Transdifferentiation of autologous bone marrow cells on a collagen-poly(ε-caprolactone) scaffold for tissue engineering in complete lack of native urothelium

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Urological reconstructive surgery is sometimes hampered by a lack of tissue. In some cases, autologous urothelial cells (UCs) are not available for cell expansion and ordinary tissue engineering. In these cases, we wanted to explore whether autologous mesenchymal stem cells (MSCs) from bone marrow could be used to create urological transplants. MSCs from human bone marrow were cultured in vitro with medium conditioned by normal human UCs or by indirect co-culturing in culture well inserts. Changes in gene expression, protein expression and cell morphology were studied after two weeks using western blot, RT-PCR and immune staining. Cells cultured in standard epithelial growth medium served as controls. Bone marrow MSCs changed their phenotype with respect to growth characteristics and cell morphology, as well as gene and protein expression, to a UC lineage in both culture methods, but not in controls. Urothelial differentiation was also accomplished in human bone marrow MSCs seeded on a three-dimensional poly(ε-caprolactone) (PCL)—collagen construct. Human MSCs could easily be harvested by bone marrow aspiration and expanded and differentiated into urothelium. Differentiation could take place on a three-dimensional hybrid PCL-reinforced collagen-based scaffold for creation of a tissue-engineered autologous transplant for urological reconstructive surgery.

1. Introduction

Autologous cells expanded ex vivo provide a possible way to restore body tissue and body function after damage. The primary advantage of using autologous tissues is the lack of immunogenic properties. Tissue-engineered transplants can therefore be designed to meet the needs of the patient without the risk of rejection. We have previously harvested normal human urothelial cells (UCs) for tissue engineering purposes by bladder washings [1]. The method has the advantage of being non-invasive, safe and reliable in patients with normal urothelium and urinary bladders. After harvesting and cell expansion, the cells were used to create autologous transplants for reconstruction of the penile urethra in patients with severe hypospadias [2,3]. So far, the algorithm for cell harvesting, in vitro handling and tissue transplantation has been established primarily for the treatment of patients with severe malformations [4]. For patients requiring reconstructive surgery of the urinary tract for restoration of function after treatment for urothelial malignancy, cell harvesting from bladder washings might be disadvantageous primarily because of an increased risk of developing new malignancies in previously
susceptible autologous urothelial tissue and, secondly, because of a lack of tissue after treatment with mutilating surgery and/or treatment with radiotherapy. A lack of tissue can also hamper cell harvesting in patients in need of reconstruction after severe traumatic injuries. In order to develop the clinical application of tissue engineering in these cases, we wished to explore the possibility of using autologous mesenchymal stem cells (MSCs) for in vitro expansion and differentiation into UCs.

Bone-marrow-derived MSCs are multipotent cells that are readily isolated and culture-expanded and can be induced to differentiate into multiple lineages as osteoblasts, chondrocytes and adipocytes [5–9]. MSCs have a low immunogenic profile and are typically not rejected after transplantation. They are regarded as safe for transplantation when grown under normal culture conditions, and there are no reports of malignant transformation or ectopic tissue generation [10–12]. The first MSC transplantation was performed over 15 years ago, and to date hundreds of patients have been treated without adverse events [13,14].

MSCs are known to interact in tissue regeneration and repair [15,16]. It has been agreed that surgery-induced inflammation and release of local factors at the site of surgery can trigger MSC mobilization and recruitment for repair. In the reports by Machiarini and co-workers, a tissue-engineered trachea was pre-seeded with autologous bone marrow mononuclear cells 36 h prior to transplantation, and the transplant was re-seeded with additional autologous bone marrow that was harvested immediately before implantation. Additionally, blood activation factors (C3a and thrombin) were added to further improve progenitor cell activation, recruitment, tissue repair and local immune suppression at the time of transplantation [17,18]. These data strongly indicate the role of MSCs in tissue formation and repair.

MSCs and haematopoietic stem cells can be isolated by bone marrow aspiration in subjects of all ages. Today, blood sampling of venous peripheral blood after growth factor stimulation is a standard procedure for harvesting haematopoietic stem cells [19–21]. Contrary, it has been difficult hitherto to isolate MSCs from peripheral blood, even after stimulation with growth factors. In the future however, the whole process could be minimally invasive from UC harvests, as well as for MSCs [22,23].

Although not extensively studied, preliminary data on bone-marrow-derived MSCs for urogenital purposes are promising [24,25]. During organogenesis, cross-talk between cells plays a key role in the developing embryo. During fetal development, the normal kidney develops from the interaction of mesenchymal cells of the metanephros, derived from the mesoderm, and cells of the endodermal lineage of the ureteric bud [26,27]. It is unknown whether cells stay allegiance their original germ cell lineage in this developmental stage. We therefore wanted to explore whether human stem cells of mesenchymal origin could be stimulated to differentiate into UCs by acting upon their microenvironment.

Advantages of co-culturing methods ex vivo include (i) the possibility of using autologous stem cells that are easy to harvest, (ii) the patient is never directly exposed to the UCs in the co-culture, and these cells can therefore be allogenic, and (iii) by avoiding the necessity of using autologous cells for conditioning the culture media, a stock of allogenic UCs can easily be kept in a freezer for use upon request.

A tissue scaffold with mechanical properties that allow surgical handling of the transplant is required in order to transplant in vitro cultured cells to the patient in a clinical setting. Collagens are structural proteins and part of the extracellular matrix in all species. Collagen-coated substrates provide cell proliferation properties, and collagen gels and sponges are used extensively as filling material in tissue defects. Pure collagen has however a limited mechanical strength. Therefore, by plastic compression and extracting water from a collagen gel, a robust, flat collagen sheet is formed that can be handled surgically [28,29]. Throughout the plastic compression that reduces the volume (more than 100-fold) in the hyperhydrated gels, the cells remain viable in the robust cell-containing collagen sheets [28]. Poly(e-caprolactone) (PCL) was selected for its high compliance compared with, for example, poly-l-lactic acid and slower rate of degradation than poly(glycolic acid) and lactic acid copolymers such a poly-o,l-lactic acid [30]. In addition, PCL nanofibres are synthetic, biodegradable and compliant and can be incorporated in the middle of the collagen sheet to meet the mechanical requirements for bladder tissue engineering [31]. This incorporation further strengthens the sheet for transplantation and surgical handling. During remodelling and incorporation inside the body, properties of elasticity will be conveyed before degradation.

As a result of advances in the fields of molecular biology and material sciences, the concept of cell guidance has gained increased attention for tissue regeneration purposes. By these means, an increased interest and knowledge in cell-to-material interaction has come to light. Biomaterials not only need to be biocompatible in respect of not being toxic or cause a foreign body reaction, but should also support important features such as cell adhesion, proliferation and differentiation. Today, significant efforts have been made in the design of advanced materials and multifunctional PCL-based scaffolds. For instance, as described by Gloria and co-workers, cell adhesion can be stimulated by modulating the surface by adding short peptides to a three-dimensional PCL scaffold or by adding iron nanoparticles into a PCL matrix [32,33]. In this study, PCL was mainly used for its mechanical properties, whereas adhesion of cells was accomplished on the top collagen layer.

To explore the feasibility of developing tissue engineering of bladder transplants also for patients without tissue resources for autologous UC harvesting, we differentiated harvested bone marrow MSCs into a UC lineage in vitro by applying different co-culturing conditions with human UCs. Differentiated cells were characterized by RT-PCR, western blot and immunohistochemistry. Differentiation was successful on plastic-compressed collagen–PCL constructs, thereby simplifying the process from autologous bone marrow harvesting to a tissue engineered construct for transplantation in a clinical setting.

### 2. Material and methods

#### 2.1. Ethics

Bone marrow was harvested after approval by the Regional Ethics Committee in Stockholm, and informed consent was obtained from all donors. MSCs were isolated from bone marrow aspirates from the iliac crest of healthy volunteer adult donors (age 37–40). The Hospital Ethics Committee approved cell harvesting of UCs and informed consent was given by the parents. UCs from three different healthy subjects (ages 1–3 years) undergoing...
catheterization for hypospadias repair or before neoimplantation owing to vesicoureteral reflux were used in this study.

2.2. Isolation and characterization of human mesenchymal stem cells

MSCs were isolated as previously described [34]. Briefly, the bone marrow was diluted in phosphate-buffered saline (PBS) to a final density of $1 \times 10^7$ cells ml$^{-1}$, and mononuclear cells were collected by density gradient centrifugation on Percoll (Amersham, Biosciences, Uppsala, Sweden) and thereafter suspended in Dulbecco’s modified Eagle’s medium–low glucose (DMEM–LG; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Sigma, St Louis, MO, USA) and 1/100 antibiotic–antimycotic solution (Invitrogen, Paisley, UK). Cells were plated at $1.6 \times 10^5$ cells cm$^{-2}$ in culture flasks and maintained at 37°C in a humidified environment containing 5% CO$_2$. After 3 days, the non-adherent cells were discarded, and the medium was changed every 3–4 days thereafter. At 70% confluence, the cells were detached with 0.05% trypsin and 0.53 mM EDTA (Invitrogen) and re-plated at a density of $4 \times 10^3$ cells cm$^{-2}$ in culture flasks. Cells in passage 3–5 were used in the experiments. Osteogenic, chondrogenic and adipogenic differentiation was ascertained as well as surface expression of CD14, CD29, CD31, CD34, CD45, CD73, CD80, CD90, CD105, CD166 and HLA class I and class II antigens in transdifferentiated MSCs as previously described [34].

2.3. Harvesting and isolation of human urothelial cells

We used a method for isolation and propagation of cells from bladder washings previously developed by our group [1]. The method is robust and requires minimal invasive access to the bladder via an indwelling catheter for the collection of loose UCs [2,4]. First, an 8F catheter was inserted through the urethra. The bladder was washed manually by filling it with sterile saline and flushing the liquid a couple of times using a 60 cm$^3$ syringe before aspiration. The procedure was repeated six to eight times, and volumes of at least 300 cm$^3$ were collected for transport to the laboratory. The bladder wash liquid was centrifuged at 300 g, and the cell pellet was re-suspended in DMEM (Invitrogen). After repeated centrifugation and re-suspension in DMEM, the bladder wash liquid was reduced to a single cell pellet. This remaining pellet was resuspended in 3 ml of standard epithelial cell growth medium (DMEM and Ham’s F12, 4:1 mixture), containing 10% FBS (all from Invitrogen), insulin (5 μg ml$^{-1}$, Humulin NPH, Eli Lilly, Indianapolis, IN, USA), hydrocortisone (0.4 μg ml$^{-1}$, Solucortef, Pfizer, New York, NY, USA), adenine (24 mg ml$^{-1}$), choleratoxin (10$^{-7}$ mol l$^{-1}$), triiodothyronine (2×10$^{-8}$ mol l$^{-1}$), transferrin (5 μg ml$^{-1}$), all from Sigma) and antibiotics (penicillin 50 U ml$^{-1}$ and streptomycin 50 μg ml$^{-1}$, Invitrogen), and seeded in a 10 cm$^2$ culture well. The culture was kept in humidified air with 5% carbon dioxide at atmospheric pressure and 37°C. After 24 h of plateau, epidermal growth factor (10 ng ml$^{-1}$, Sigma) was added to the growth media. The media were changed three times a week. After two to three weeks, when cell colonies had grown to a size covering more than 50% of the bottom area of the well, cells were passaged to a 25 cm$^2$ flask (passage 1) for further expansion. We used the UCs at passages 2–6 for our experiments. The phenotype of the cultured UCs was confirmed by immune staining of cytokeratin-18 (CK-18) and uroplakin IIIa (UpIIIa).

2.4. Induction of human bone mesenchymal stem cells to urothelial cells

Third-passage MSCs were used in the experiments. We used two different methods to induce MSCs to UCs. Both methods are based on a 14 day long exposure of conditioned medium (CM) from the cell type of desired destination, namely UC. Changes in morphology were monitored under the microscope every day. In the first method, MSCs were cultured with UC-derived CM. CM was collected from the UCs every 12 h when they were at 70–80% confluence. The collected media were centrifuged at 500 g for 5 min, and the supernatant was collected and then filtered through a mesh with a 0.2 μm pore size in order to remove any remaining cells. Fresh DMEM with 10% FBS was added to the CM in a 4:1 ratio. MSCs cultured in a filtered medium mixture, but without previous exposure to UCs, were used as controls.

In the second method, MSCs (2500–3000 cells cm$^{-2}$) were co-cultured indirectly with UCs (6000–7000 cells cm$^{-2}$) in a separate cell culture insert (24 mm dish, Transwell, Corning 3412, Tewksbury, MA, USA). UCs were placed in the top chamber and separated from MSCs in the bottom chamber by a barrier membrane with a 0.4 μm pore size. MSCs co-cultured with allo- genic MSCs were used as a control. As previously described, the cells were cultured in a standard epithelial cell medium, which was changed every 3 days.

2.5. Transdifferentiation of mesenchymal stem cells in plastic compressed collagen–PCL knitted fabric scaffolds

The scaffold consisted of two outer layers of collagen type I and an inner layer of PCL knitting. The knitting was prepared and characterized as previously described [31]. In brief, the PCL 0.25 μm monofilament knitting with stockinette structure was prepared with 35 needles per inch, and a needle gauge of 75 was used to give a compliant and highly porous fabric. For the collagen, eight parts of rat tail collagen type I (2.06 mg ml$^{-1}$ protein in 0.6% acetic acid, First Link Ltd, Wolverhampton, UK) was mixed with one part of DMEM and neutralized with 2.5 M NaOH before one part of alpha-MEM (Invitrogen) was added. The bottom layer of collagen was poured into half of a 3 × 2 cm and 1 cm thick mould and incubated for 10 min at 37°C. PCL was placed above the semi-rigid hydrogel, and additional collagen was poured on top and the construct was incubated for another 20 min to finalize the gel formation. To achieve plastic compression, the construct was placed between two 110 μm thick nylon meshes, and two 400 mm thick stainless steel meshes with gauze pads placed on the bottom. A loading plate of 120 g was placed on top for 5 min to press out the water that formed a hybrid scaffold of less than 0.5 mm thickness.

MSCs were cultured on top of the hybrid construct (at 2500–3000 cells cm$^{-2}$) for 14 days with CM. Positive and negative controls were UCs and MSCs cultured on the hybrid scaffold in standard urothelium medium. Routine histology and immunocytochemistry were performed as described above.

2.6. Determination of urothelial lineage gene expression

Cell differentiation into the UC lineage was determined by measuring specific gene transcripts in the induced MSCs by RT-PCR after 14 days of induction. Total cellular RNA was isolated from cells using the RNeasy mini kit (Qiagen, Hilden, Germany). cDNA was synthesized using the revert-aid first strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. Transcripts were analysed using the following PCR conditions: 94°C for 30 s, 55°C for 30 s and 72°C for 30 s for 35 cycles. In all PCR experiments, a 10 min extension step at 72°C was executed. The sequences of primers and expected product sizes are specified in Table 1. The reaction products were analysed by electrophoresis using a 1.5% agarose gel. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control.
Table 1. Details of primers used for gene expression analysis along with their expected product size.

<table>
<thead>
<tr>
<th>gene</th>
<th>GenBank ID</th>
<th>primer</th>
<th>size (bp)</th>
</tr>
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<tr>
<td>cytokeratin-18</td>
<td>NM_000224</td>
<td>F:GAGCCATCCAGAACGAGAAG</td>
<td>392</td>
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<tr>
<td></td>
<td></td>
<td>R:AGGCCCTGATCTGATCTCCA</td>
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<tr>
<td>UpIIIa</td>
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<td></td>
<td></td>
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<tr>
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<td>F:CTCTGACTTCAAGGCGACA</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:TCCTCTCTTCTCTTGGGC</td>
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2.7. Urothelial lineage protein expression by western blot

Protein expression was determined by western blot in order to assess differentiation of MSCs into induced UCs after 14 days of induction. Induced MSC lysates were prepared and subjected to western blot for detection of urothelial lineage-specific gene expression. Briefly, cells were washed with cold PBS, and lysates were obtained by homogenizing cells in tissue protein extraction reagent with halt protease inhibitor cocktail (Thermo Scientific). Equal amounts of protein (50 µg) were separated using SDS–PAGE (15%, Bio-Rad, Richmond, CA, USA) and transferred to a polyvinylidene difluoride membrane (GE Healthcare, Uppsala, Sweden). After blocking unspecific bindings with 5% non-fat dry milk, the membrane was incubated overnight with primary antibodies: UpIIIa (1:500; Santa Cruz, Dallas, TX, USA), CK-18 (1:500; Vector, Burlingame, CA, USA) and β-actin (control; 1:5000; Sigma) at 4°C with constant motion by shaking. Blots were stained with anti-mouse IgG conjugated to horseradish peroxidase (1:2000; Dako, Glostrup, Denmark) and visualized with an enhanced chemiluminescence system (GE Healthcare, Uppsala, Sweden).

2.8. Expression of urothelial lineage-specific proteins by immunocytochemistry

Protein markers of induced cells were further investigated by immunocytochemistry. CK-18 and UpIIIa protein were used as epitopes and markers for differentiated UCs. Induced cells were seeded on diagnostic slides (Thermo Scientific), and allowed to adhere for 24–48 h. Cells were fixed in 4% paraformaldehyde for 24 h and washed three times with PBS at room temperature. The immunocytochemistry was carried out using the Vectastain elite ABC kit (Vector). Endogenous peroxidase was inactivated with 0.3% hydrogen peroxide for 15 min. After washing with PBS, primary anti-human CK-18 monoclonal antibody (Vector) or UpIIIa (1:500; Santa Cruz) or various cytokeratins (MNF-116, Pancytokeratins 5, 6, 8, 17 and 19; Dako, Glostrup, Denmark) was added, and slides were incubated in a humidified chamber overnight at 4°C. After being cultured for three weeks in osteogenic medium, the cells were able to deposit abundant mineralized matrix as demonstrated by staining with alizarin red S (red staining) and Von Kossa (black staining; electronic supplementary material, figure S1b). Flow cytometry demonstrated a typical antigen-presenting pattern and fulfilled the criteria for MSCs [34]. Antigen expression was positive (more than 90% positive cells) for CD73, CD90, CD105 and HLA class I and negative (less than 5% positive cells) for CD14, CD31, CD34, CD45, CD80 and HLA class II (electronic supplementary material, figure S1).

3. Results

3.1. Isolation and characterization of mesenchymal stem cells

MSCs were successfully isolated and expanded for characterization and used in the experiments. Cultured MSCs presented with a homogeneous population and a phenotype with typical mesenchymal spindle-shaped morphology (electronic supplementary material, figure S1a). After induction under adipogenic conditions, the isolated cells changed morphology and produced intracellular droplets which were demonstrated by staining with oil red O (electronic supplementary material, figure S1b). After being cultured for three weeks in osteogenic medium, the cells were able to deposit abundant mineralized matrix as demonstrated by staining with alizarin red S (red staining) and Von Kossa (black staining; electronic supplementary material, figure S1c,d). Flow cytometry demonstrated a typical antigen-presenting pattern and fulfilled the criteria for MSCs [34]. Antigen expression was positive (more than 90% positive cells) for CD73, CD90, CD105 and HLA class I and negative (less than 5% positive cells) for CD14, CD31, CD34, CD45, CD80 and HLA class II (electronic supplementary material, figure S1).

3.2. Isolation and characterization of urothelial cells

The UCs used in the experiments formed colonies and expanded in a monolayer with growth characteristics and cell morphology, with respect to the shape and size, typical of UCs (figure 1a). Immunostaining for cytokeratin and uroplakin confirmed a urothelial phenotype.

3.3. Morphology and protein expression of induced mesenchymal stem cells

MSCs were initially spindle-shaped and non-colony-forming but grew close together in a compact, linear manner (figure 1b). The morphology changed into a polygonal epithelium-like shape resembling UCs (figure 1a) after 14 days of culture in either CM (experiment 1, figure 1c) or in co-culture (experiment 2, figure 1d). In all cultures, cells had a homogeneous morphology with no changes resembling other cell lineages such as adipogenic or osteogenic characteristics, as previously presented in the characterization of MSCs (electronic supplementary material, figure S1). In the control group, MSC morphology did not change (figure 1b).
The aim of tissue engineering is to replace and restore tissues or organs owing to a congenital malformation or after a traumatic or therapeutic loss. Autologous in vitro cultured UCs have been used successfully in cell-based tissue engineering for the treatment of severe hypospadias [4]. However, a lack of autologous urothelial tissues for cell harvest might limit this application in certain cases. An alternative to using UCs could be to harvest autologous MSCs that could be expanded and induced into a UC lineage in vitro.

MSCs are multipotent cells that reside in different niches all over the body. They are good candidates for tissue engineering owing to their favourable properties and ease of isolation and propagation, high differentiation potential and a non-immunogenic and anti-inflammatory profile in combination with a low oncogenic risk [5,11,35,36]. At present, in vitro and in vivo studies have demonstrated that MSCs can differentiate beyond mesodermal lineages and into endodermal and ectodermal lineages [6–9]. In addition, isolation of MSCs from bone marrow is a well-established method accredited with standards for good manufacturing practice and is currently used in clinical trials [36–38].

The MSCs in this study were initially thoroughly characterized and displayed typical MSC characteristics in terms of cell expansion patterns in vitro and fibroblastic morphology, as well as expression of surface markers and the functional capacity to differentiate along osteogenic and adipogenic lineages. We have thus proved the multipotential differentiation capacity that could be used in our transdifferentiation experiments [34].

In this study, we show that human adult bone-marrow-derived MSCs can be induced and display UC-like properties, proved by morphology and expression of urothelial-specific transcripts and proteins. Other groups have demonstrated UC differentiation by exposure to a specific microenvironment. In the studies by Ning et al. [24], fetal urinary and bone marrow tissues were used for the experiments. Our studies further support that also post-natal UCs have the potential to stimulate allogenic adult MSCs to differentiate into urothelial-like cells [25]. So far, stem cells from adipose tissue [39] and bone marrow [25] have been studied and showed that differentiation into urothelial-like cells is possible.

Using UCs from bladder washings is an easy and robust method that is minimally invasive besides transurethral catheterization [1,40]. Importantly, in long-term quality controls after extensive in vitro culture up to 12 passages, we have not found any structural alterations in the chromosomes, and therefore the method is considered to be safe and reliable [41]. Theoretically, in cases where the patients’ own UCs could be used for the transdifferentiation of MSCs, UC harvesting could be undertaken without requiring general anaesthesia. Another advantage includes cells not subjected to enzymatic treatment in vitro. In previous studies, UCs from open biopsies of adult or fetal bladders or ureters have been used for the induction of MSCs [24,25,39]. In a clinical setting, we believe that our less invasive method of harvesting from bladder wash would substantially facilitate the recruiting of UC donors. Our results demonstrate that UCs secrete substances that stimulate mesenchymal cells to differentiate into a UC lineage. Theoretically, this suggests that bone marrow MSCs, if circulating in the blood stream, also could act as cellular resources for wound healing in the urinary bladder. By permitting indirect interaction with UCs, excreted factors, soluble in the growth medium, were allowed to interact with bone marrow-derived MSCs in a paracrine and autocrine mode. The molecular mechanism behind the induction process has yet to be understood and further studies, including changes
in gene and protein expression, need to be further explored for this purpose. Potential growth factors have been discussed, and a higher expression of platelet-derived growth factor and vascular endothelial growth factor has been measured in the CM setting [25,39]. Other potential mechanisms such as intercellular cross-talk, as seen during organogenesis in the embryo, might also be of importance.

The extracellular environment strongly influences the differentiation capacity of progenitor or stem cells. For this reason, and for future clinical applications, we therefore wanted to confirm that human adult bone marrow MSCs could be induced to form UCs also on a collagen carrier to produce tissues for surgical handling in transplantation procedures. We used a hybrid scaffold that was developed to satisfy important characteristics for application in the human urinary system: tensile strength, barrier function and high-grade compliance [30,42]. The transplant (hybrid scaffold with autologous cells) is constructed to enable take, survival of transplanted cells and ingrowth of capillaries and extracellular matrix directly after implantation [29]. This initial step is enabled by the collagen component after partial dehydration [28]. Sometime after transplantation, part of the transplant (the collagen) is biodegraded to allow compliance, but still acts as a support for a prolonged time before degradation (the PCL core) [30].

In this study, we show that the hybrid scaffold provides good attachment and proliferation of cells. In previous studies, we have clearly demonstrated that cell adhesion and proliferation are supported on the collagen surface of the PCL scaffold, including mechanical details of the scaffold [31]. Cell adhesion to collagen is well established and previously used successfully in different clinical settings. The challenge

![Figure 2. Immunocytochemistry characterization of urothelial transdifferentiation of MSCs induced in conditioned medium (CM) or co-culture with UCs after 14 days of induction.](http://rsif.royalsocietypublishing.org/)

![Figure 3. Analysis of MSCs induced by conditioned medium (CM) or co-culture in culture insert with UCs by RT-PCR and western blot after 14 days of induction.](http://rsif.royalsocietypublishing.org/)
that we resolved in our PCL–collagen scaffold was the interaction between the collagen and the PCL that allows reproducibility and large-scale fabrication. The hybrid is easy to make, has high tensile strength, with a thickness that can be regulated, and is readily handled surgically [31]. MSCs could easily attach and differentiate into urothelial-like cells on top of the scaffold and thereby form a confluent epithelial cell layer. Hence, this demonstrates the feasibility of this method in a clinical setting.

For clinical applications, any human UCs could be stored and stocked in freezers in order to be available when patient MSCs are isolated and available for transdifferentiation on the transplant approximately two weeks before reconstructive surgery. The study construct clearly demonstrates that soluble substances from differentiated human UCs act as signals to stimulate MSCs to differentiate into UC lineage. As the mechanisms are the same in both methods and signalling takes place through the medium, not by direct cell-to-cell interaction, we did not expect to find any differences between the two methods. In accordance to this, we did not see any differences in the capacity for induction of the UC lineage in the two methods used, neither by conditioned media nor culturing in a culture well insert. The experiment was not constructed to answer this question, but rather to exclude the possibility of contamination of allogenic UCs in the induced MSC culture. Both methods were successful, and both methods could be used for the induction of UCs on the hybrid scaffold. Preparation of a complex three-dimensional transplant with CM might be easier whereas co-culture would be less labour intensive in two-dimensional cultures.

In conclusion, human MSCs could easily be harvested by bone marrow aspiration, expanded and differentiated into UC-like cells by local action of factors excreted from normal human UCs. The differentiation can take place on a three-dimensional hybrid PCL-reinforced collagen-based scaffold for clinical purposes. The molecular mechanisms are still unknown and should be further studied, including in vivo animal studies, before a clinical application could be considered. However, this study confirms previous findings of a potential role for MSCs in reconstructive urology and further develops the approach for a clinical application.

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