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Vaginoplasty using autologous *in vitro* cultured vaginal tissue in a patient with Mayer–von-Rokitansky–Küster–Hauser syndrome

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Mayer–von-Rokitansky–Küster–Hauser syndrome (MRKHS) is characterized by vaginal agenesis with variable Müllerian duct abnormalities. The Abbè–McIndoe technique is considered a valid treatment option for vaginoplasty but no consensus has been reached on what material should be used for the neovagina canal wall lining. We report the first case of autologous vaginal tissue transplantation in a 28-year-old women with MRKHS. The patient was subjected to a 1 cm^2 full-thickness mucosal biopsy from the vaginal vestibule. Following enzymatic dissociation, cells were inoculated onto collagen IV-coated plates and cultured for 2 weeks. The patient was subjected to a vaginoplasty with a modified Abbè–McIndoe vaginoplasty with 314 cm^2 autologous *in vitro* cultured vaginal tissue for the canal lining. At 1 month from surgery, the vagina appeared normal in length and depth and a vaginal biopsy revealed normal vaginal tissue. The use of autologous *in vitro* cultured vaginal tissue to create a neovagina appears as an easy, minimally invasive and useful method.

Keywords: autologous transplant; Mayer–von-Rokitansky–Küster–Hauser syndrome; vaginoplasty

Introduction

Mayer–Rokitansky–Küster–Hauser syndrome (MRKHS) (Mayer, 1829; von Rokitansky, 1938; Küster, 1910; Hauser and Schreiner, 1961) is characterized by vaginal agenesis with variable Müllerian duct abnormalities and normal secondary sexual characteristics. The incidence is estimated to be 1 in 4000–10 000 live-born girls (Rock and Azziz, 1987). Although the aetiology of this syndrome remains unclear, a multifactorial mode of inheritance appears to be the most likely explanation (Schaltz *et al.*, 2005).

The creation of the vagina can be accomplished over prolonged periods by progressive dilatation with coitus (D'Alberton and Santi, 1972) or using graduated dilators (Frank, 1938); however, most physicians report using surgery. Several surgical techniques have been developed for the creation of the neovagina (Baldwin, 1904; William, 1964; Vecchietti, 1965; Burger *et al.*, 1989, Fedele *et al.*, 1994; Hendren and Atala, 1994), but most authors adopt the Abbè–McIndoe vaginoplasty (Abbè, 1898; McIndoe and Banniser, 1938). The procedure consists in the creation of a canal in the potential neovaginal space that is subsequently covered by a full-thickness skin graft. Several variations regarding the material adopted for the canal lining have been proposed (Davydov and Zhvitiashvili, 1974; Dhall, 1984; Ashworth *et al.*, 1986; Jackson and Rosenblatt, 1994; Templemen and Hertweck, 2000).

We present the first case in which *in vitro* autologous vaginal cell cultures obtained from biopsies from the vaginal vestibule were used for the epithelization of the neovaginal walls.

Case report

A 28-year-old woman was referred to our department for sexual dysfunction and infertility. Gynaecologic history revealed primary amenorrhoea for which she had never sought medical attention. The woman exhibited normal secondary sexual characteristics, hormonal analyses were within normal ranges and her karyotype was 46, XX. Pelvic examination revealed a vaginal agenesis. Ultrasound examination and MRI revealed the agenesis of the uterus and of the left adnexa (atypical MRKHS) (Schmid-Tannwald and Hauser, 1977). After appropriate counselling regarding the methods, as well as possible complications, a modified Abbè–McIndoe operation with autologous vaginal tissue transplant was decided upon. A 1 cm^2 full-thickness mucosal biopsy from the vaginal vestibule was carried out in the operative theatre (Fig. 1). The fresh biopsy was immediately transported to the

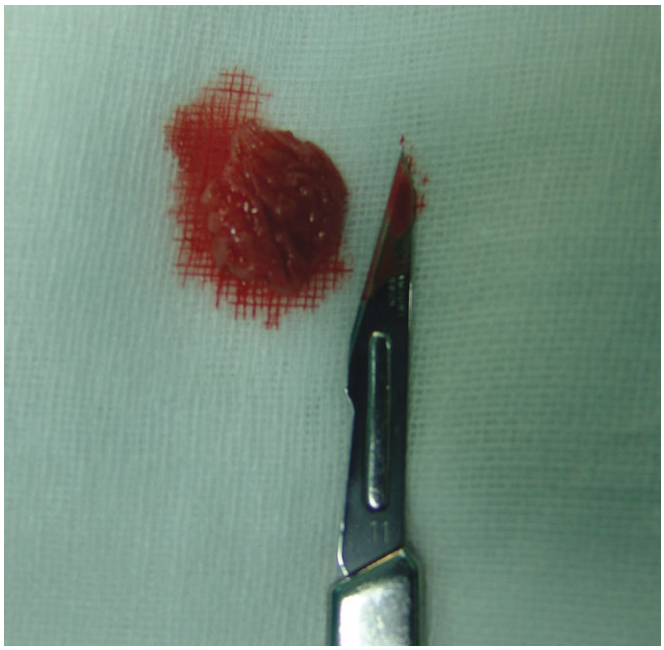

Figure 1: A 1 cm^2 full-thickness mucosal biopsy obtained from the vaginal vestibule

Department of Experimental Medicine of our Institution for histological analysis and cell extraction for tissue culture.

Following enzymatic dissociation, the keratinocyte suspension was inoculated onto four collagen IV (10 μg/ml)-coated culture plates. Cells were seeded at cell density of 2.5×10^5 cells/plate and maintained in chemical defined medium MCDB 153 (EpiLife, Cascade Biologics, Inc., Portland, OR, USA) with medium change twice a week. After a week of culture, cells reached 70–80% of confluency (Fig. 2a and b inset) being cultured for additional 8 days in order to obtain the fully differentiated mucosal tissue (Fig. 2c and d inset). Autologous reconstructed vaginal tissues reached 314 cm^2 when they were harvested from the culture plates by incubation with dispase II (2.5 mg/ml) (Fig. 2e), washed in PBS and mounted on 2 mg/10 cm^2 hyaluronic acid embedded gauze to maintain the orientation of the mucosal tissue and transferred to the operative theatre (Fig. 2f and g).

Before surgery, the patient was treated with antibiotics prophylaxis and a bowel enema. In the operative room, the patient was placed in a lithotomic position with a Foley catheter. A midline incision at the vaginal introitus was made, and a 10 cm canal was made between the bladder and the rectum using a blunt digital and scissor dissection reaching the pouch of Douglas (Fig. 3a). Complete haemostasis was obtained with electrocautery. The tunnel was covered with the gauze with the cell stratum facing the canal walls (Fig. 3b). A 2 cm in diameter and 12 cm in length vaginal mould covered by a condom was placed in the neovagina. The mould was fixed to the perineum with stitches bilaterally. No stitches were used to keep the gauze in place. Operation time was 18 min. Estimated blood loss was <100 ml. The gauze, the Foley catheter and the mould were kept in place

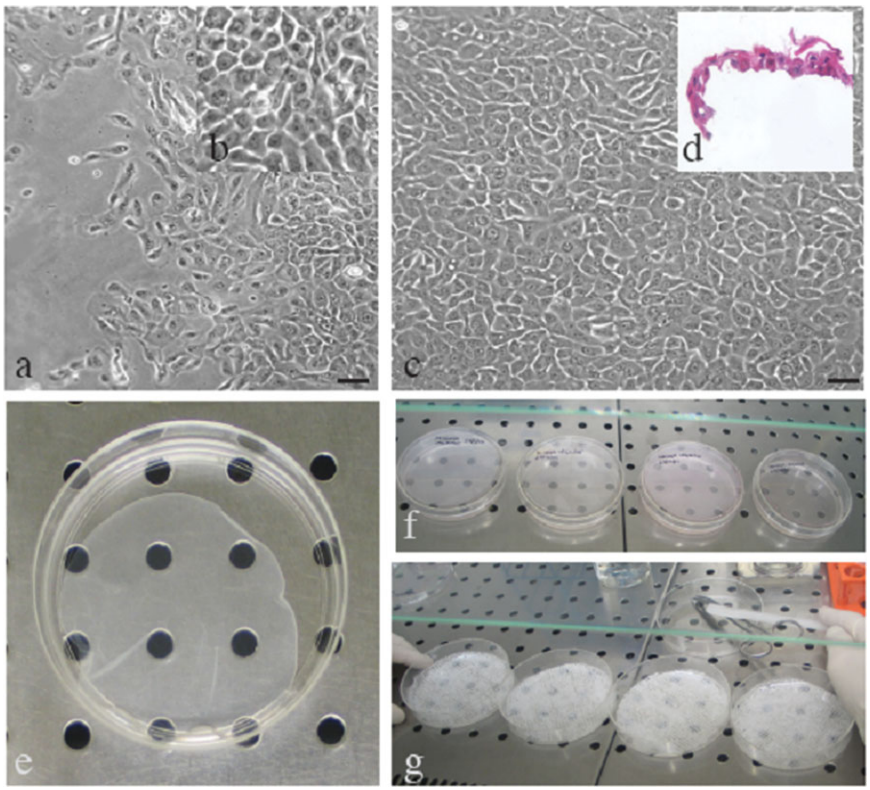

Figure 2: Vaginal cell extraction and in vitro tissue culture (**a**, **b**) 70–80% of confluence of autologous keratinocyte cultures obtained from vaginal biopsy (Bar 10 μm); (**c**, **d**) post-confluence epithelial cell cultures after 15 days, haematoxylin stained frozen section of *in vitro* reconstructed vaginal tissue (Bar 10 μm); (**e**, **f**) detached tissues after Dispase II treatment; (**g**) application of hyaluronic acid embedded gauze

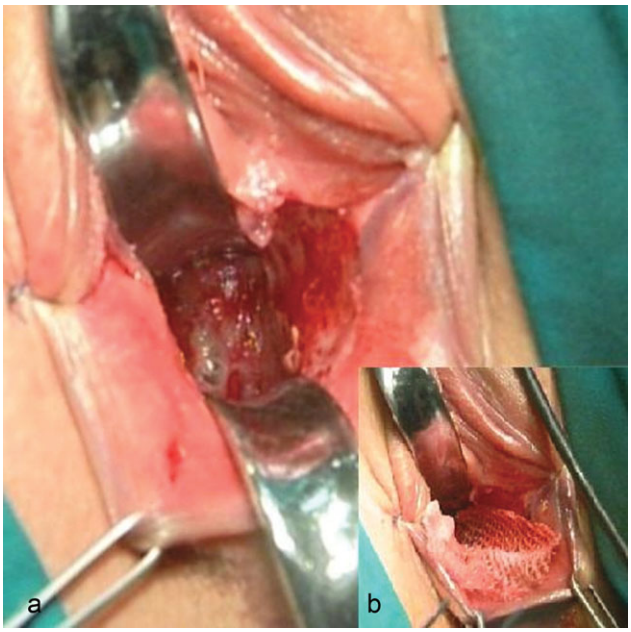

Figure 3: Modified Abbe–McIndoe vaginoplasty (**a**) A canal reaching the pouch of Douglas was made between the bladder and the rectum using blunt digital and scissor dissection. (**b**) The tunnel was covered with the gauze with the cell stratum facing the canal walls

for 5 post-operative days. At this point the estimate percentage of take was over 90%. The patient was instructed to wear at night the vaginal mould for the successive 6 weeks. Colposcopy and a vaginal biopsy confirmed normal epithelium (Fig. 4). At four months of follow-up, no stricture, no foul-smelling vaginal discharge or sensation of vaginal dryness was reported and sexual intercourse was referred by both partners as satisfactory.

Conclusions

This is the first case of *in vitro* cultured autologous vaginal tissue transplantation. The ideal treatment of the MRKHS remains a matter of debate. Although non-surgical techniques for the creation of a neovagina (D'Alberton and Santi 1972, Frank, 1938; Edmonds, 2001; Roberts *et al.*, 2001) have been reported, and in motivated patients represent the gold standard treatment, the duration of treatment which varies from 2 months to up to 2 years have generally led physicians to prefer surgery.

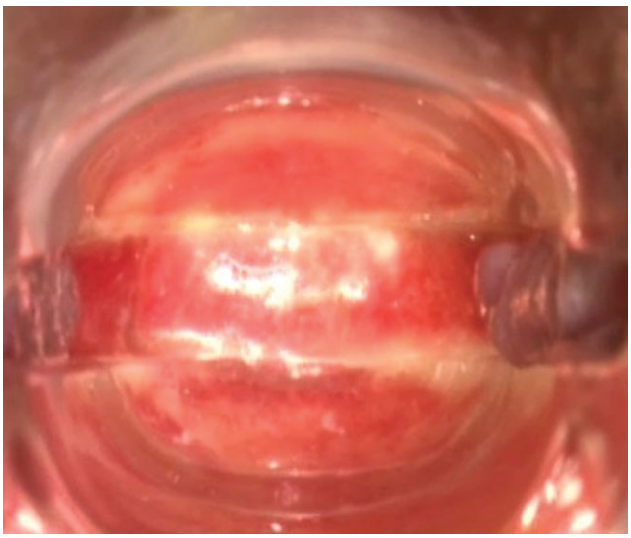

Figure 4: Colposcopy showing normal vaginal epithelium

A valid alternative for the creation of the neovagina is the Abbè–McIndoe vaginoplasty. This consists in the creation of a vaginal canal created by dissecting the potential neovaginal space, which is then subsequently covered by a skin graft (Abbè, 1898; McIndoe and Banniser, 1938). The main disadvantages connected with this procedure are the surgical complexity and the scar that remains in the donor site.

Several modifications of this technique, especially regarding the material adopted for the canal lining, have been proposed. These include the use of the peritoneum (Rotman, 1972; Davydov and Zhvitiashvili, 1974), the amnion (Dhall, 1984; Templeman *et al.*, 2000), allogenic epidermal sheets (Carranza-Lira *et al.*, 1999), Interceed (Jackson and Rosenblatt, 1994) and autologous buccal mucosa (Lin *et al.*, 2003). The use of the peritoneum has the disadvantage of being relatively complex and requires the opening of the peritoneal cavity. In addition, the shifting of the labia minor in the vagina has been reported (Karim *et al.*, 1995). The use of allogenic tissue such as the amnion and allogenic epidermal sheets carries the inherited disadvantage of allograft rejection and the risk of transmission of infective disease. The use of acellular absorbable sheets has recently been attempted in limited series (Jackson and Rosenblatt, 1994, Motoyama *et al.*, 2003, Noguchi *et al.*, 2004) with high success rates. In these cases epithelization occurs from the vaginal vestibule and usually requires several months.

A modified Abbè–McIndoe technique that appears promising is the use of autologous meshed buccal mucosa (Lin *et al.*, 2003). Disadvantages connected with this technique are the morbidity connected with the removal of the mucosa from the donor site and the relatively long time necessary to achieve a functional vagina. This time period is probably necessary for the meshed mucosa to colonize the entire neovaginal wall.

In the present case, 314 cm^2 of *in vitro* cultured vaginal tissue was used for the epithelization of the canal wall. The decision to adopt the vaginal vestibule as donor site was carried out in order to minimize patient's discomfort and avoid permanent scars. Furthermore, it appeared sensible to carry out an orthotopic transplant as compared with a heterotopic one. Autologous tissue does not carry the risk of infection or allogenic tissue rejection. In addition, in this case, the mould was applied for a reduced time period. We hypothesize that the short time necessary to achieve a normal epithelium was due to the possibility of having enough tissue to cover the entire canal surface. In fact, most techniques, which adopt autologous tissue grafts, adopt mesh of tissue to different ratios before being transplanted. The vaginal mould was adopted in order to maintain in place and to increase the pressure on the gauzes. After removing the mould and gauze, epithelization of the entire canal could be seen throughout the entire

neovaginal wall and this suggests that epithelization occurred from the transplanted tissue and not as with other techniques from the vaginal vestibule. Before an attempt was carried out to grow *in vitro* vaginal tissue in mice (Iguchi *et al.*, 1983), as this was the first case reported in the literature in human, we preferred to maintain a foreign body at night and avoid sexual intercourse for a relatively long time period in order to prevent the formation of a vaginal septum and/or strictures.

This case suggests that autologous *in vitro* cultured vaginal tissue and successive transplant is feasible and safe. The excellent results obtained by this patient warrant further investigation. *In vitro* grown vaginal tissue will also serve for other conditions such as vaginal trauma squeal or vaginoplasty in patients' subjected to pelvic exenteration.

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