

Case Report

Cultured Bilayered Skin Allograft for Vaginal Construction

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Abstract. *Objective:* This is the first report of live human cultured bilayered skin allograft (taken from another person) (LHCBSA) to line a dissected space to create a vagina.

Case: A 19-year-old with Mayer-Rokitansky-Küster-Hauser syndrome (MRKH syndrome) of vaginal and uterine agenesis had a space dissected and lined with LHCBSA. Although the lining devitalized within 2 weeks, there was a rapid ingrowth of vaginal mucosal cells from the vaginal dimple with an excellent long-term result.

Conclusion: This is the first report that LHCBSA is able to stimulate vaginal mucosal cell growth for a neovagina. It is possible that it might stimulate other surface tissue lining to cover adjacent raw areas such as bladder or esophagus.

Key Words. living cultured bilayered skin allograft—MRKH syndrome

Introduction

Mayer-Rokitansky-Küster-Hauser (MRKH, also called Rokitansky) syndrome is congenital absence of the vagina and uterus. It occurs in about 1 of every 5000 females. In place of a vagina, there is a 2-cm patch of vaginal dimple mucosal cells where the vaginal opening should be. The diagnosis is usually made in late puberty when there is investigation of primary amenorrhea. The etiology is unknown and is assumed to be multifactorial.^{1–3}

If the vaginal dimple is pliable and not tender and if the patient is motivated, the use of vaginal dilators is usually recommended.⁴ If this procedure is not done or if it is ineffective, the most common surgical procedure in the United States is the McIndoe

procedure.^{1–3} A space for the neovagina is dissected and lined with a split-thickness autologous skin graft taken from the patient. A vaginal stent is used for several months after surgery to prevent stricture. The lining remains skin and the long term results are satisfactory.^{1–3}

Case Report

The patient was a 19 year-old with MRKH syndrome. Her family came from a centuries-old, isolated, inbred Jewish community in Uzbekistan. Her maternal aunt's daughter has the same diagnosis.

The patient also had a congenital coloboma, an absent left kidney, moderate facial acne, increased facial hair, and mild alopecia. She suffered from enuresis that she controlled with nasal desmopressin, which she did not reveal until after surgery. Laboratory evaluation showed a chemical hyperandrogenism. Adrenocorticotrophic hormone stimulation disclosed congenital adrenal hyperplasia resulting from partial 21-hydroxylase deficiency. Polycystic ovaries were detected by pelvic ultrasound.

The patient had the general physical and psychological appearance of a normal young woman, but she had never menstruated. The vulva was normal. There was a 1-finger-width dimple where the normal vagina introitus should be. The uterus was not palpable on rectal examination. Multiple attempts to use the Frank technique of dilator pressure invagination were unsuccessful.⁴ The patient refused to have the standard split-thickness skin graft to avoid a donor site scar, even though it would have been taken from the buttocks. After lengthy discussions, she gave informed consent to have a LHCBSA lining even though it had never been done, and even though it might not be successful and another operation might have to be performed. The surgeons were AA and

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HB, who had vast experience using LHCBSA for delayed healing of skin ulcers of the legs.

Live human cultured bilayered skin allograft (Apligraf, Organogenesis, Canton, MA) is supplied as a skin substitute. It is derived from human neonatal male foreskin tissue under aseptic conditions.

The epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum; the dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. Although matrix proteins and cytokines found in human skin are present in Apligraf, Apligraf does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, or hair follicles.

The foreskin donor's mother is tested for human viruses, including antibodies to human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), human T-lymphotropic virus type 1 (HTLV-1), hepatitis C virus (HCV), hepatitis B surface antigen (HbsAg), and syphilis. The fibroblast and keratinocyte cell banks, which are the source of the cells from which Apligraf is derived, are tested for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, karyology, isoenzymes, and tumorigenicity. The final product is tested for morphology, cell viability, epidermal coverage, sterility, mycoplasma, and physical container integrity. Product manufacture also includes reagents derived from animal materials, including bovine pituitary extract. All animal-derived reagents are tested for viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use, and all bovine material is obtained from countries free from bovine spongiform encephalopathy (BSE).

Apligraf is used with standard therapeutic compression for chronic leg ulcers due to venous insufficiency and for dermal diabetic foot ulcers. It is contraindicated in clinically infected wounds and in patients with known allergies to bovine collagen or to the components of the shipping medium. It has to be kept under controlled temperature of 68°F–73°F (20 °C–23 °C). It should be placed on the wound bed within 15 minutes of opening the package. It is supplied as a circular disc 75 mm in diameter and 0.75 mm thick. The shelf life is 10 days.

Preliminary laparoscopy revealed a left müllerian duct, a normal left ovary, and a normal left fallopian tube. On the right side there was a 4 × 2 cm thickening of the Müllerian duct, representing a vestigial hemiuterus. There was a round ligament connecting the vestigial uterus to the ovary, but no fallopian tube. Vaginal dissection followed the laparoscopy. Biopsy of the vaginal dimple showed vaginal squamous mucosal cells with microscopic condylomata. A large space was developed by bilateral incision of the medial edge of the levator muscles. Many

patches of the allograft were sewn together with fine absorbable sutures to cover an inflatable vaginal stent 14 cm in length and 4.5 cm in width. The allograft was inserted into the vaginal space with the dermis in apposition to the raw space surface. Two heavy braided silk retention sutures were placed from side to side of the labium majus to keep the stent immobile. The patient was kept on bed rest for 1 week, after which the stent was replaced with a second stent that was covered with a new allograft layer. The introitus was 2 fingers wide and more than 10 cm deep. A week later, the second stent was replaced with a 12-cm-long, 4-cm-wide, soft vaginal stent. The allograft skin was decomposing while the vaginal mucosa of the vaginal dimple was rapidly growing inward.

Three months later, small vaginal adhesions were lysed. Biopsies from the neovagina vault showed fibrovascular tissue with severe acute and chronic inflammation. There was no evidence of the graft. Nine months postoperatively, cytology revealed normal mucosal cells. The neovagina had re-epithelialized with vaginal squamous cell mucosa, which was pliable and soft. The patient reported her first coitus. The routine use of hard acrylic vaginal stents to prevent strictures had been reduced progressively and discontinued at that time. There was no need to dilate the neovagina.

Seven years after surgery, the patient reported that she continued to have intercourse frequently with orgasm. The vagina and its mucosa were soft, pliable, and easily extended to 9 cm in depth, with a loose 2 finger-width. The patient and her husband requested a referral for a gestational surrogate.

Discussion

There have been many methods of surgical creation of a vagina, including dissection of a space without using a lining and keeping it open with a stent. This procedure has not been popular because of the time required for re-epithelialization before stricture formation. Many publications have reported a lining of oxidized regenerated cellulose, human cadaver dermal collagen matrix lattice, full-thickness skin graft (from the patient), and autologous (from the patient) intestine graft.^{1–3} The Vecchiotti operation to pull in the vaginal dimple (rather than the invagination of Frank dilators⁴) has been popular in Europe and is being done by laparoscopy. The initial apparatus of an abdominal constant tension spring was not approved by the US Food and Drug Administration. The new version has just been approved.¹⁶

This is the first report of the use of LHCBSA (Apligraf, Organogenesis, Canton, MA) to line a neovagina. In our case, the vaginal space was made wider than

usual and an initial wider soft stent was used in anticipation of possible postoperative stricture, which did not occur. In addition, 2 applications of LHCBSA were used instead of the usual 1 application. Such use is “off label,” and if done requires an experienced surgeon.

The advantages were no postoperative stricture, and a capacious, soft, nontender, pliable vagina made of the patient’s own vaginal mucosal cells. This conclusion is based on 1 case done under ideal circumstances.

The consensus is that LHCBSA stimulates the skin surrounding nonhealing leg ulcers by many individual growth factors and cytokines, which it contains and which are associated with wound healing⁵ together with antimicrobial activity and proteolytic enzyme creating a biologically active matrix in the wound.⁶

Originally, it was thought that in vitro culture of an LHCBSA graft lost its antigenicity and would not be rejected by the host. Recent reports agree that the allograft does not survive more than a few weeks.^{7,8,9} This finding suggests that there might be a loss of immediate acute rejection, but late rejection persists and occurs slowly as the adjacent skin grows in to cover chronic leg ulcers.

Another theory is that rather than the loss of acute antigenic rejection, there is a delay of acute rejection because of circulatory isolation of the allograft.⁷ The LHCBSA has no blood vessels, and therefore the circulation cannot bring in leukocytes and circulate antigens. There are no Langerhans cells to carry processed antigen to stimulate an immune rejection. The keratinocytes develop only HLA-DR genes and class II MHC molecules when they are stimulated by interferon γ secreted from infiltrating T-cells. Eventually there is rejection, because class I MHC molecules are expressed in all nucleated cells and present endogenous antigen.^{9,10} Serial in vitro culture of skin results in loss of antigen-presenting cells (Langerhans cells, lymphocytes, macrophages). These cells are needed for activation of allogenic T-cells and rejection. The fibroblasts and keratinocytes of LHCBSA do not express HLA class II and common costimulatory molecules and therefore do not activate unprimed allogenic T-cells. Allogenic cells are eventually silently replaced by host cells.^{7,11}

It is possible that recently living tissue might have healing properties. The Edwin Smith Papyrus (1600 BC) described military acute fractures and flesh wounds of the head and neck and advised “bandage him with fresh meat (cattle) the first day.” A medical commentary by David T. Mininberg, MD was “the raw meat prescribed in the treatment contained enzymes that are useful for cleaning a wound”¹². Fresh human amnion fetal membrane sac has been used to

line the vaginal dissection for MRKH syndrome. It was discontinued for fear of transmission of HIV.

“Off-label” LHCBSA use has been used successfully for acute surgical wounds; general large, high-grade chronic wounds¹³; complicated general and nonsurgical wounds in children with difficult abdominal skin defects¹⁰; leg and sternal acute and complicated surgical wounds; heel ulcers⁶; epidermolysis¹⁴; and gingival wounds.¹⁵

The other unusual features of our case included inbred familial occurrence, and the first report of histologic condylomata of the vaginal dimple, presumably from human papilloma virus during an attempt at coitus.

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