

Lytic Effects Of Water On Bladder Cancer Cells – Implications For Continuous Bladder Irrigation After TURBT

R. NANDURKAR¹, P. SLUKA¹, M. LI^{1,2}, H. WARDAN¹, I. D. DAVIS^{1,2}, S. SENGUPTA^{1,2}

1. Eastern Health Clinical School, Monash University

2. Eastern Health

INTRODUCTION

- Recurrence of NMIBC after TURBT is observed in 40-80% of patients
- Intraoperative spillage of cells can lead to tumour cell re-implantation and local recurrence
- Intravesical chemotherapy is effective at reducing recurrence, but is underutilised due to high cost and toxicity
- Bladder irrigation with water as opposed to saline may be a comparable alternative to chemotherapy as it causes osmotic cytolysis in addition to mechanical dilution to remove remaining viable cells

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AIMS

1. To determine the time course of osmotic effects of water on bladder cancer cells *in vitro* and *in vivo*
2. To determine whether water is able to lyse cancer cell lines other than bladder cancer *in vitro*
3. To determine what degree of contamination by urine or blood nullifies the effects of water's osmolytic properties

METHOD

IN VITRO

- Two bladder cancer cell lines (HT1197, HT1376) were exposed to water, 0.9% saline or 1.5% glycine
- Cell counts were made at regular intervals for 5h
- Cell counts in triplicate were performed with trypan blue exclusion
- These experiments were repeated for colorectal (LIM2405, KM23), ovarian (COV434), and renal (SKRC52) cancer cell lines

IN VIVO

- 21 patients received 3h or saline or water irrigation post-TURBT
- Hourly 200mL washout samples were collected for cell counting at 0, 1, 2, and 3 hours post-TURBT
- Selected patient samples were sent for immunohistochemistry (IHC)

RESULTS

IN VITRO (Figures 1 & 2)

- Water caused 100% cell lysis within 20 minutes
- Both saline- and glycine-irrigated samples had viable cells remaining at 5h
- Similar results were achieved on all 4 other cancer cell lines
- Gradation study showed that an increase to 0.045% NaCl would impact the osmotic functions of water

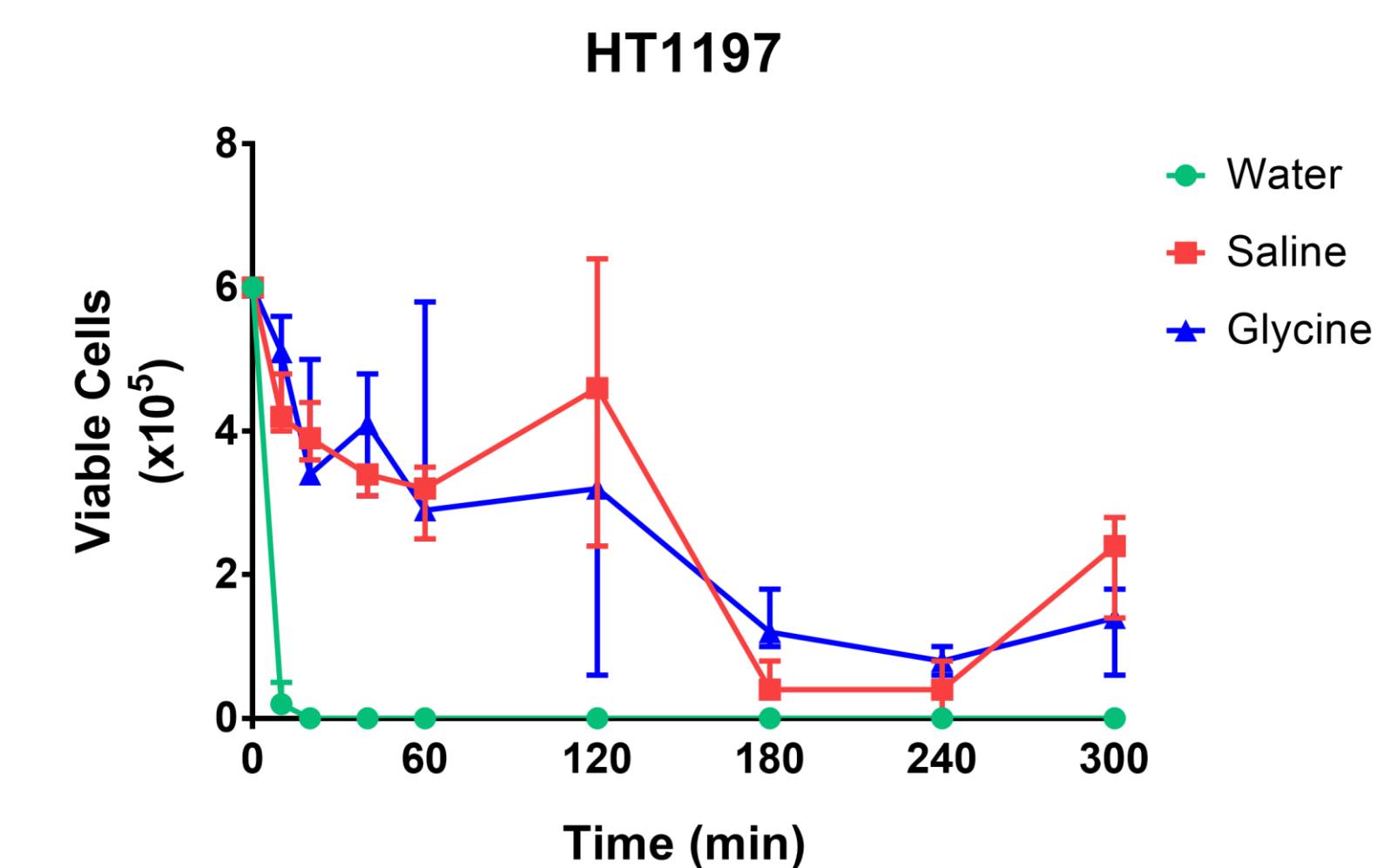


Figure 1. Viable cell numbers for bladder cancer cell line HT1197 when exposed to water, saline or glycine for up to 5 hours.

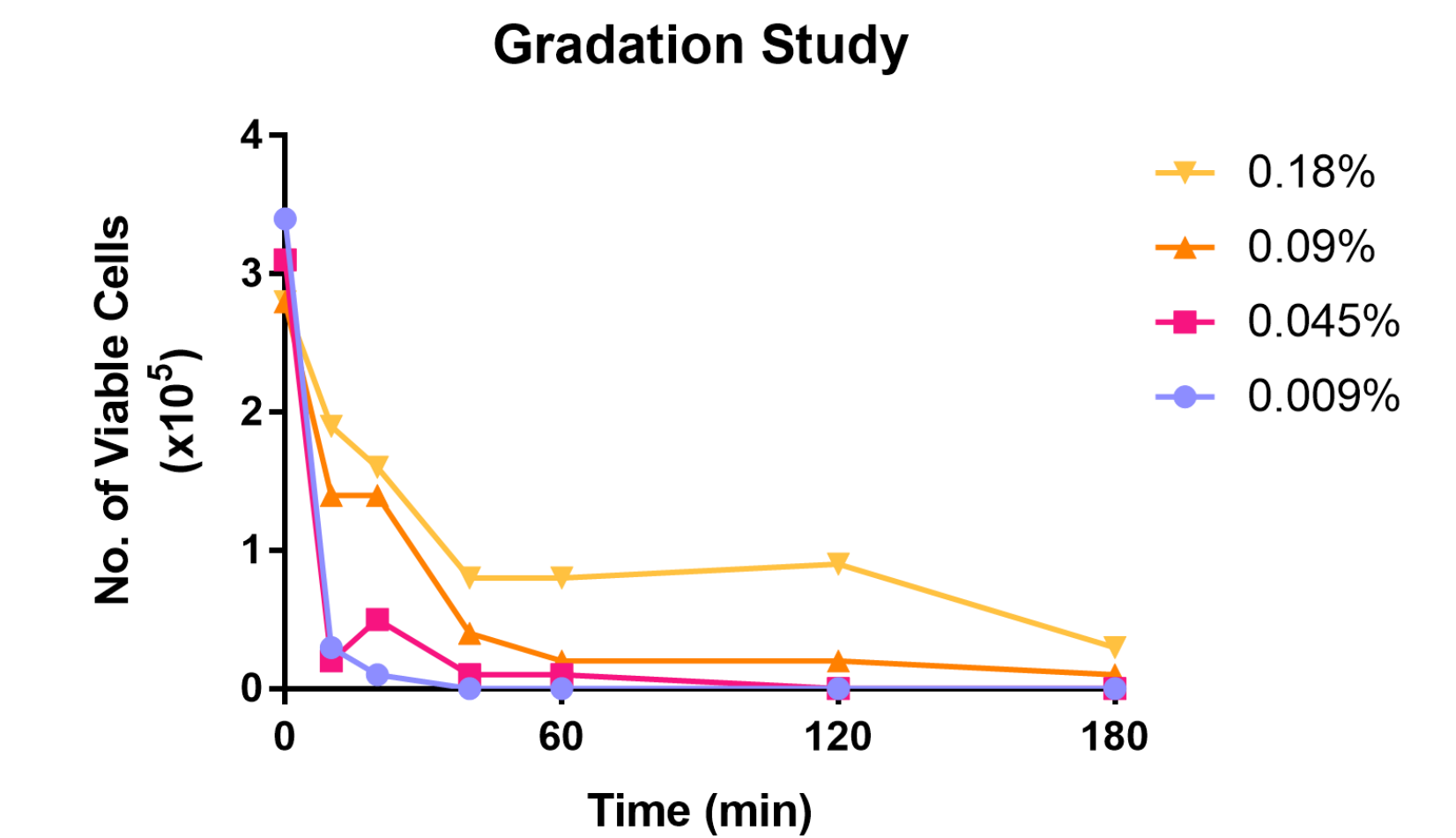


Figure 2. Viable cell numbers for bladder cancer cell line HT1197 when exposed to decreasing concentrations of NaCl. Data represents median values of n = 3 measures

IN VIVO (Figure 3)

- 14 patients received water irrigation *in vivo*
 - Time 0 (median) = 14 x 10³ cells
 - 1 hour onwards (median) = 0 cells
- 9 patients received saline irrigation *in vivo*
 - Time 0 (median) = 40 x 10³ cells
 - 3 hours (median) = 20 x 10³ cells
 - Median cell count never reached zero

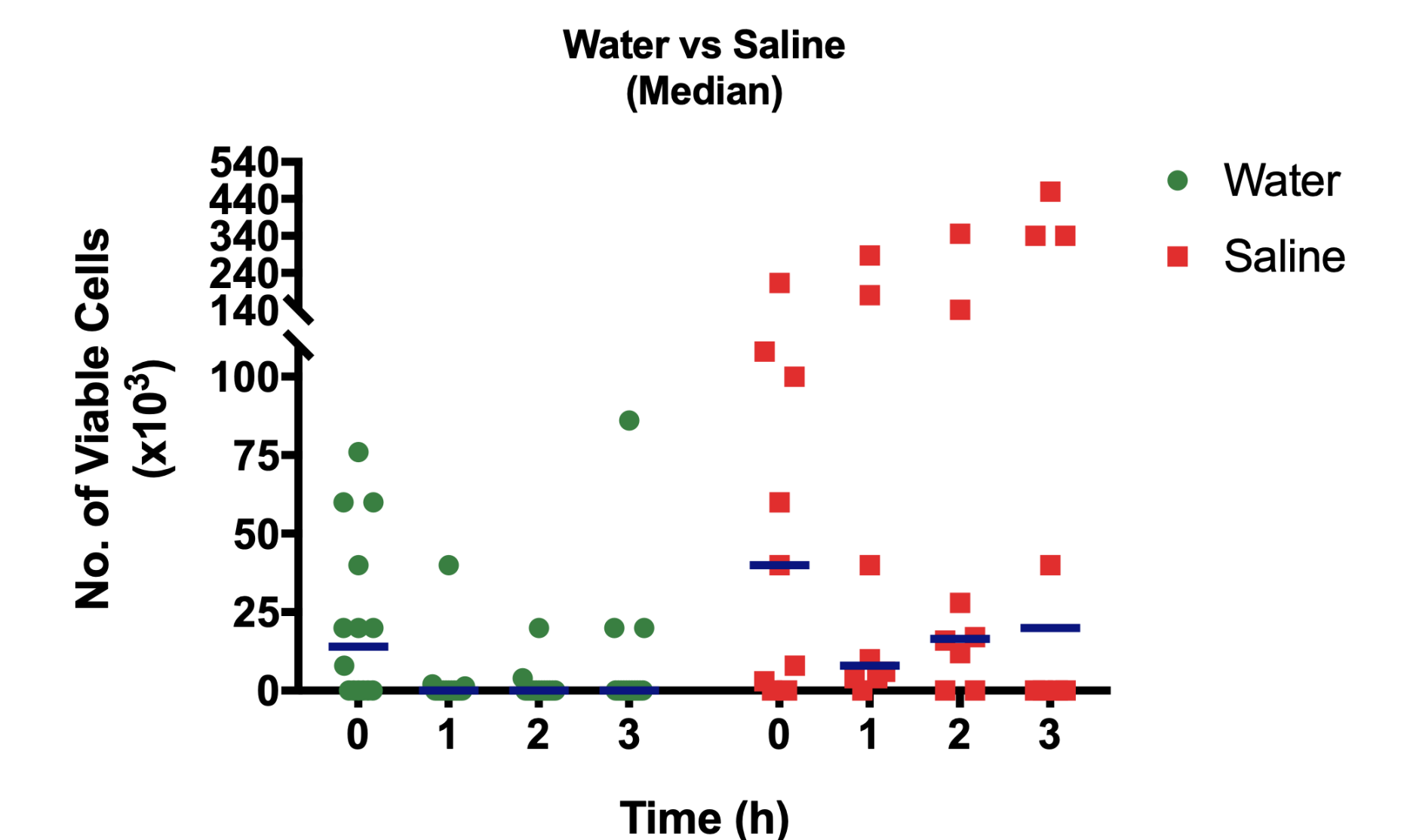


Figure 3. Water vs saline in vivo

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RESULTS CONT.

- IHC (Figures 4 & 5) on *ex vivo* samples showed significant numbers of bladder epithelial cells in saline-irrigated patients in contrast to water-irrigated patients
- Cytokeratin 7 (CK7) showed bladder epithelial cells in brown

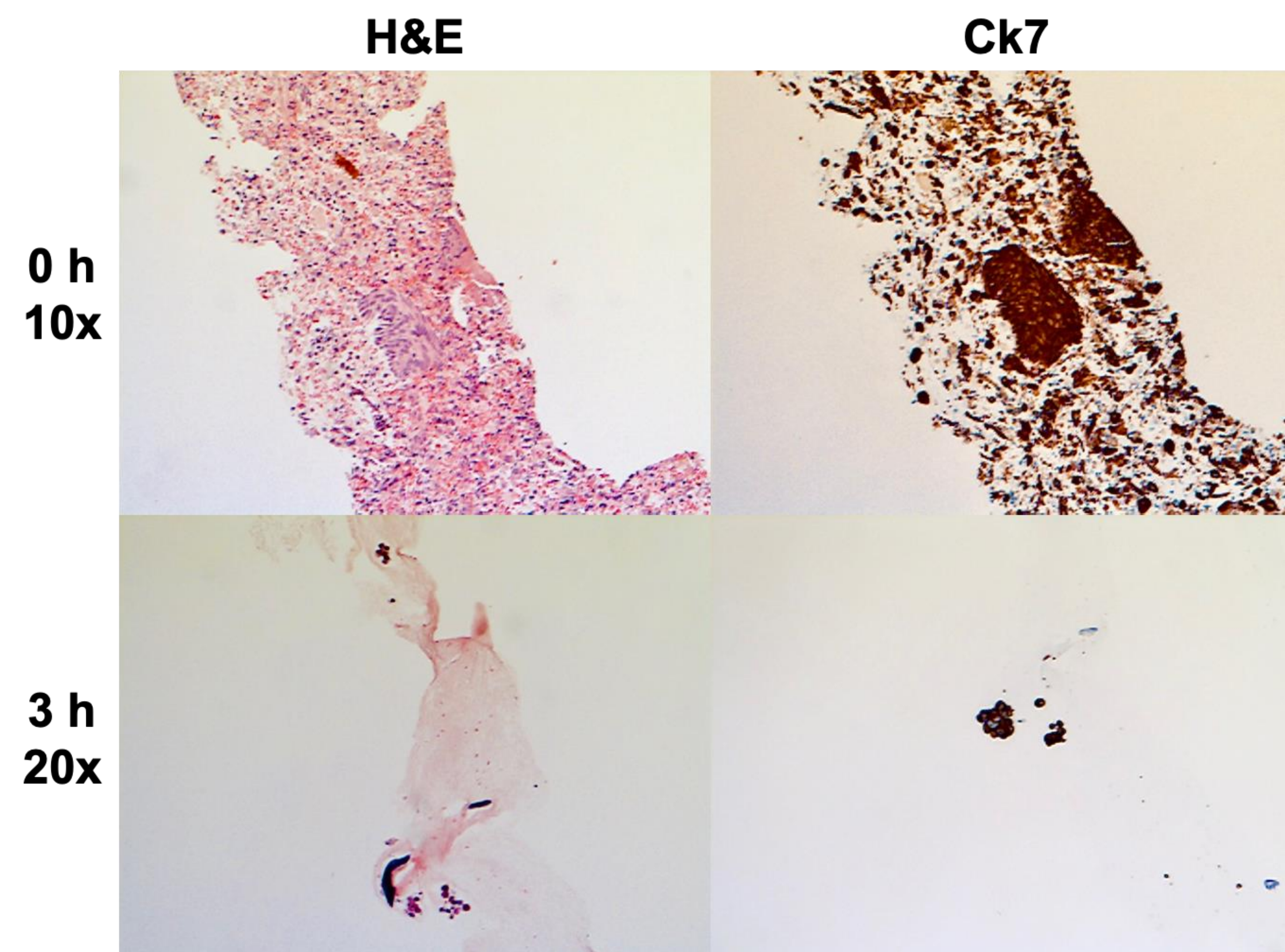


Figure 4. IHC for a saline-irrigated patient

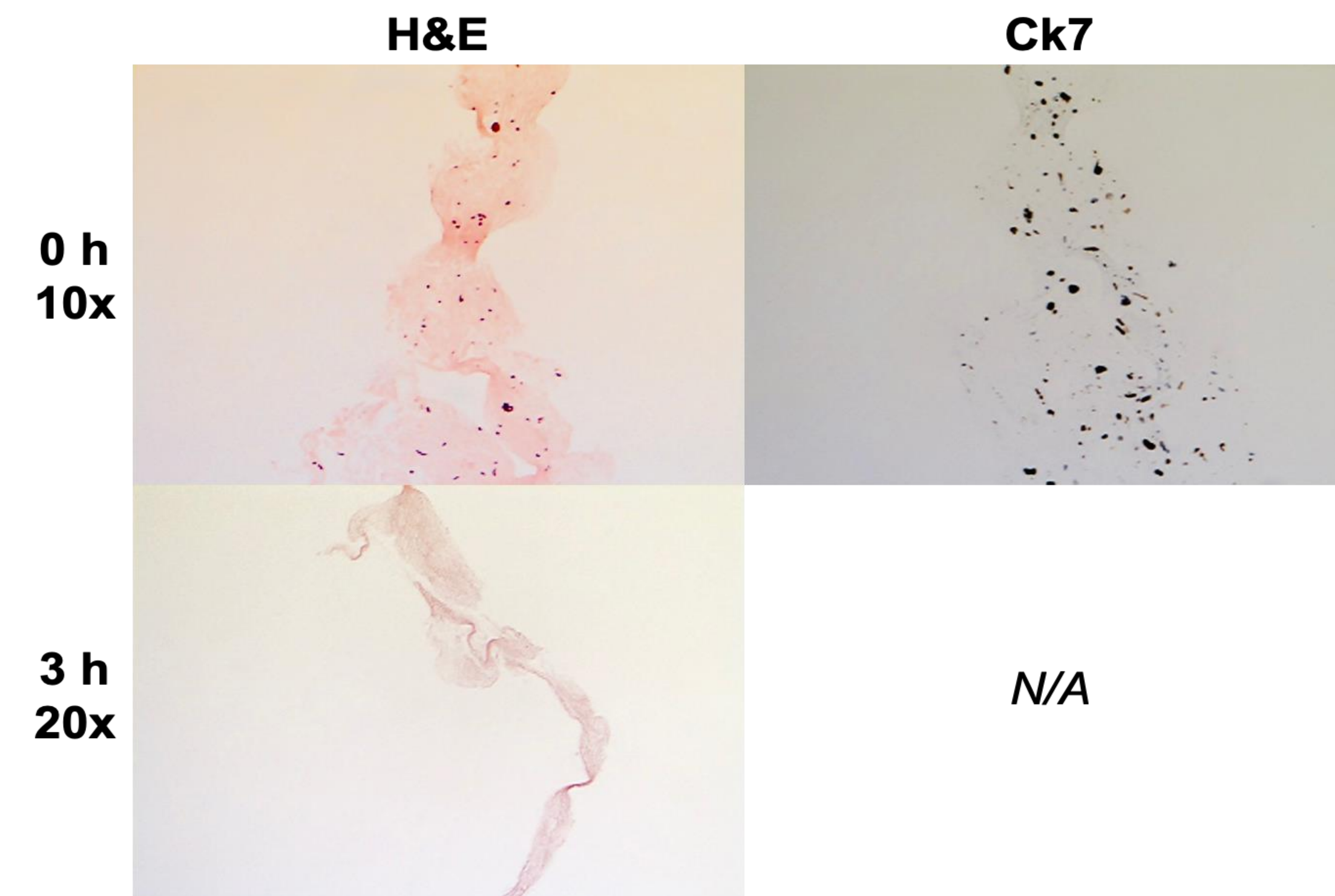


Figure 5. IHC for a water-irrigated patient

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CONCLUSIONS

Water is able to rapidly lyse bladder cancer cells *in vitro* and *in vivo*. A short 3-hour period of water irrigation post-TURBT may be an effective intervention to reduce bladder cancer recurrence

- Water is able to lyse viable cells in at least 4 other cancer cell lines
- An increase from 0% NaCl (pure water) to 0.045% NaCl was sufficient to alter the efficiency of cytolysis

ACKNOWLEDGEMENTS

The clinical part of this project is supported by an ANZUP Below-the-Belt grant. We would also like to thank Monash University for their generous scholarship awarded to Ms Nandurkar.

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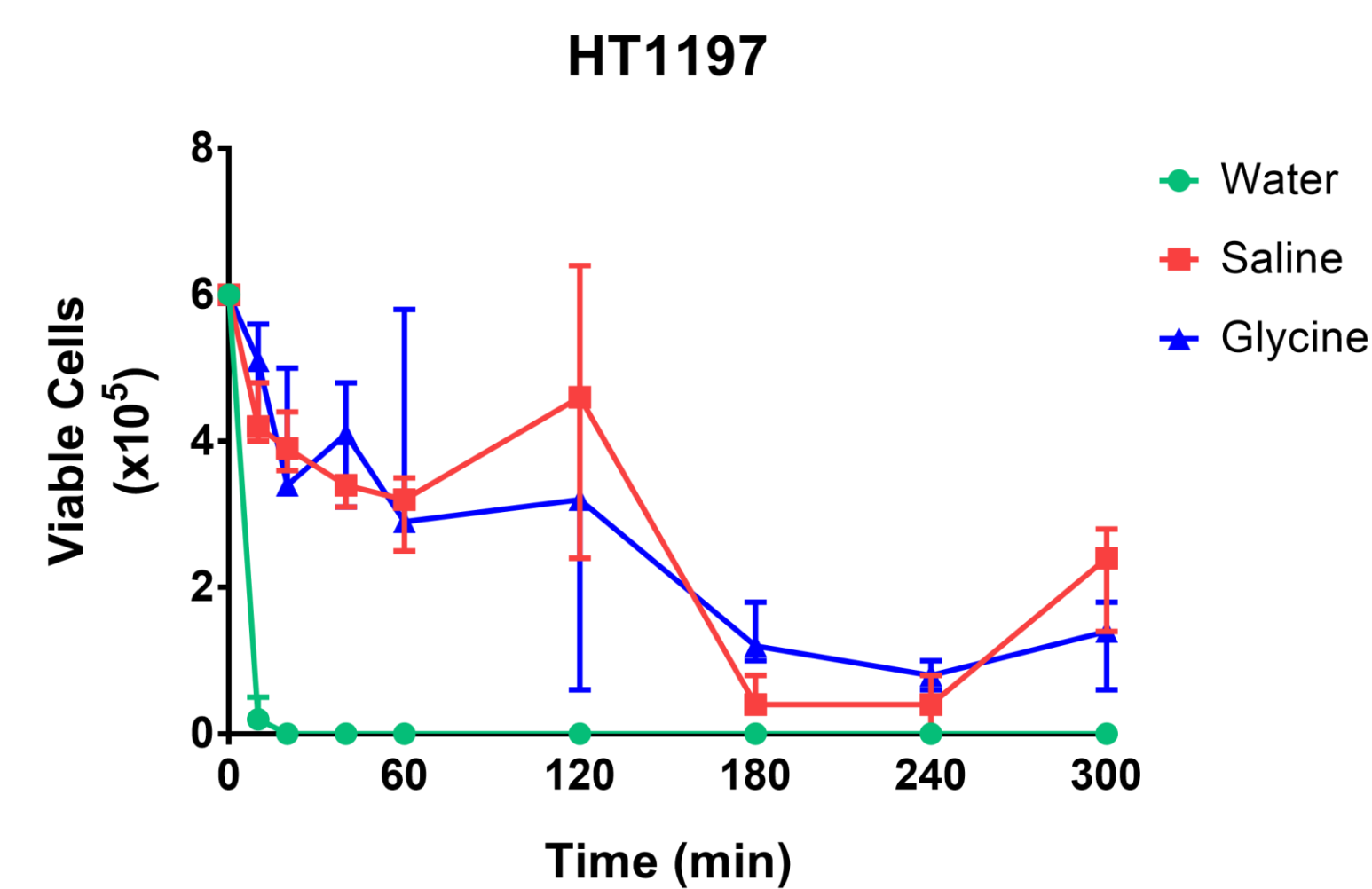


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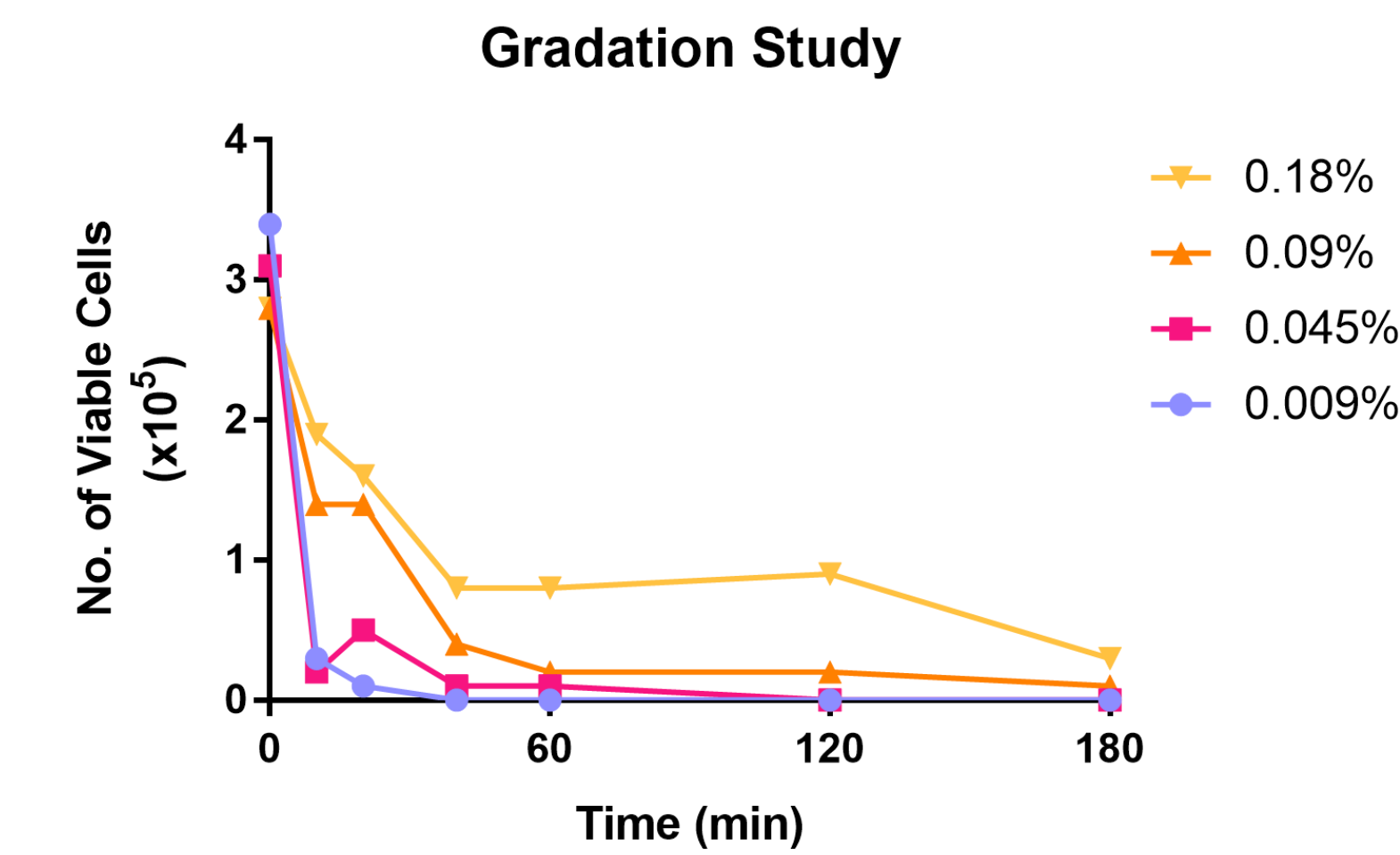


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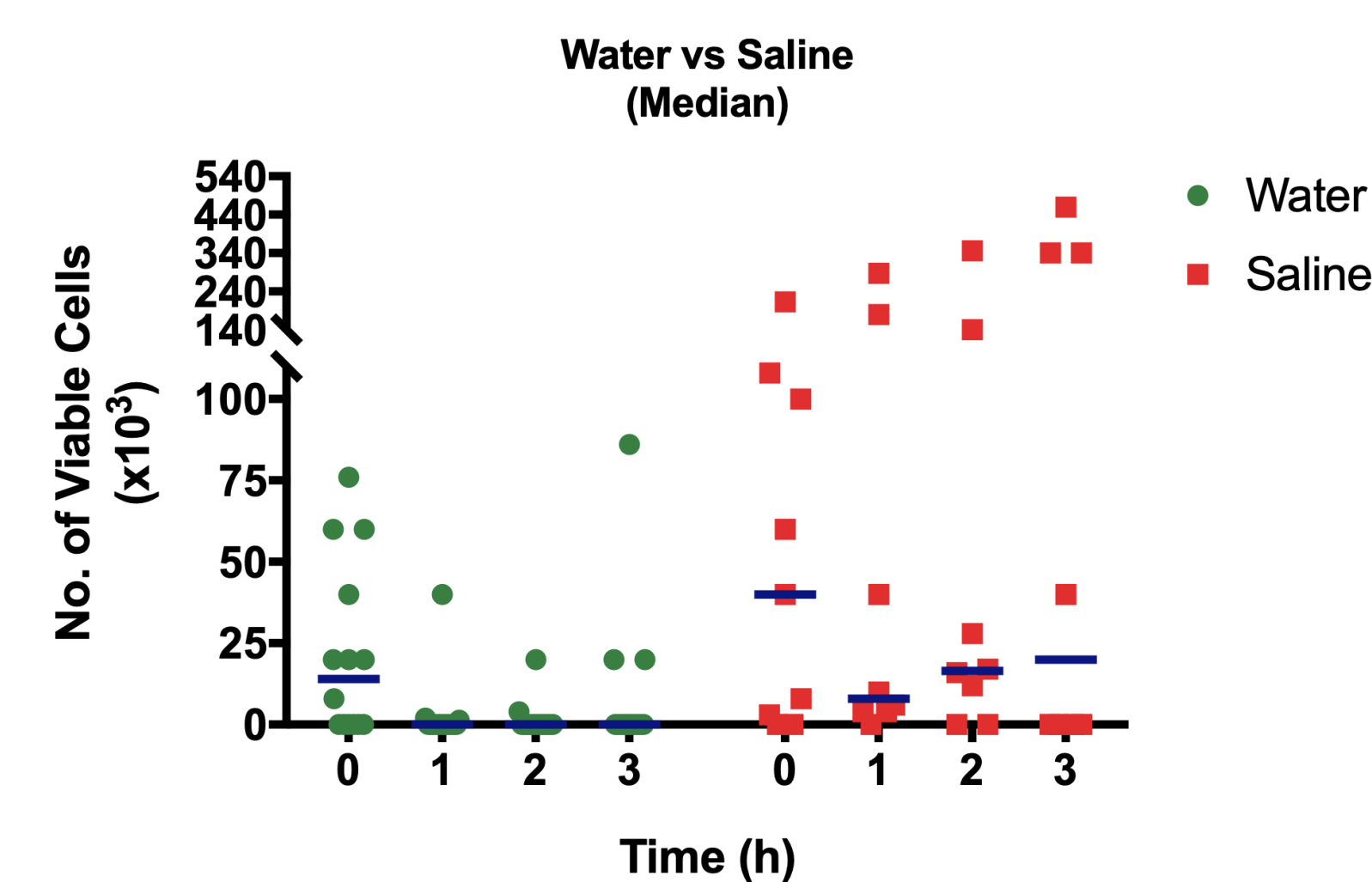


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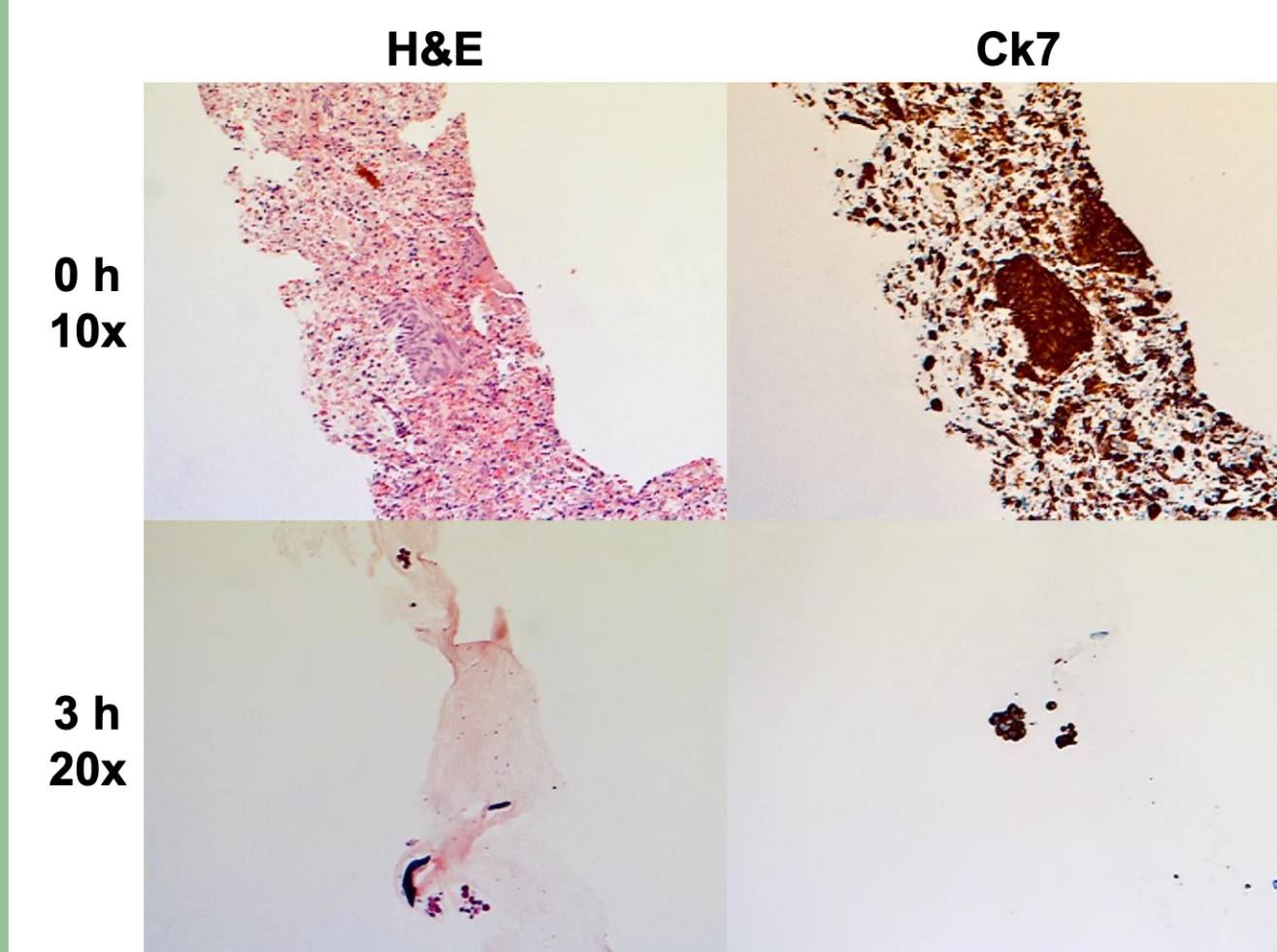


Figure 4. IHC for a saline-irrigated patient

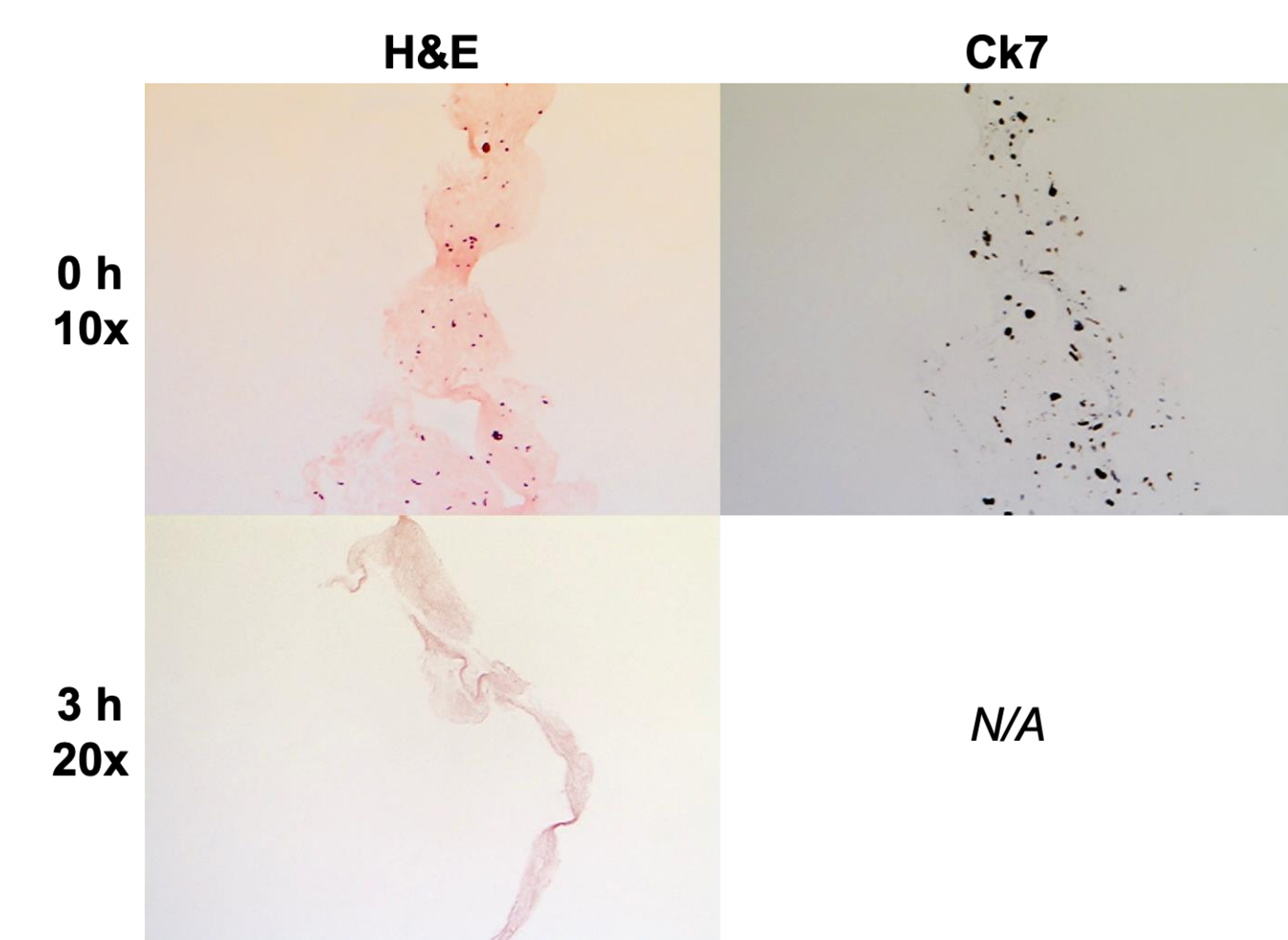


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