Mesenchymal stem cell transplantation improves biomechanical properties of vaginal tissue following full-thickness incision in aged rats

Ofra Ben Menachem-Zidon,1 Michal Gropp,1 Benjamin Reubinoff,1,3,* and David Shveiky2,3
1The Sidney and Judy Swartz Stem Cell Research Center, The Goldyne Savad Institute of Gene Therapy, Hadassah Medical Center and Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
2Division of Female Pelvic Medicine and Reconstructive Surgery, Hebrew University of Jerusalem, Jerusalem, Israel
3Department of Obstetrics and Gynecology, Hadassah Medical Center and Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
*Correspondence: benr@hadassah.org.il
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SUMMARY
Pelvic organ prolapse (POP) is common among post-menopausal women and is associated with bladder, bowel, and sexual dysfunction. Surgical repair with the patients’ native tissues is sub-optimal with high reoperation rates, potentially due to diminished age-related healing. We demonstrate that systemic transplantation of mesenchymal stem cells (MSCs) improves healing of full-thickness vaginal incision in the vaginal wall of old rats, as suggested by both histological and functional analysis. Transplanted MSCs homed and survived at the surgical vaginal site. Attenuation of the injury-induced inflammatory response, increased angiogenesis, and reduced matrix metalloproteinase 9 expression were observed at the surgical site of transplanted rats. Most importantly, the functional biomechanical properties of the healed vagina, at day 30 post-injury, were improved in MSC-transplanted, compared with sham-operated non-transplanted, old rats. These results may pave the way to further translational studies toward clinical transplantation of MSCs adjuvant to POP repair for the improvement of surgical outcome.

INTRODUCTION
Pelvic floor disorders (PFDs) include pelvic organ prolapse (POP) (Dieter et al., 2015; Jelovsek et al., 2007), urinary incontinence (Aoki et al., 2017), and sexual dysfunction (Fatton et al., 2020). PFDs impair women’s quality of life and are associated with limited physical and social activity, leading to poor self-image and depression (Larouche et al., 2020; Moroni et al., 2019).

Aging is considered a major risk factor for POP (Barber, 2016; Vergeldt et al., 2015). As the general population grows older, the incidence of POP is expected to rise (Bruseke et al., 2016; Dieter et al., 2015; Wu et al., 2009) and so is its burden on healthcare (Giannini et al., 2018; Hong and Ding, 2019). According to recent estimates, the prevalence of symptomatic POP is 6%–9.6% in women aged >20 years (Pang et al., 2021) and 30%–40% in post-menopausal women (Tinelli et al., 2010). The cornerstone of treatment of POP is surgery. The lifetime risk for a woman to undergo surgical treatment for POP is 12% (Rizvi and Chughtai, 2017; Smith et al., 2010). Unfortunately, recurrence of prolapse after surgery is not rare, with reoperation rates reaching 20%–30% (Friedman et al., 2018). In an attempt to improve cure rates and reduce the rate of reoperation, synthetic vaginal mesh grafts have been developed. While initial outcomes were promising, a surge of reports on adverse events emerged (Dällenbach, 2015; Schmidt and Taylor, 2021), leading to multiple FDA warnings and resulting in a product recall in 2019 (Winkelman et al., 2019). Therefore, new conceptual therapeutic approaches are needed to improve the surgical outcome of native-tissue vaginal reconstruction.

The sub-optimal success of surgery may be attributed to the impaired wound-healing processes of vaginal tissue in aged patients. Wound healing, which involves a complex interaction between inflammation and tissue remodeling, is strongly affected by age (Gosain and DiPietro, 2004; Gurtner et al., 2008; Wilkinson and Hardman, 2020). Aging is associated with a reduced quality of wound healing, resulting in scar tissue with reduced biomechanical properties (Ashcroft et al., 1997; Gurtner et al., 2008; Wilkinson and Hardman, 2021).

Several animal models of POP have been previously described (Abramowitch et al., 2009; Mori da Cunha et al., 2021); however, they do not fully authentically mimic the human condition, especially with regard to aging. In order to assess vaginal tissue healing following pelvic floor surgery, we developed a rodent model for vaginal surgical injury and demonstrated impaired wound healing and biomechanical properties in aged rats (Ben Menachem-Zidon et al., 2020; Shveiky et al., 2019).

Mesenchymal stem cells (MSCs) were shown to have a beneficial effect on skin wound-healing processes in both preclinical (Eylert et al., 2021; Falanga et al., 2007; Isakson et al., 2015) and clinical studies (García-Bernal et al., 2021; Huang et al., 2020). MSC transplantation was specifically examined in the context of PFDs, using rodent models for vaginal distention (Dai et al., 2015; Mori da Cunha et al., 2018; Sadeghi et al., 2016) and anal sphincter injury (Salcedo et al., 2013), as well as in multiparous ewes (Emmerson et al., 2019), and a ovarietomized rhesus macaque...
Figure 1. Homing and survival of transplanted MSCs following full-thickness vaginal incision

(A and B) Low power images of H&E staining show the location of the injury site, just opposite to the urethra in sham (A) and MSC-transplanted (B) old rats at day 3 post-transplantation.

(C) Bar graphs displaying the maximal distance between epithelial edges of the wound (μm) in old and young rats at 3 days post-transplantation.

(D and E) A higher magnification of the incision sites of sham (D) and MSC-transplanted (E) rats are shown.

(F) Immunofluorescence image of a section of a sham-operated rat at day 3 post-injury, stained with anti-cytokeratin and counterstained with DAPI.

(G) A dual-color immunofluorescence image showing cytokeratin staining and PKH26\(^+\) cells in an old MSC-transplanted rat at day 3 post-transplantation.

(H and I) DAPI-stained vaginal section from an old MSC-transplanted rat at day 30 post-injury showing a venule/arteriole (H), which is composed of GFP-labeled cells (I).

(J) A merged image of (H) and (I).

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model (Zhang et al., 2021) characterized with vaginal wall weakness. These studies showed homing, survival, and beneficial effects of transplanted MSCs on the tissue. However, the role of age, a highly relevant factor to clinical reality, has not yet been studied.

We previously examined in young rats the effects of MSC transplantation on healing of vaginal surgical injury. Using our model, we showed that systemic transplantation of bone-marrow-derived MSCs following full-thickness vaginal surgical incision was associated with homing of the transplanted cells to the vaginal injury site, with creation of new vascular-like structures by direct differentiation into endothelium (Ben Menachem-Zidon et al., 2019). The aim of the current study was to proceed another step forward, toward exploring the effects of MSC transplantation on inflammatory parameters, vascularization, and biomechanical properties of vaginal tissue following full-thickness surgical incisions in old rats.

RESULTS

Homing and survival of transplanted MSCs in the vaginal injured site

Young and old virgin Sprague Dawley (SD) rats underwent a full-thickness vaginal incision. The incision included both the vaginal epithelial layer and the fibromuscular tissue underneath. Fluorescence-activated cell sorting (FACS) analysis showed that 95.2% ± 1.3% of the cells expressed PKH26 (n = 5) and 97.6% ± 0.96% of the transduced cells expressed GFP (n = 5) (Figure S1). Healing was analyzed and compared at sequential time points between the intranovously (i.v.) transplanted and sham-operated groups. On day 3 post-injury, the incision was almost fully bridged antevesially (i.v.) transplanted and sham-operated groups. On day 3 post-injury, the incision was almost fully bridged. Evaluation of the maximal distance between epithelial edges of the wound on day 3 showed a significantly smaller gap (p < 0.01) in old MSC-transplanted rats compared with sham non-transplanted controls (Figure 1C). Wound closure was not significantly different between young MSC-transplanted rats and young sham-operated rats. Survival of transplanted MSCs was also monitored in both young and old rats. The number of labeled MSCs per animal in the vaginal tissue surrounding the injury site in old rats did not significantly differ from young rats at 3 (old = 852.5 ± 67.4; young = 874.5 ± 134.5), 7 (old = 294.8 ± 35.4; young = 254.2 ± 29.5), and 30 (old = 145 ± 9.8; young = 154.3 ± 13.2) days post-transplantation (Figure S2). At 30 days post-transplantation, similar to our previous published findings in young rats (Ben Menachem-Zidon et al., 2019), the transplanted cells gave rise to either post-capillary venules and precapillary arterioles (vessels with 10–50 μm diameter) (Figure S3) or venules or arterioles (vessels with 50–100 μm diameter) (Figures 1H–1J) expressing CD31 (Figures 1K–1N). These results indicated a beneficial effect of transplanted MSCs on wound closure in old rats.

The effect of MSC transplantation on the inflammatory response at the vaginal injury site

We assessed inflammation by immunofluorescence staining for CD68, a pan-macrophage marker. Sections were stained and analyzed for macrophages expressing CD68 around the injury site. Representative images of macrophages around the injury site from sham-operated rats (Figure 2A) and MSC-transplanted rats (Figure 2B) on day 7 post-injury demonstrated the decreased inflammatory response of old MSC-transplanted rats. Quantification of macrophage numbers in old and young rats on days 3, 7, and 30 post-transplantation showed a significantly higher number of CD68+ cells in old, compared with young, sham-operated rats (Figure 2C). In both old and young rats, MSC transplantation was associated with significantly lower numbers of CD68+ cells at days 3 and 7 post-injury (Figure 2C). At day 30, a significantly lower number of CD68+ macrophages was observed in transplanted, compared with sham-operated, old rats, while a significant difference was not seen in young rats (Figure 2C). A multivariate analysis demonstrated that the number of macrophages adjacent to the injury site was significantly affected by age, MSC transplantation, and the length of time following injury. The interaction effect was also significant, suggesting that the effect of MSC transplantation differed between old and young rats (more prominent in old rats) and depended on the length of time post-injury. To further characterize the inflammatory response, sections were co-stained for CD68 and tumor necrosis factor α (TNF-α). The percentage of CD68+ cells co-expressing TNF-α from total CD68+ cells was significantly (p < 0.01) lower, as early as day 3, in old and young MSC-transplanted rats.
compared with sham-operated rats. In the old rats, this difference was also significant \((p < 0.01)\) on days 7 and 30 post-injury (Figure 2L). Taken together, these results showed that MSC transplantation attenuated the inflammatory response in young and old rats at days 3 and 7 post-injury. Furthermore, in old rats, this effect was also observed at 30 days post-injury.

The effect of MSC transplantation on MMP9 expression following injury
Matrix metalloproteinase 9 (MMP9) is an important enzyme regulating collagen metabolism and other extracellular matrix (ECM) protein degradation (Bonnans et al., 2014; Van Doren, 2015). Analysis of the expression of MMP9 in the vagina was performed by immunofluorescence staining. While MMP9 was not expressed in the naive vagina of young and old rats (data not shown), we found that it was rapidly induced 3 days following injury in all treatment groups. On day 3 post-transplantation, sham-operated rats displayed disorganization of the vaginal tissue at the injury site (Figures 3A–3C), with a strong and scattered expression of MMP9 (Figure 3C). MSC-transplanted old rats displayed an organized, healed vaginal tissue (Figures 3D and 3E) with decreased expression of MMP9 (Figure 3F). Quantification of MMP9+ cells (Figure 3G) on day 3 post-injury revealed significantly higher numbers of MMP9+ cells in old, compared with young, sham rats \((p < 0.05)\) as well as significantly higher numbers of MMP9+ cells in sham, compared with MSC-, treated young \((p < 0.01)\) and old rats \((p < 0.01)\) (Figure 3H). Double staining of CD68 with MMP9 demonstrated that CD68 macrophages were not expressing MMP9 but were in very close proximity to MMP9+ cells (Figures 3I–3L). These results showed that vaginal injury induced MMP9 expression in young and old rats that was more pronounced in old, compared with young, rats. Furthermore, MSC transplantation significantly decreased MMP9 expression both in young and old rats.

The effect of MSC transplantation on the number of blood vessels on day 30 post-surgery
To assess the effect of MSC transplantation on tissue vascularization, vaginal sections from naive, sham, and MSC-transplanted rats in both young and old rats were stained with the endothelial marker CD31. The total number of blood vessels was quantified on days 3, 7, and 30 in old and young rats. This analysis showed a significantly higher total number of blood vessels in MSC-transplanted rats (Figure 4B) compared with controls (Figure 4A) on days 7 and 30 (Figure 4C) in both young and old rats. The total number of blood vessels did not significantly differ between young and old rats in all study groups. To further assess the contribution of transplanted MSCs to blood vessel formation, and to analyze whether it is affected by age, we quantified the CD31+ blood vessels that included cells co-labeled with PKH-26 in their wall. Quantification of CD31 blood vessels that included PKH-26 cells in their walls showed a similar percentage of blood vessels: 30.7% ± 5.3% and 32.7% ± 3.1% from total CD31 blood vessels in young and old rats, respectively. A representative image of a blood vessel, composed of MSCs, expressing CD31 is presented in Figures 4D–4F and S3. These results showed that MSC transplantation increased total blood vessels in MSC-transplanted young and old rats.

The effect of MSC transplantation on biomechanical properties of the vaginal tissue
On day 30 post-transplantation, the vagina was dissected and prepared for biomechanical evaluation. Sham rats, both young and old, displayed lower maximal tensile strength when compared with naive rats. MSC-transplanted rats displayed significantly higher maximal tensile strength than sham-operated rats in both young and old rat groups \((p < 0.05)\). In fact, tissue recovery following MSC transplantation reached the tensile strength values of naive rats (Figure 5A). Stress-strain curves (Figures 5B and 5C) were plotted, and Young’s moduli were calculated for each sample (Figure 5D). The Young’s modulus of old...
Figure 3. The effect of MSC transplantation on MMP9 expression following vaginal surgical incision

(A and B) Sections from sham-operated rats at day 3 post-injury were stained with DAPI (A) and anti-cytokeratin (B).
(D and E) Representative images from MSC-transplanted old rat at day 3 post-transplantation stained with DAPI (D) and cytokeratin (E). The dashed white line marks the location of the vaginal injury site. The white squares in (B) and (D) define the lamina propria just adjacent to the epithelial layer (marked by cytokeratin staining) near the injury site.
(C and F) A higher magnification of the area within the white squares in (B) and (E), respectively, stained with anti-MMP9.
(G) A higher magnification of MMP9-expressing cells.
(H) Bar graphs displaying quantification of MMP9+ cells on day 3 post-injury.

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respective *NS, non-significant*), while MSC-treated rats played shorter stretching time (17 ± 7.5 s, respectively) compared with young rats, respectively (Figure 5E). Looking at the length of time at high load, vaginal tissue from sham-operated rats, both old and young, was stretched for a significantly shorter time at high load (10.4 ± 6.3 s and 14.4 ± 4.6 s for old and young rats, respectively) compared with MSC-treated rats (23.1 ± 7.6 and 33.5 ± 21.3 s) for old and young rats, respectively (p < 0.01) (Figure 5F).

**DISCUSSION**

In this study, we demonstrated that systemic transplantation of bone-marrow-derived MSCs after vaginal full-thickness incision in old rats was associated with survival of the MSCs at the injury site for 30 days. MSC transplantation attenuated the inflammatory response, increased angiogenesis, and attenuated MMP9 immunolocalization at the injury site. Most importantly, transplantation improved biomechanical properties of the vagina, resulting in stronger healed vaginal tissue.

Our findings that MSC transplantation attenuated age-related inflammatory response correlated with reports on skin injury in old rodents (Gaur et al., 2017; Mazini et al., 2020; Silina et al., 2020). Similar to studies on the skin (Jo et al., 2021), we demonstrated the potential of MSCs to affect age-related inflammation in the healed tissue following full-thickness vaginal incision by attenuating macrophage number and their expression of the pro-inflammatory cytokine TNF-α. The attenuation of macrophage number was more prominent in MSC-transplanted old, compared with young, rats. The ability of MSCs to attenuate the inflammatory response in skin pathology was also demonstrated in clinical trials (Cheng et al., 2016; Gaur et al., 2017). Thus, MSC transplantation may offer a targeted treatment approach to attenuate age-related inflammatory response in the injured vagina.

MMP9 is a key enzyme involved in the degradation of both collagen and elastin in the ECM (Löffek et al., 2011; Page-McCaw et al., 2007). Therefore, its expression was previously examined in women with POP (Zhu et al., 2020). While some studies reported an increase in MMP9 expression in the vaginal tissue of women with POP (Alarab et al., 2014; Dviri et al., 2011; Wu et al., 2012), others suggested no such increased activity (Ghersel et al., 2019). A significant increase in vaginal MMP9 expression was evident in rodents following vaginal delivery, vaginal distention injury (Rahn et al., 2008), and in the POP transgenic mouse model, which do not express the ECM protein fibulin (Fbln5<sup>−/−</sup> mice) (Chin et al., 2016). It was shown that local administration of the MMP inhibitor in Fbln5<sup>−/−</sup> mice (Budatha et al., 2011, 2013), as well as in rats following obstetrical injury (Florian-Rodriguez et al., 2019; Hamner et al., 2020), rescued injury-induced loss of vaginal tissue strength and had positive site-specific effects on collagen distribution within the vagina. In accordance with these observations, we demonstrated the induction of MMP9 expression at day 3 following vaginal injury. Its expression was significantly more prominent in the injured vagina of old, compared with young, rats.

The connective tissue of the vaginal wall remodels to adapt to changes in biomechanical stresses by ECM metabolism. Remodeling is controlled by an interplay between MMP9 expression, which degrades the ECM, and its inhibitor-tissue-derived inhibitors of metalloproteinases (TIMPs). Several studies identified an imbalance between MMPs and TIMPs in women with POP, which resulted in an accelerated process of collagen degradation that exceeded its synthesis (Guler and Roovers, 2022; Rahajeng, 2018). Hence, it may be hypothesized that the observed MSC-induced reduction of MMP9 expression is associated with decreased degradation of vaginal ECM components, resulting in improved biomechanical properties of the treated vagina.

It was previously shown that TNF-α stimulates MMP9 production by other cells (Bahar-Shany et al., 2010; Campos et al., 2014; Leber and Balkwill, 1998; Zhou et al., 2009). In addition, it was shown that TNF-α mediates the activation of MMP9 through down-regulation of TIMP-1 (Han et al., 2002; Nee et al., 2007). Hence, the attenuating effect of MSC transplantation on the percentage of TNF-α<sup>+</sup> macrophages, which we demonstrated, may have a role in the regulation of MMP9 expression. Further studies are needed to confirm this potential mechanism.

A major finding of our study was that MSC transplantation improved biomechanical properties of the healed vagina in old rodents (Gaur et al., 2017; Mazini et al., 2020; Silina et al., 2020). Our findings that MSC transplantation attenuated age-related inflammatory response correlated with reports on skin injury in old rodents (Gaur et al., 2017; Mazini et al., 2020; Silina et al., 2020). Similar to studies on the skin (Jo et al., 2021), we demonstrated the potential of MSCs to affect age-related inflammation in the healed tissue following full-thickness vaginal incision by attenuating macrophage number and their expression of the pro-inflammatory cytokine TNF-α. The attenuation of macrophage number was more prominent in MSC-transplanted old, compared with young, rats. The ability of MSCs to attenuate the inflammatory response in skin pathology was also demonstrated in clinical trials (Cheng et al., 2016; Gaur et al., 2017). Thus, MSC transplantation may offer a targeted treatment approach to attenuate age-related inflammatory response in the injured vagina.

(I–K) Staining for DAPI (I), CD68 (J), and MMP9 (K) in the lamina propria. (L) A higher magnification of a merged image is demonstrated. Scale bars: (A, B, D, and E) 500, (C, F, and I–K) 100, (L) 50, and (G) 25 μm. Mean ± SD are presented (H). Two-way ANOVA performed in (H), followed by post hoc analysis between specific groups using Bonferroni correction. *p < 0.05, **p < 0.01. n = 5 rats/group.
Figure 4. The effect of MSCs on the number of blood vessels on day 30 post-injury

(A and B) Representative images of CD31+ blood vessels in the lamina propria of an old MSC-transplanted rat (A) and a sham-operated rat (B).

(C) Bar graphs displaying the total number of blood vessels (including postcapillary venules, precapillary arterioles, venules, and arterioles) per microscopic field in old and young rats at 3, 7, and 30 days post-transplantation.

(D–F) Representative images of a postcapillary venule/precapillary arteriole that is CD31+ (D) and includes PKH-26+ cells in its wall (E) and is counterstained with DAPI is presented in the merged image (F).

Scale bars: (A and B) 100 and (D and E) 50 μm. Mean ± SD are presented (C). Three-way ANOVA performed in (C), followed by post hoc analysis between specific groups using Bonferroni correction. **p < 0.01. n = 5 rats/group.
Figure 5. The effect of MSC transplantation on biomechanical properties of the vaginal tissue
(A) Bar graphs displaying the maximal tensile strength (N) of vaginal tissue by study group.
(B and C) Stress-strain curves of old (B) and young (C) rats’ vaginal tissue of each study group. Mean stress (Pa) values ± SD are presented for each experimental group.
(D) Bar graphs displaying Young’s modulus (KPa) values of all experimental groups.
(E) Bar graphs displaying the time (seconds) of stretching before tear of vaginal tissue from each group.
(F) Bar graphs displaying the distribution of length of time at peak stress, until loss of tensile strength by study group, representing viscoelasticity.
Mean ± SD are presented in (A)–(F). Two-way ANOVA performed in (A) and (D)–(F), followed by post hoc analysis between specific groups using Bonferroni correction. **p < 0.01. n = 9 rats/group.
vagina and reversed age-related effects, including reduced maximal tensile strength 30 days post-injury. In addition to measuring tensile strength, we measured viscoelastic properties of the healed vagina by stress-relaxation analysis, plotting stress against time and measuring the time elapsed at peak stress under continuously increasing strain. We chose to use the stress-relaxation characteristic, as we found it most representative of the post-operative state in clinical life where the pelvic floor tissue is holding against a continuous intra-abdominal pressure and gravity over time (Wang et al., 2012).

Tissue elasticity as represented by Young’s modulus varied between old and young rats at baseline; however, it was not altered by MSC transplantation. This may suggest that the improved tissue healing, caused by MSC transplantation, is not associated with stiffer scar tissue formation and that the original age-determined tissue elasticity is preserved.

Local transplantation of MSCs may provide clinical advantages compared with systemic delivery, as it avoids their systemic dissemination and potential undesired side effects (Musial-Wysocka et al., 2019). However, our previous studies (Ben Menachem-Zidon et al., 2019) and those of others (Dai et al., 2015; Kasap et al., 2019; Mori da Cunha et al., 2018; Ripperda et al., 2017) showed that local injection of MSCs into the vagina resulted in poor survival of the transplanted cells. We therefore transplanted the MSCs systematically and showed their homing and long-term survival in the injured vagina. Future developments of delivery systems of MSCs may allow simple and safe local transplantation combined with cell survival and the beneficial healing effects of systemic transplantation.

EXPERIMENTAL PROCEDURES

This study was approved by the Hebrew University Animal Care and Use Committee. SD female rats were held in the specific pathogen-free unit in Hadassah Hebrew University Medical School with food and water ad libitum. Young rats were 10 weeks old, with an average weight of 200 g when operated on, and old rats were 12 months old with an average weight of 500–600 g. The rats were divided into the following six groups (n = 9 rats/group): young and old naive rats, which did not undergo any surgical procedure; nor receive treatment; young and old sham rats, which had vaginal incision was performed opposite to the urethra on both the BW), a standardized 9 mm posterior midline sagittal full-thickness vaginal incision performed and domitor (75 mg/kg BW [body weight] and 0.5 mg/kg BW), a standardized 9 mm posterior midline sagittal full-thickness vaginal incision was performed opposite the urethra on both the young and old rats. Upon completion of the procedure, administration of atipamezole (1 mg/kg) was given to reverse the anesthetic effects, and 2*10^6 MSCs were systemically transplanted into the tail vein immediately following the full-thickness vaginal incision. Rats were monitored daily after the incision. According to the protocol, in case of excessive bleeding, rats were excluded from the experiment. For histological analysis, including inflammatory response, rats were sacrificed at 3, 7, and 30 days post-transplantation (n = 7–9 rats/group/at each time point). For biomechanical studies, rats were sacrificed at 30 days post-transplantation.

Dissection of vaginal tissue and histological evaluation
Rats were sacrificed with an overdose of CO_2. In scarified rats, the entire vagina including the adjacent extra vesicle urethra were dissected en bloc and carefully embedded in paraffin, preserving its anatomical orientation (Figure S4). Six μm sections were prepared and used for all histological parameters. H&E staining was performed on every ninth section to identify and map the grafts’
Biomechanical testing

Biomechanical properties of the vaginal tissue were tested using a dynamic mechanical analyzer (TA Instruments, New Castle, DE, USA). The load cell used was of 22 N with a resolution of ±0.1 N. Thirty days after injury, young and old rats (n = 9 in each group) were euthanized. The vaginal specimen was longitudinally cut at the midline of the anterior wall along the urethra, forming a rectangular shape with the healed wound in the middle. A standardized rectangular specimen sized 1 x 2 cm was prepared for biomechanical testing. The specimens were kept moist by spraying sterile warm (37°C) saline solution prior to and during testing. Each specimen was loaded onto the mechanical analyzer using a smooth plastic clench, and tension was gradually increased until the breaking point was reached. The induced force was tracked at a 5% strain rate up to the breaking point of the subject tissue, and we observed changes in the stress-strain curve.

The following parameters were analyzed: (1) maximal tensile strength, represented by the peak force (N) applied to each sample just before tearing. (2) A stress-strain curve was drawn for each specimen to measure elastic properties. Stress (Pa) was defined as the force applied to the tissue divided by the cross-sectional area of the specimen. Strain was defined as the percentage of change in length of the specimen compared with the initial length between the clamps. (3) Young’s modulus is the slope of the stress-strain curve, representing elasticity. The Young’s moduli were calculated from the initial linear section of the stress-strain curves. (4) Viscoelastic properties: the total stretching time (seconds) until tearing and the time elapsed at maximal load under a continuously increasing strain (time in high load) were measured. This is a dynamic measure of tissue strength, looking at the ability of the tissue to keep its biomechanical properties under strain over time.

Statistical analysis

Normality and equal variances tests were performed to ensure the appropriate use of parametric tests. Data are presented as mean ± SD and include the data points to show the level of variability. Three-way or two-way ANOVA was performed on this data. Post hoc analysis between specific groups was performed using Bonferroni correction. p values of <0.05 were considered significant.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2022.09.005.

AUTHOR CONTRIBUTIONS

O.B.M.-Z., D.S., and B.R. designed the experiments and wrote the paper; O.B.M.-Z., D.S., and M.G. conducted the experiments; O.B.M.-Z., D.S., M.G., and B.R. approved the final draft of the paper.

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CONFLICTS OF INTEREST

B.R. is a member of the journal’s editorial board. B.R. is a founder, holds shares, and is the chief scientific officer of CellCure Neuroscience, Ltd. The company did not fund the study presented in this manuscript and has no interest in its results.

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