] The pretreated scaffolds were placed between dartos and Buck's fascia, without covering the urethra, after penile degloving. Out of 84 patients who entered the study, 70% were completely satisfied. Mean penile girth augmentation was 3.15 cm in flaccid and 2.47 cm in erect state. However, 21 of our patients, who underwent this procedure with biodegradable scaffolds, requested repeated treatment for further gain in penile girth, due to dissatisfaction with achieved dimensions after the first surgery and desire for additional enhancement. Despite the significant gain in girth after the first penile enhancement surgery, their penile dysmorphophobia led to repeated surgery with the aim to improve the achieved girth after primary surgery. To date, the use of cosmetic surgery to enlarge the penis remains highly controversial. There is a

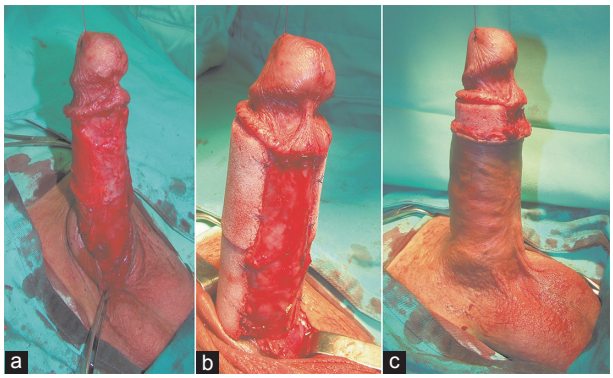

Figure 1: Repeated penile girth enhancement using biodegradable scaffolds, with biopsy of previously formed tissue. (**a**) Penile skin is dissected carefully. New layer of tissue is visible. (**b**) Scaffolds are placed onto penile body next to the urethra. (**c**) Penile girth is equally enhanced.

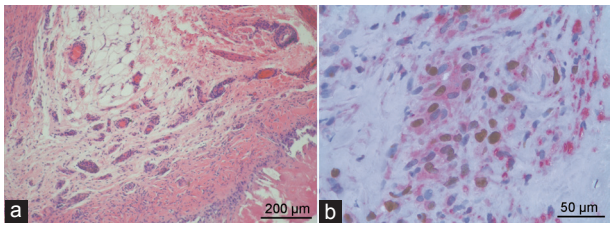

Figure 2: Light microscopic findings of tissue samples collected during surgery. (**a**) Hematoxylin-eosin-stained biopsy tissue sample shows connective tissue with a small group of adipocytes surrounded with connective tissue fibers, small blood vessels, and inflammatory cells. (**b**) Double staining for Ki-67 (brown) and Vimentin (red) shows that the majority of proliferative cells are fibroblasts (red).

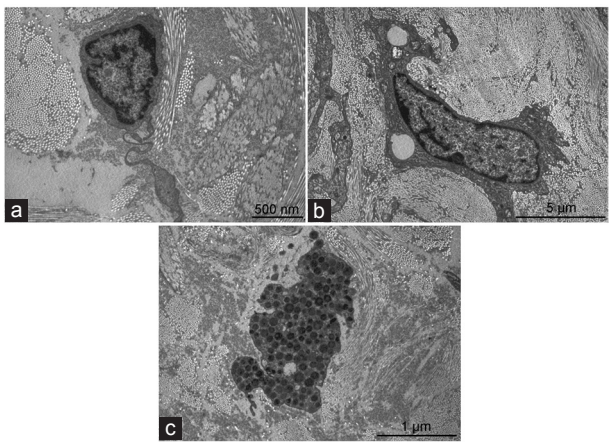

Figure 3: Electron microscopic findings of tissue samples collected during surgery. (**a**) Electron microscopic micrograph showing fibroblast surrounded with bundles of collagen fibers. (**b**) Fibroblast with lipid droplets surrounded with parallel bundles of collagen fibers. (**c**) Electron micrograph of mast cell with abundance of large, dense granules.

Table 1: Preoperative patient data and postoperative outcomes

Patient number	*Age (year)*	*Stage 0, F/E (cm)*	*Stage I, F/E (cm)*	*Stage II, F/E (cm)*	*Stage I to Stage 0, F /E (cm)*	*Stage II to Stage I, F/E (cm)*	*Stage I to Stage II, Period (month)*	*Follow-up (month)*	*Satisfaction, (1–5)#*
1	23	8.4/10.6	11.6/13.2	12.9/13.8	3.2/2.6	1.3/0.6	14	24	5
2	27	9.4/10.9	11.7/13.2	12.6/14.0	2.3/2.3	0.9/0.8	14	45	4
3	26	9.3/10.9	11.5/13.1	12.5/14.2	2.2/2.2	1.0/1.1	23	27	5
4	37	7.7/9.6	11.5/12.4	13.0/13.8	3.8/2.8	1.5/1.4	12	61	5
5	32	9.1/10.8	11.6/13.1	12.6/13.9	2.5/2.3	1.0/0.8	17	36	4
6	22	9.6/11.2	12.6/13.4	13.4/14.1	3.0/2.2	0.8/0.7	18	13	3
7	36	10.0/11.8	12.2/14.1	12.9/14.6	2.2/2.3	0.7/1.5	22	66	3
8	25	9.1/10.7	11.9/12.5	13.2/13.7	2.8/1.8	1.3/1.2	13	40	5
9	28	7.6/10.0	9.8/12.6	11.5/13.8	2.2/2.6	1.7/1.2	16	35	4
10	22	9.4/11.2	11.9/13.2	12.7/14.2	2.5/2.0	0.8/1.0	22	48	5
11	24	8.7/10.5	11.1/12.8	12.8/14.1	2.4/2.3	1.7/1.3	26	55	5
12	30	8.5/10.2	11.1/12.5	12.0/13.3	2.6/2.3	0.9/0.8	15	16	4
13	27	8.0/10.3	10.6/12.8	11.8/13.6	2.6/2.5	1.2/0.8	30	41	4
14	22	10.0/11.1	12.4/13.0	13.0/14.3	2.4/1.9	0.6/1.3	19	14	3
15	37	9.6/11.0	12.8/13.2	13.5/14.2	3.2/2.2	0.7/1.0	26	52	5
16	27	8.6/11.1	10.9/13.6	12.4/14.4	2.3/2.5	1.5/0.8	19	47	4
17	23	9.7/10.9	12.2/13.7	13.1/14.5	2.5/2.8	0.9/0.8	25	21	5
18	34	8.6/11.2	10.9/13.4	12.3/14.1	2.3/2.2	0.7/0.7	28	33	3
19	29	9.5/10.7	11.4/12.9	12.2/13.8	1.9/2.2	0.8/0.9	14	38	5
20	26	10.2/11.9	12.4/14.0	14.0/14.7	2.2/2.1	1.6/0.7	32	28	5
21	31	8.2/10.6	10.7/13.0	11.8/13.8	2.5/2.4	0.9/0.8	15	48	4
Mean±s.d.	28.0±4.9	9.0±0.8/10.8±0.5	11.6±0.8/13.1±0.5	12.7±0.6/14.0±0.4	2.6±0.4/2.3±0.3	1.1±0.4/1.0±0.3	20.0±6.0	37.5±15.1	4.3±0.8

#Satisfaction: 1-very dissatisfactory, 2-dissatisfactory, 3-good, 4-very good, 5-excellent. Gain after first surgery: in flaccid 1.9-3.8 cm, and in erection 1.8-2.8 cm. Gain after second surgery: in flaccid 0.6-1.7 cm, in erection 0.6-1.5 cm. Stage 0: preoperative measures; Stage I: girth after first surgery; Stage II: girth after second surgery; F: flaccid; E: erectile; s.d.: standard deviation.

lack of any standardization of all described procedures with poorly defined indications and outcome measures.

In the present study, we measured the penile girth before and after the second girth enhancement procedure using biodegradable scaffolds and investigated the relationship between the penile body and adjacent tissues. Finally, we performed histological examinations of the newly formed penile structures, which play an important role in evaluating the safety of biomaterials. One of the main questions was: why should penile enhancement surgery be repeated and is this reasonable with respect to penile girth? We found significantly better girth enhancement in men with repeated surgery compared to the gain in girth after the first procedure ($P < 0.001$). However, the surgeon should consider a psychological clearance prior to confirmation of repeated penile enhancement procedure.

One of the main characteristics of the biodegradable scaffolds is their tendency to become almost completely absorbed following implantation. The scaffold is gradually replaced by local tissue ingrowth and is eventually no longer needed. Another benefit is the predisposition of the scaffolds to support cell ingrowth, in part by potentiating native cell–cell interaction. One of the reasons for cell ingrowth potential is that the scaffolds are composed mostly of matrix proteins, which have powerful abilities to promote and direct the ingrowth of various cell types.[13–15]

During the repeated procedure in our group, we obtained samples of newly formed tissue, 12 to 32 months after previous penis girth enhancement with PLGA pretreated scaffolds. Microscopic evaluation showed the presence of vascularized connective tissue with an abundance of collagen fibers, fibroblasts, and inflammatory cells, indicating active neovascularization and fibrillogenesis. Mast cells were found in all analyzed tissue samples. Mast cells are known to participate in three phases of wound healing: the inflammatory reaction, angiogenesis, and extracellular-matrix reabsorption. The activated mast cells control the key events of the healing phases: triggering and modulation of the inflammatory stage, proliferation of connective cellular elements, and final remodeling of the newly formed connective tissue matrix.[16,17] The gross organization of the scarce and loose connective tissues in surgically removed tissue is normally characterized by a few widely spread fibroblasts with a small quantity of fibrillar collagen and a few collagen fibers visible among muscle fibres.[18,19] Most of the lipid-laden fibroblasts could be recognized as myofibroblasts. The significance of the droplets for the deposition of interstitial materials or for the synthesis of prostaglandins is discussed.

Our present and previous reports confirmed good biocompatibility of scaffolds. Complications included local infection, local skin necrosis, and seroma due to limitation of available compliant skin after the first procedure. However, all of the complications were successfully treated conservatively.[5,6] Histological examination demonstrated newly generated tissue characterized by significant cell number, collagen content, and ingrowth of small blood vessels. Regardless of which type of procedure is being sought, the patient should be aware that there is no universally accepted protocol for either type of surgery. Most of the reported case studies have been in a small experimental population with short follow-up. The patients should also be informed of the numerous complications that can result from such procedures, which include but are not limited to, poor cosmesis, further shortening, and sexual dysfunction. In our group, all men appraised the outcomes after the second surgery as satisfactory and excellent, and very good results were obtained in more than 80%. Another study by Jin *et al*.,[13] who treated 69 patients, showed similar results with satisfaction in more than 90%.

Tissue-engineered materials appear to be the best materials for penile girth enhancement; however, the relatively immature technologies and complicated cell cultures limited their practical application. Our main findings included significantly increased girth enhancement after each of the two procedures with biodegradable scaffolds, in both flaccid and erect penises. These results were permanent in all patients after more than 3 years of follow-up. Limitations could include a lack of a control group of men who underwent different procedures for penile enhancement. One of the questions is that could possibility of using the scaffolds be without seeding of fibroblasts. Furthermore, the question remains whether the increased girth was related to a successful surgery or to a good choice of procedure. In addition, there is a lack of other studies with similar indications, surgical techniques, type of girth enhancement, and length of follow-up. According to our experience, until new and more objective and reproducible data are available, we can accept these procedures as investigational and patients should be discouraged from undergoing these invasive treatments.

Techniques in tissue engineering continue to improve and more clinical work must be done before most of us are fully comfortable with using these techniques. Although the optimal result of girth enhancement would be increased symmetry and uniform girth with preserved function of the penis, achieving these goals using the current available techniques is a great challenge. Further studies and long-term follow-up are also needed for this procedure.

AUTHOR CONTRIBUTIONS

MLD was responsible for the study concept and design and drafted the manuscript. UB carried out interpretation of data and helped draft the manuscript. BS performed data acquisition and analysis, participated in the study design, and helped draft the manuscript. TKS carried out the electron microscopy analysis and interpretation of ultrastructural findings. TM performed immunostaining and interpretation of data. MB performed the statistical analysis and helped with data acquisition. VK coordinated the study and helped in drafting the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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