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Sustainable healthcare: A future for all

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Cold Atmospheric Plasma in the Treatment and Sensitisation of Radiation Therapy in Extensive Skin Field Cancerisation.

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BACKGROUND

Skin cancer is the most common cancer diagnosis in Australia of which cutaneous squamous cell carcinomas (cSCCs) are the major cause of morbidity and mortality, particularly in Queensland[1]. Radiotherapy (RT) is the primary treatment for cSCC in patients that are not surgical candidates, however the application is limited by skin toxicity[2], and some patients develop multiple skin cancers in large area of sun-exposed skin in a disease known as extensive skin field cancerisation (ESFC; Figure 1). Cold atmospheric plasma therapy delivered as a Plasma Activated Medium (PAM) is emerging as a powerful new cancer therapy.[3] CAP is generated through the ionisation of a gas such as argon at room temperature (Figure 2) with a device such as the kINPen® (developed by neoplas GmBH). The reactive oxygen and nitrogen species (RONS) generated by CAP can be captured in a fluid medium such as culture media or a hydrogel and is termed a plasma-activated medium (PAM). PAM allows for more versatile treatment delivery and is particularly attractive as a topical therapy in the management of skin conditions. PAM can be applied topically to skin cancers and has the potential to be combined with RT. We added different concentrations of PAM to a standard dose of ionising radiation (IR) in cultured A431, KJD and SCC25 SCC cells.

PROJECT AIM

To determine the efficacy of PAM alone or in combination with radiation therapy in the treatment of cSCC.

METHODOLOGY

Cell lines representing cSCC were plated at 5000 cells per well in 96 well black glass bottom plates and incubated at 37°C overnight. The next day cells were treated with various concentrations of 10PAM[4] (0, 20, 50, 70 and 100%; Figure 2) and 1 hour later, half the samples were treated with 5Gγ irradiation. The plates were incubated overnight, and cell viability was measured at 24 hours post-PAM treatment. To measure cell viability, cells were stained with an optimised concertation of Hoechst 33342 and Propidium Iodide (PI) and imaged on the InCell 6500. Cell analysis was performed using the IN Carta analysis software. Statistical Two-way ANOVA was performed using Graphpad Prism.

RESULTS AND ANALYSIS

There was a dose-dependent decrease in cell viability following RT (5Gγ) (Figure 3A, B, C). Cell viability also significantly decreased with higher concentrations of PAM (statistically significant differences in comparison 0% PAM are indicated). Combining different concentrations of PAM (0-100%) with RT (5Gγ) led to an enhancement of cell death in all 3 cSCC cell lines, however, the additive effects appeared more pronounced when used with lower concentrations of PAM (e.g. 20%). These observations will require further validation with other low doses.

CONCLUSION AND FUTURE DIRECTIONS

This data suggests that PAM at low doses (20%) in combination with RT (5Gγ) could be useful in enhancing cSCC treatment. Further studies will employ use of lower doses of PAM, additional cSCC models and ex-vivo cultured cSCC tumouroids and organoids.

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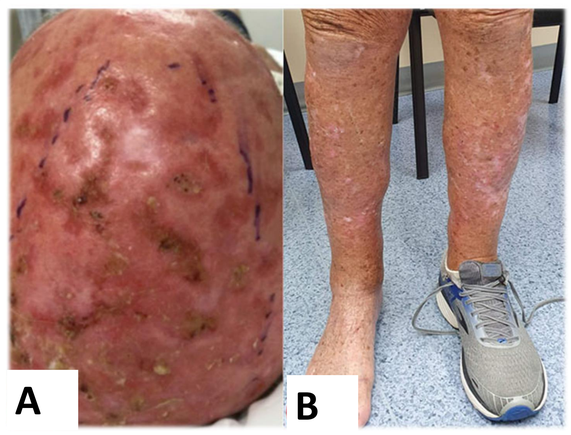

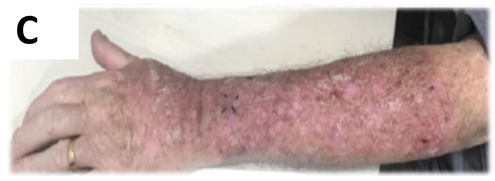

Figure 1: Large areas of sun-exposed skin demonstrating field cancerisation on (A) scalp, (B) lower limb and (C) forearm.[5]

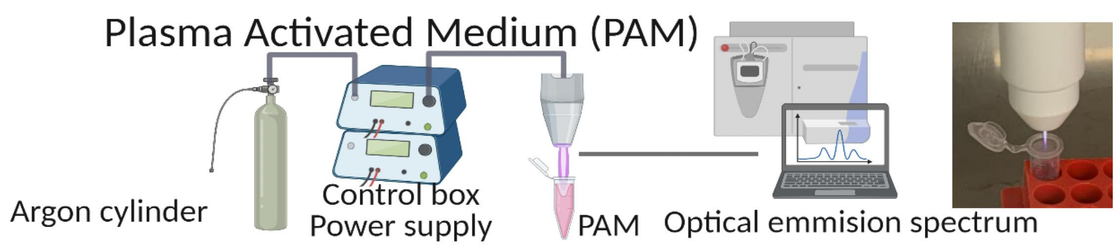

Figure 2: Schematic graph of the CAP device set up. Model presentation of the CAP generation with the kINPen 09 for PAM generation.

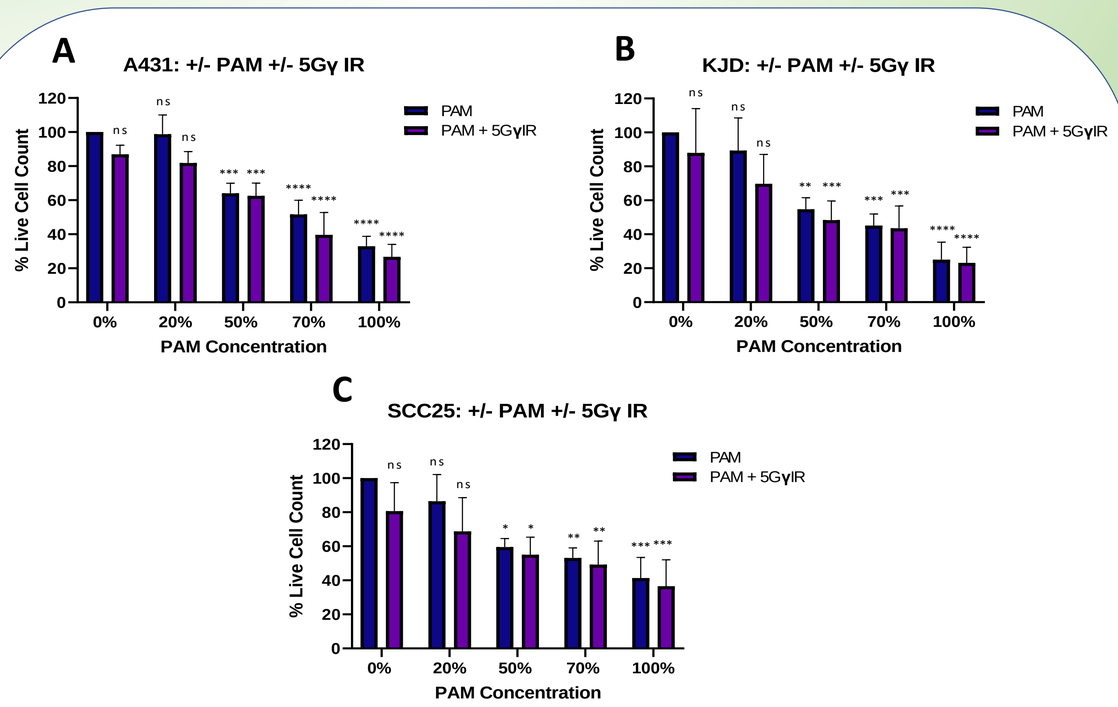

Figure 3: PAM and RT (IR) effects on **A)** A431, **B)** KJD and **C)** SCC25 cells over 24 hours. PAM was added 1hr prior to RT (5Gγ) and Live Cell Count determined by Live-Dead Cell analysis. Representative of 3-4 repeat experiments.

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