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ORIGINAL ARTICLE

Use of Urinary Bladder Matrix, a Bioactive, Acellular Scaffold, in Transplant Donor Scars and Androgenetic Alopecia: Initial Clinical Experience

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Introduction: Emerging regenerative medicine technologies have yet to be utilized in hair restoration surgery. Urinary bladder extracellular matrix (UBM) has shown promise in a wide variety of applications, inducing site-specific remodeling of injured tissue. This case series describes one clinician's experience in the first use of this regenerative material in hair restoration surgery.

Materials and Methods: Twenty subjects underwent one of several protocols: (1) Treatment of 3-mm biopsies within donor site scars with UBM versus untreated controls, (2) Removal of a strip of donor site scar tissue and treatment of the site with UBM, (3) Soaking of occipital hair follicle grafts in a solution of UBM, or (4) Soaking of beard or temple hair follicle grafts.

Results: At 6 months, hair was observed in biopsy sites that contained UBM, including donor scar areas that would not normally regrow hair, in contrast to the control biopsy sites. Similar results were observed for the strip excisions. UBM-soaked occipital, beard, and temple hair grafts consistently yielded higher than expected numbers of hair follicles.

Conclusions: These cases provide anecdotal support for the hypothesized benefits of UBM regenerative technology in restoration therapy for men and women with androgenetic hair loss.

Androgenetic alopecia is the most common cause of hair loss affecting 50% of men and 20% to 53% of women by 50 years of age.¹ This patterned form of hair loss is caused by genetic and hormonal factors and occurs in a highly predictable location in men²⁻⁴ and is more diffusely and less patterned in women.⁵ Hair transplantation is effective in replacing terminal hair follicles in hair loss areas. While this

procedure is generally effective, the challenge for patients with severe hair loss is depletion of donor follicles before the area of hair loss is fully covered. Scar formation at the donor site can also occur. Therefore, the ability to remove hair from one area and transplant into another, while allowing the donor hair to grow back would be highly useful, as would the ability to reduce the incidence of donor scars, which are typically refractory to surgical excision. While researchers continue to make progress in this area, no treatment to date has emerged to (1) eliminate donor scars completely or (2) regenerate hair follicles in both the donor site or on the bald scalp.

The field of regenerative medicine seeks to restore normal structure and function to damaged tissues.⁶ Investigators are working to bioengineer the hair follicle.⁷ In contrast to many drug therapies, in situ regenerative therapy, such as extracellular matrix (ECM) as a bioscaffold for tissue reconstruction, hypothetically does not rely on a single target receptor or pathway for its action. Minimally processed ECM isolates contain a diversity of structural proteins and associated bioactive molecules, including growth factors that may act as potent modulators of cell behavior.⁸ Other components of ECM include collagens, proteoglycans, and glycoproteins.⁹ While all tissues have an underlying ECM, only those from the urinary bladder, skin, the submucosa of the small intestine, and the pericardium have been used clinically.^{8,9} Urinary bladder ECM (UBM) is the only organ that has an intact epithelial basement membrane, the anatomic site for epithelial cell development and residence. The porcine UBM product used in this study was prepared using methods that preserve the basement membrane structure.¹⁰ The degradation of naturally occurring ECM has also been associated with release of growth factors and peptides, many of which have

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been shown to maintain bioactivity.^{11,12} The growth factors contained in UBM include vascular endothelial growth factor, basic fibroblast growth factor, epidermal growth factor, transforming growth factors alpha and beta 1, keratinocyte growth factor, bone morphogenic protein, insulin-like growth factor, hepatic growth factor, and platelet-derived growth factor.^{8,9,13} These growth factors have been demonstrated to maintain bioactivity following UBM preparation and e-beam sterilization.^{13,14} Characterization studies have shown that UBM is primarily composed of collagen I, II, III, IV, and VII and contains glycosaminoglycans, fibronectin, laminin, and elastin.^{10,14}

ECM scaffolds have been described as degradable reservoirs of naturally occurring growth factors.⁶ Porcine-derived urinary bladder extracellular matrix (MatriStem, Acell Inc, Columbia, Md) is an FDA-approved, resorbable bioscaffold material that has been successfully used for the repair of musculotendinous structures, lower urinary tract reconstruction, dura mater replacement, repair of full and partial thickness skin wounds, and vascular and esophageal reconstruction.⁶ Because the scaffold is acellular, UBM has been hypothesized to not contain major antigenic stimuli that could otherwise cause an adverse immune response.¹⁵ Two hundred thousand patients have been implanted with a xenogeneic UBM scaffold for musculoskeletal and other conditions with no cases of xenogeneic pathogen transmission.⁹ It has also been used in the veterinary field for equine and canine musculoskeletal conditions.^{6,16}

To the author's knowledge, regenerative medicine technology has not been utilized for surgical hair replacement. The objective of this study was to

evaluate the effectiveness of UBM in facilitating hair growth in subjects with surgically refractory donor scars, and to evaluate the effect of UBM on hair numbers in transplanted grafts when transplanted with plucked donor hair.

Materials and Methods

The overall case study involved 18 male and 2 female subjects. They were divided into different groups as listed in the upcoming text. Lower occipital donor sites existed for all male subjects in case study 1 and 2. All UBM material for injection was manufactured and supplied by ACell Inc and marketed under the trade name MatriStem. Prior to packaging, the UBM was milled into a fine particulate, yielding particles ranging from 50–250 μm .¹⁷ The particulate UBM was then terminally sterilized by e-beam irradiation.

All patients underwent a detailed consultation and signed an informed consent release. Patients were treated as described in the following studies. Following procedures, patients were instructed to refrain from washing their hair for at least 48 hours. Sites were examined at 10 days to assess healing and infection. Patients were reexamined for hair growth at 6 months. Results were documented by digital photography (12 Mps).

Case Study 1: Effects of UBM on Punch Biopsy Wound Healing

The purpose of this case study was to determine if UBM facilitated hair growth in the male donor scar areas previously diagnosed as refractory to surgical removal. In 4 male subjects, six 3-mm punch graft



Figure 1. Patient with donor scar area with blue marks indicating sites of punch biopsies to be taken.

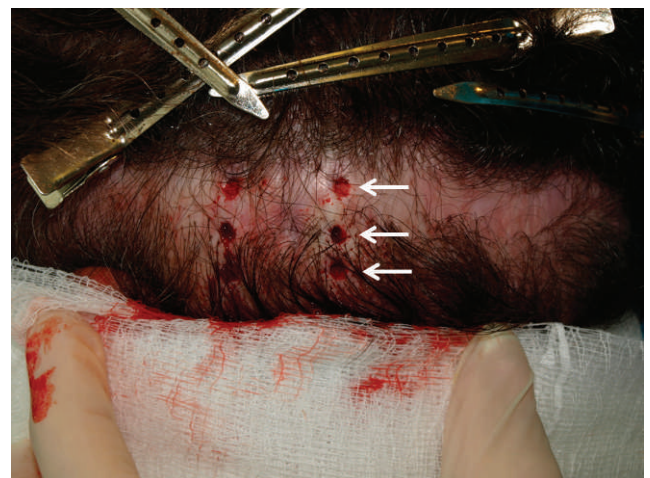


Figure 2. Day 1. Three punch biopsy sites were created: 1 in the donor scar area, 1 in an area with donor scar and hair, and 1 within the hair. The vertical 3 on the left are controls and the 3 on the right were packed with urinary bladder extracellular matrix.

samples were biopsied from the old donor scar region. Two rows of 3 sites were made in each side of the scalp (Figure 1). The 3 sites included (1) a punch completely in the scar area, (2) a punch in which the inferior half of punch touched and included donor hair, and (3) a punch completely within virgin hair bearing donor area. One row of 3 sites was used as a control with no UBM. The second row/column of 3 sites functioned as the test sites for UBM.

Hemostasis was achieved prior to administration of the UBM. In the first row, approximately 5 mg of the powdered formulation of UBM was packed into each of the punch graft sites until the wound was filled. In the second row of punch biopsy sites, control wounds were not treated with UBM. All sites were then covered and sealed with Aquaphor.

Case Study 2: Effects of UBM on Donor Site Scar Healing

A second case series was performed to determine if donor site scars previously diagnosed as refractory to excisional removal could be reduced if UBM was added to the wound just prior to loose closure. Four male subjects (50 to 55 years of age) with donor sites determined to be refractory to improvement with excisional repair were used in this study. The donor site scar was cleaned and a sterile surgical field prepared. A strip of donor scar tissue 18 cm long \times 2.5 cm wide was removed by surgical excision with a scalpel. Once bleeding was controlled with infrared coagulation, the wound was partially closed with 2-0 Vicryl using subcutaneous sutures pulled loosely to create approximately 5 to 7 small football-shaped pockets. Before full closure, 50 mg of sterile UBM powder was



Figure 3. ACell in keloid scarred donor area. Prior resections only formed more keloids (top left). Keloids punched out and ACell applied into the left 4 punch sites (top right). Follow-up 5 months later (bottom left). Note: Only the left side of keloid donor area was treated. (Note absence of recurrent keloid and regrowth of hair in previously scarred area.)



Figure 4. Patient with significant donor scar before excision and application of urinary bladder extracellular matrix.

aliquoted into the pockets. The wound edges were then closed using 2-0 nylon suture (Ethicon).

Case Study 3: Effect of UBM on Graft Hair Proliferation

The purpose of this case study was to determine the effect of soaking hair follicle grafts in UBM solution. Four men (54 to 65 years of age) with androgenetic balding were enrolled. Two women (ages 50 and 55 years) with thinning hair in their temple or scalp regions were enrolled. Six donor hair strips (9 cm long \times 2.5 cm wide) were surgically excised from the lower occipital region of each male subject's scalp. One donor strip (approximately 10 cm \times 8 mm) was cut from each female subject's scalp. Donor strips were

soaked in a UBM solution (60 mg UBM/mL 0.9% sterile injectable saline) for 20 minutes. Under sterile conditions, the strips were removed from solution and cut into individual donor grafts (2.5 mm long \times 0.3 mm wide). The hair population of each graft was counted under microscopy (\times 10) using a trichoscope with an average 4 hairs/graft. Grafts were then placed in recipient sites, which included the hairline and adjacent bald area.

Case Study 4: Effects of UBM on Plucked Beard Hair Transplantation

The purpose of this case study was to determine the effects of UBM on plucked beard or temple hair



Figure 5. Same patient 6 months after donor site excision with application of urinary bladder extracellular matrix. Note robust hair growth in donor scar area.



Figure 6. Female patient with temple hair loss before transplantation (left). One hundred twenty-seven miniaturizing hairs were counted prior to transplantation. One month (middle) and 6 months (right) after transplantation with hair surgically excised from the lower occipital region of the scalp, soaked in urinary bladder extracellular matrix (UBM) and then cut into individual grafts before placement. One hundred fifty single UBM-soaked terminal grafts grew 550 terminal hairs. Miniaturizing hairs were not included in this count.

growth when placed into either donor scar area or area of baldness. Six male subjects (38 to 56 years of age) were enrolled in this study. Two had male pattern baldness and 4 presented with donor site scars. Donor hair was plucked from both their beards and temple and soaked in UBM solution (60 mg UBM/mL 0.9% sterile injectable saline) for 20 minutes prior to placement in the recipient sites.

Results

Case Study 1: Effects of UBM on Punch Biopsy Wound Healing

The result in case 1 would determine whether UBM would facilitate hair growth in the donor scar area and whether the presence of hair was required. Six months following the biopsy wound preparation, hair regrew in control sites that fully or partially resided in haired areas. However, no hair growth was observed in the control biopsy sites that resided completely within the scar region. Hair regrew in all sites that contained UBM as long as hair was included in the punch biopsy site based on the punch site location (Figures 1 through 3). No hair was included (punch was in area of complete scar) nor grew in the exclusively scarred region. These results provide anecdotal support for the hypothesis that UCM facilitates hair growth in donor scar areas, which normally would not regrow hair.

Case Study 2: UBM Facilitates Hair Growth in Donor Site

We next tested the hypothesis that UBM would facilitate hair growth in a donor scar area that was previously refractory to surgical excision or any other treatment. Anecdotally, the scars appeared smaller than the original. In addition, we wish to report that multiple prior attempts at reducing these scars by both

surgical reduction or by prior transplantation into these scars resulted in disappointing results. Instead, within 6 months of excisional removal of one patient's donor scar region with UBM treatment, the patient had robust hair growth (Figures 4 and 5).

Case Study 3: UBM Soaked Hair Grafts Grow More Hair

In patients with thinning of temple or scalp hair, 150 single hair UBM-soaked grafts transplanted from the occipital region yielded 550 terminal hairs 4 to 6 months after transplantation (Figure 6), with similar hair growth in patients with hair transplanted to the scalp region (Figures 7 and 8). These data suggest that UBM facilitates follicular development with hair duplication in these patients. Previous analyses have demonstrated that in any transplant procedure there may be up to 15% dormant hairs included with grafts.¹⁸ If this had been fully the case here, then we would have reasonably expected approximately 195 terminal hairs where 150 hairs were transplanted. The fact that 550 terminal hairs were counted (approximately a 375% increase in the number of hairs transplanted) indicates, in all likelihood, that a duplication process of hairs rather than dormant hair growth is occurring.

Case Study 4: UBM Facilitates Growth of Plucked Beard Hair Transplanted to Donor Scar Region

All male patients grew robust hair in their donor scar region when transplanted with 55 plucked beard or temple hairs that were soaked in UBM solution (Figures 9 and 10), which yielded more than 150 growing hairs in the scar region by count. Counts were easy as there had been no hair in the scar region previously. These data further suggest that UBM facilitates follicular development, hair duplication, and hair growth in recipient scarred regions of the scalp.

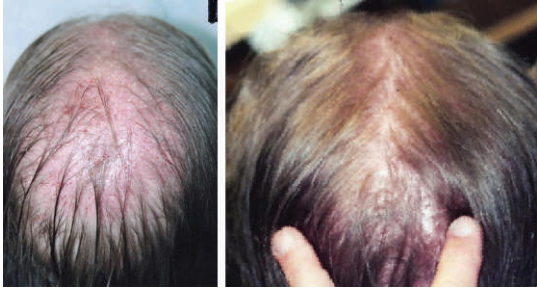


Figure 7. Female patient before (left) and 6 months after transplantation with urinary bladder extracellular matrix-soaked grafts from the donor region (right).

Discussion

The case series provide anecdotal support for the hypothesized benefits of UBM regenerative technology in hair restoration. It appears that UBM facilitates hair regrowth in donor scar areas when (1) other active hair follicles are present in the area (case study 1 and 2), and (2) when plucked beard hair is used as donation material (case study 4). The scalp heals uniquely by biologic creep, or the formation of new skin but without hair follicles, which can lead to donor scar formation.¹⁹

The physiology of follicular development is helpful in formulating a hypothetical mechanism of action for these effects of UBM on hair regrowth in the donor scar area. Two follicular progenitor cell types are necessary and sufficient for follicle formation: the bulge epithelial cells (located in the follicular bulge region) and the papilla mesenchymal cells (located in the

bottom of the follicle).²⁰ Both cell populations have been demonstrated to have stem cell properties.^{20,21} If these trichogenic cells are dissociated and injected into immune-deficient mouse skin, new follicles will form rapidly.²² This may explain why transplanting plucked beard hair is successful in growing hair in the transplantation site.²³ By plucking beard hair, both cell types remain in the donor area and are also carried on the plucked hair to its transplantation site. Once transplanted, the cells associated with the plucked hair grow a new follicle. These clinical data suggest that “injecting” dissociated follicular stem cells in man in the form of a beard hair with attached cells, when mediated by the potential function of the UBM scaffold, also results in the folliculogenesis similar to that which occurs in mice.

Three of the case studies support a similar idea, that when these trichogenic, or progenitor, cells are present, along with UBM, new follicles form. In case study 1, hair grew in the donor scar area only when UBM was present or when other hair was present in the vicinity. In case study 2, hair grew in the donor scar area when UBM was used during the skin closure of the surgical excision with hair in the adjacent edges. In case study 4, hair grew in the donor scar area when implanted with plucked beard hair that was soaked in UBM. These 3 case studies have in common that the described stem cells would be present in the area in which UBM was used, leading to hair regrowth. Although the molecular mechanisms of folliculogenesis in these

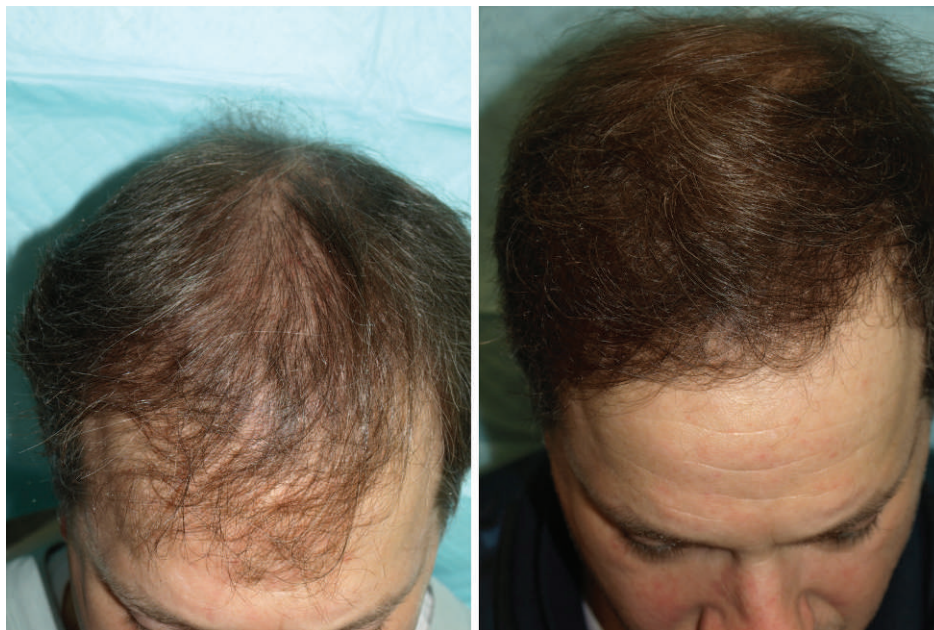


Figure 8. Male patient before (left) and 4 months after (right) transplantation with urinary bladder extracellular matrix-soaked grafts from donor region. (Patient came in early because he was surprised at the amount of growth this early.)



Figure 9. Plucked beard hair with urinary bladder extracellular matrix (UBM). Donor scar area with incisions for transplantation of UBM-soaked donor hair plucked from the patient's beard (left). Placement of transplant hairs plucked from the beard and then soaked in UBM (middle). Final placement of transplanted hairs (right).

cases is not fully understood, one hypothesis could be that UBM at the transplant site recruits these endogenous follicular stem cells from the adjacent area to form follicles. And, in the case where trichogenic cells are present on the plucked hair, UBM may participate in or modulate signaling pathways that trigger the cells to form follicles. UBM recruitment of circulating stem cells, previously demonstrated,²⁴ and interaction with local environmental cues may also play a role in folliculogenesis.

Case study 3 data suggest that UBM also stimulated more than one hair follicle to develop from a single transplanted hair. In this study, women and men were transplanted with single donor hairs from the occipital region that were soaked in UBM prior to transplantation. In each case, an average of approximately 150 terminal hairs were transplanted, yet 6 months later approximately 550 terminal hairs were growing in the transplanted area, suggesting that UBM stimulated

more follicles to grow than merely the one that was transplanted. This is not surprising if UBM is functioning to recruit and signal local and circulating stem cells that may then initiate folliculogenesis.

This series of case studies is the first report of UBM, a regenerative technology, facilitating hair growth in both donor scar areas and in areas of hair loss in both men and women. Although more scientifically rigorous, appropriately powered studies are warranted based on this initial pilot study, these data clearly suggest that UBM technology is a useful tool in restoration therapy in men and women with androgenetic hair loss.

Conclusion

Hair transplantation procedures continue to be sought by those with androgenetic hair loss. Loss of the patient's donor area, before complete coverage of hair loss area, and donor scar formation remain problematic. In this case series, UBM facilitated hair regrowth in the donor scar area and increased follicle numbers in the transplant site from one transplanted hair. Although more studies are necessary to further understand the role of UBM in folliculogenesis, it appears that UBM may facilitate hair regrowth in androgenetic hair loss.

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Figure 10. Robust growth in donor scar region 6 months after transplantation with plucked beard hair soaked in urinary bladder extracellular matrix solution.

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