Journal of Plastic, Reconstructive & Aesthetic Surgery (2016) xx, 1–4

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JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

CORRESPONDENCE AND COMMUNICATION

Irrigation of cutaneous squamous cell carcinoma wounds to prevent local recurrence

Dear Sir

Surgical excision of non-melanoma skin cancers is the mainstay of treatment, and a common procedure performed by clinicians from various backgrounds and training levels. Despite efforts to excise squamous cell carcinomas (SCCs) with a margin of normal tissue, some tumours are incompletely excised or cells are "seeded" into the wound allowing local recurrence. Following surgical oncology procedures many operators irrigate wounds or body cavities with water rather than saline. The logic being that water will induce an osmotic shift of fluid into the cells, causing them to lyse. There is little evidence to support this practice.

Work with human material was carried out in compliance with the UK Human Tissue Act (2004) and approved by the National Research Ethics Service (08/H0306/30).

SCC13 is cell line established from a facial cutaneous SCC, from a 56-year old female.[1] Cells were labelled with a lentivirus yellow fluorescent protein (YFP)/luciferase reporter and grown in triplicate (100 cells per dish) to form colony forming assays (CFAs). Cells were also added on top of de-epithelialised dermis (DEDs) (100 cells per DED), as an *ex-vivo* model, to simulate the operative wound environment. After 30 min, media was removed and plates irrigated with either FAD standard keratinocyte culture medium, sterile Milli-Q water, 0.9% saline or a 10% betadine solution. After 5 min all irrigation fluids were rinsed and replaced with standard FAD culture medium.

Following 2 weeks in standard culture conditions CFAs were fixed with 4% PFA and stained with 0.1% Toluidine Blue (Figure 1). DEDs were imaged using a UV lamp to detect the YFP label (Figure 2), then treated with D-luciferin to activate the luciferase reporter. Luciferase activity was imaged and quantified using the Xenogen In-Vivo Imaging System (IVIS) 200 (Supplied by Perkin Elmer, USA) (Figure 2). The mean and standard deviation for each group was calculated and then un-paired t-tests were performed using GraphPad Prism 6 software to determine statistical significance of differences between groups. Results were considered significant if the p-value equalled less than 0.05.

Water irrigation significantly decreased ability to form colonies on plastic, compared with FAD culture medium or 0.9% saline ($p < 0.005$), however, substantial growth was still present (Figure 1). The relative levels of growth were similar when cells were grown on in the *ex-vivo* DEDs model, however there was no significant difference between the samples irrigated with FAD culture medium, 0.9% saline or water. Imaging the DEDs with a UV lamp showed well developed colonies. The Xenogen IVIS allowed visualisation and accurate quantification of colonies (Figure 2). Only the 10% betadine solution showed complete absence of cell growth on either plastic ($p < 0.0001$) or DEDs ($p < 0.0001$).

Irrigation with water may reduce the ability of SSC cells to survive, but it does not completely remove them, risking local disease recurrence. Irrigation with a betadine solution, however, absolutely prevents cells from surviving, greatly reducing the risk of recurrence. The DEDs model replicates the surgical environment when spilt SCC cells rapidly adhere to exposed dermis, making them less susceptible to the mechanical forces of irrigation, and the non-physiological osmotic pressures of water irrigation.

Malignant cells are by their nature more resilient than normal cells. The "Warburg hypothesis" demonstrates that cancer cells grow preferentially in non-physiological conditions of hypoxia, low pH and can survive varying temperatures.[2] The cancer stem cell hypothesis suggests that only a small number of cells within a tumour may be capable of sustaining tumour growth, but that these cells may be even more impervious to environmental changes or therapies. Tumour cells which metastasize must be able to migrate via the lymphatic or vascular system and survive anoikis: the process of programmed cell death that normal cells undergo when detached from their extracellular matrix.[3] This study shows that a brief exposure to water is insufficient to trouble these hardy cells.

http://dx.doi.org/10.1016/j.bjps.2016.05.020

Please cite this article in press as: Goldie SJ, Lyons SK, Irrigation of cutaneous squamous cell carcinoma wounds to prevent local recurrence, Journal of Plastic, Reconstructive & Aesthetic Surgery (2016), http://dx.doi.org/10.1016/j.bjps.2016.05.020

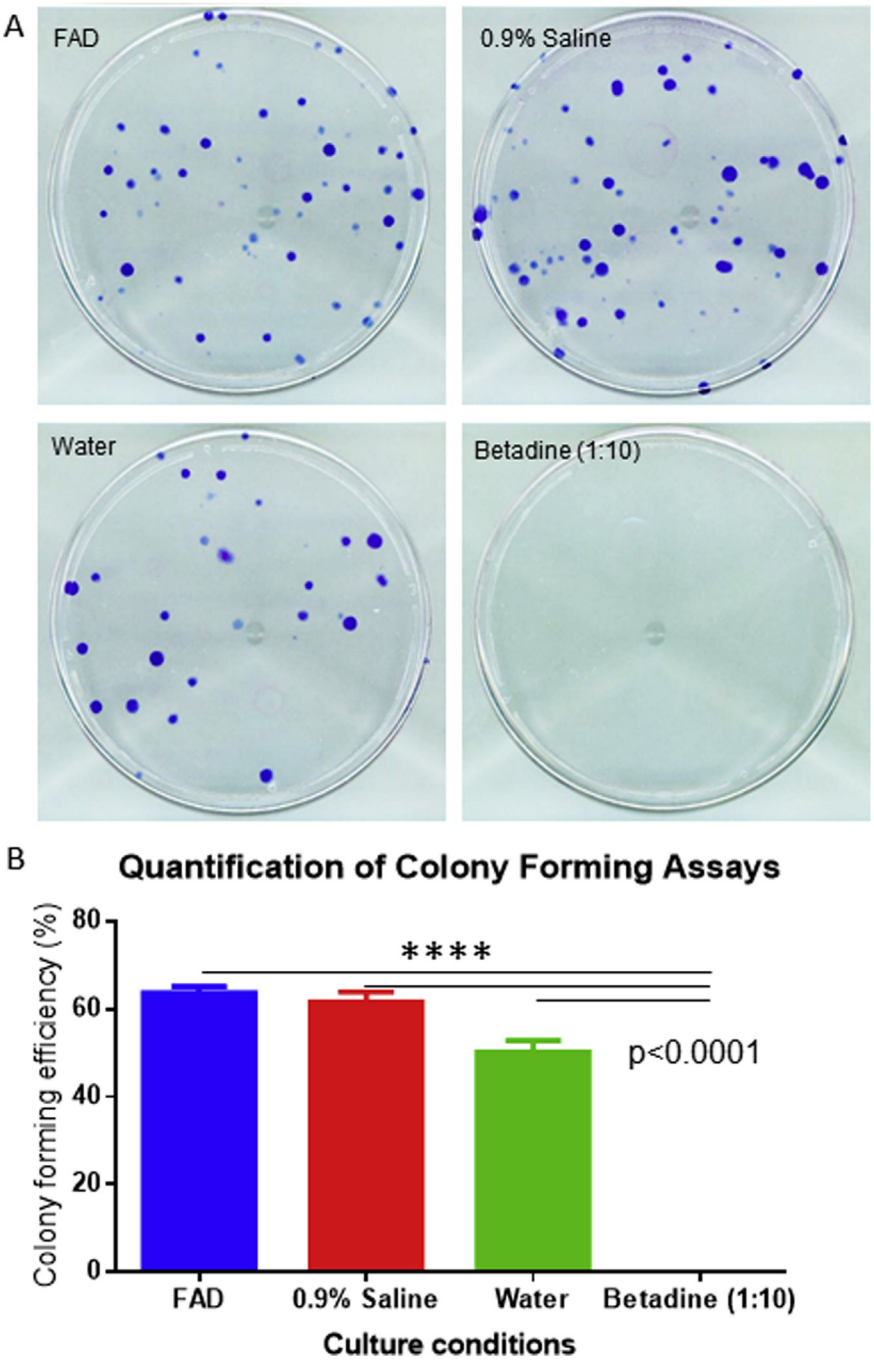

Figure 1 A — Representative plates from CFAs. Plates irrigated with FAD culture medium controls compared with 0.9% saline, sterile water and betadine (1:10) solution (n = 3 per condition, 100 cells per well); B — Quantification of CFAs (colony forming efficiency %), presented as mean and standard deviation. Differences between the betadine solution and the other conditions were extremely significant ($p < 0.0001$).

Collier et al., found that irrigation with water did nothing to prevent recurrent tumours in their rat model, whereas solutions of monoxychlorosene and mechlorethamine reduced local recurrence rates to 5% and 3% respectively.[4] However, these toxic solutions caused irritation, and delayed wound healing, so did not enter clinical practice. Our findings are concordant with data from head and neck cell lines irrigated *in vitro*. Lodhia et al., found a modest effect on survival when cells were irrigated with water, but 99% of cells were killed when plates were rinsed with 5% povidone-iodine or 1.5% hydrogen peroxide solution.[5]

This study benefits from the use of human SCC lines being tested on human dermal samples; perhaps a more realistic model than previous animal studies. However, the experiments still do not completely emulate the clinical

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