# Regeneration of the upper urinary tract using mESC-derived ureteric buds

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## Background:

- Kidney disease and its related complications are an important public health problem worldwide.
- Pluripotent stem cell (PSC)-derived kidney organoids are a recent advance in the field of regenerative medicine, and may facilitate drug testing, disease modelling and generating tissue for renal regeneration or replacement.
- There is intense interest in turning organoids into complete kidneys for transplant.
- Less explored is the idea of transplanting organoids into diseased kidneys, to replace damaged tissue.
- This approach would depend on an ability of organoid collecting ducts to join those of the existing kidney.

## Methods:

To test whether PSC-derived ureteric bud/ collecting ducts can connect with existing collecting duct trees of a host kidney, we have done:

- Differentiation of mESCs into ureteric bud (iUB) using methods described by Taguchi et al,. 2017, and to visualize these iUBs we employed a HoxB7-GFP mESC line.
- Induction of an injury into either the CD or the ureter of a cultured E11.5 mouse kidney.
- Grafting the iUBs adjacent to the ureter or the collecting duct at the site of the induced injury.

## Results:

- The iUBs grafted in the metanephric mesenchyme showed branching and induced nephron formation, as natural immature collecting duct trees do, and they connected to the host mouse collecting duct system.
- Those grafted by the ureter connected to it and expressed uroplakin, a marker specific for urothelium, and acquired a smooth muscle coat.

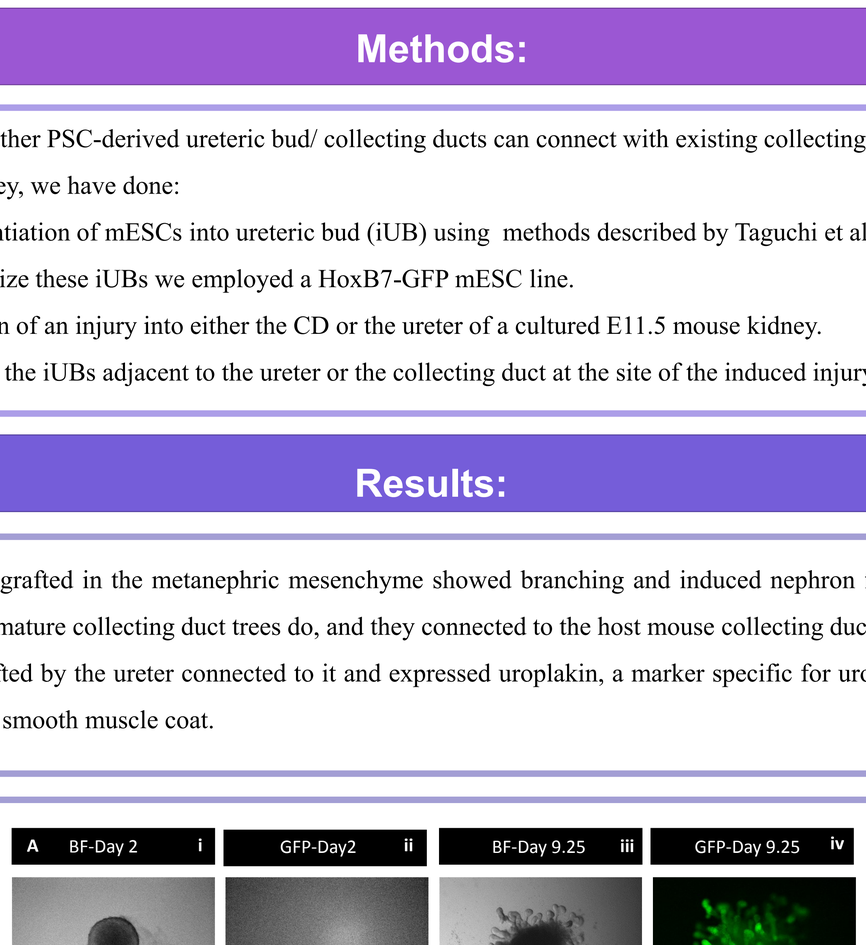


***Figure 1. Induction of the iUB differentiation using HoxB7-GFP mESC line. (A) i.*** *Bright field image showing embryoid body formation on day2 after induction of the differentiation.* ***ii*** *.GFP image of A.i showing no hoxB7- GFP reporting.* ***iii***. *Bright field images of the iUB spheroids on day 9.25 of induction showing neumerous UB like structures.* ***iv.*** *GFP image of A.iii expressing the HoxB7-GFP+ ub like structures.* ***(B) i.*** *Bright field image showing a single iUB bud isolated from day 9.25 spheroids and cultured in 3D gel with ramogens day 0.* ***ii.*** *Day 4 iUB in 3D gel, showing branching.* ***iii.*** *Bright field image showing day 7 iUB in 3D gel showing extensive branching.* ***iv.*** *GFP image showing day 7 iUBs in 3D gel expressing HoxB7-GFP, scale bar 200 µm. (C) Imunoflourescent images of day 9.25 spheroids showing positive UB markers CK8,Pax2,Ecadherin,PCK and Calbindin scale bar 100 µm .*

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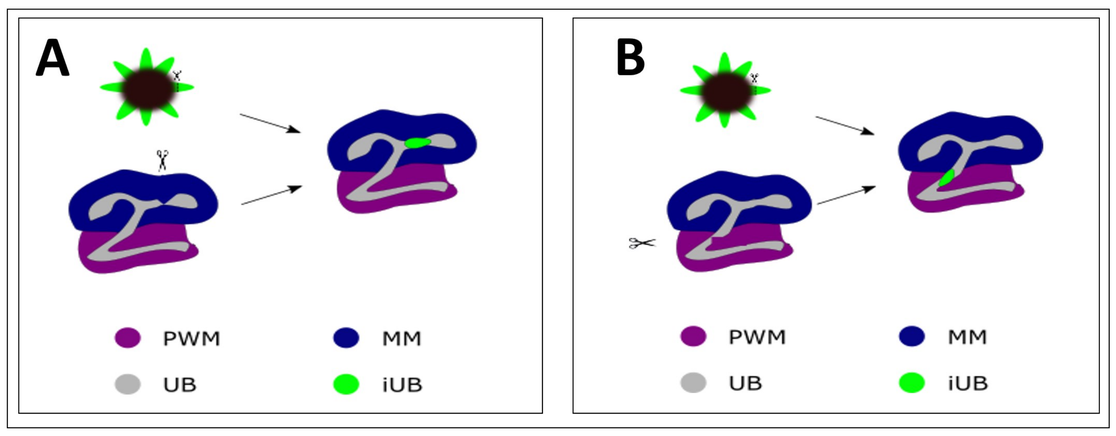


***Figure 2. The iUBs can be induced to connect with either the CDs or the ureters of a host kidney. (A,B)*** *illustration showing the steps of the connection experiment in both metanephric mesenchyme and peri-wolffian mesenchyme respectively.*

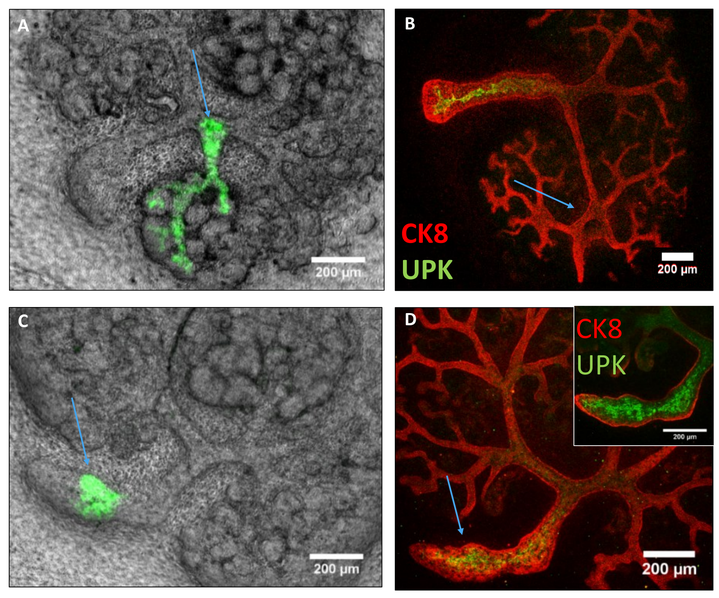


***Figure 3.*** **The fate of the iUB differentiation into either CD or ureter is controlled by the surrounding mesenchyme.** ***(A)*** *Combined bright field and GFP image showing the iUB connected to the collecting duct tree of a host kidney, the iub reports HoxB7-GFP and behaves like a CD, showing branching and nephron formation.* ***(B)*** *Imunoflourescent image of the iUB connected to the collecting duct tree (CK8 expression), No UPK is seen in the graft.* ***(C)*** *Combined bright field and GFP image of the iUB connected to the ureter, the iUB reports HoxB7-GFP.* ***(D)*** *imunoflourescent image of the iUB connected to the ureter showing urothelial continuity and UPK expression.*

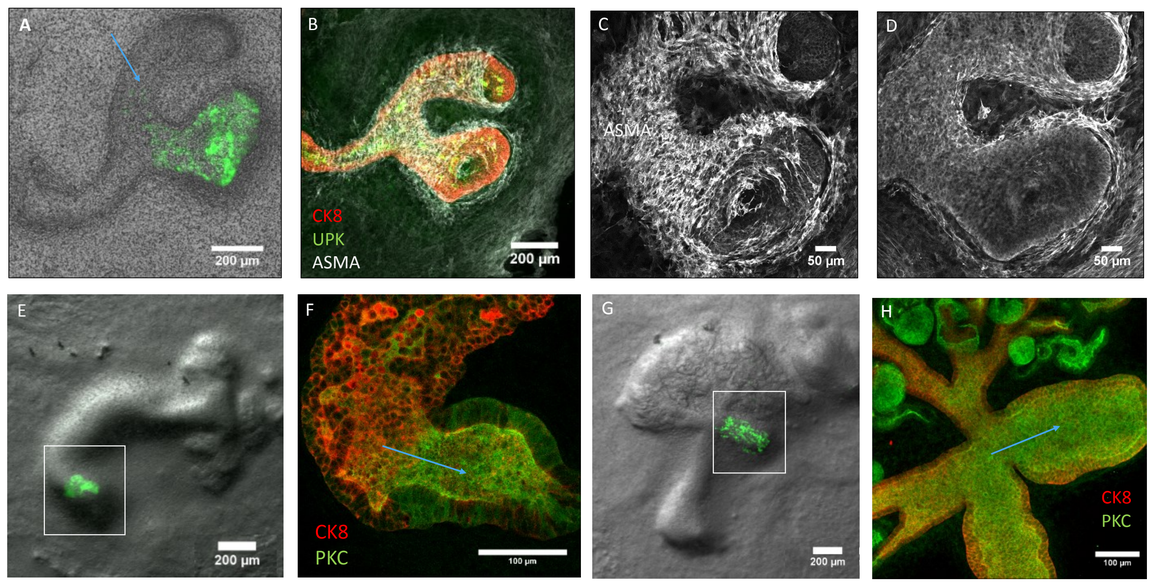


***Figure 4. The connected grafts share an opened lumen with the kidney CD and/or the ureter. (A).*** *Combined bright field and GFP image of iUB connected to the shaft of a ureter, the iUB reports hoxB7-GFP.* ***(B)*** *Imunoflourescent stain of the connected iUB showing CK8, UPK and ASMA expression in the graft as in the ureter****.(C,D)*** *Magnified image of the ASMA stain showing multilayer smooth muscle coat around the connected graft****. (E)*** *Combined bright field and GFP image of iUB connected to the lower end of ureter of a donner kidney, the graft is reporting HoxB7-GFP****.(F)*** *Staining of the apical domain PKC showing connected lumen between the iUB graft and the ureter.****(G)*** *Combined bright field and GFP image of iub grafted at the pelvi-ureteric junction of donner kidney, the graft is reporting HoxB7-GFP.* ***(H)*** *The apical marker PKC imuneflourescent stain showing connected lumen between the graft and the PUJ of the donner kidney.*

## Conclusion:

We consider this work as a first step towards integrating organoids with host kidney tissue for regenerative purposes.

**Abbreviations**: iUB (induced Ureteric bud), MM (metanephric mesenchyme), PWM (peri-wolffian mesenchyme), CD (collecting duct), PKC (phospho kinase c), PUJ (pelvi-ureteric junction),