Effects of pH Neutral, Super-Oxidized Solution On Human Dermal Fibroblasts *In Vitro*

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BACKGROUND: Super-oxidized solutions (SOSs) have been shown to be efficient antimicrobials and disinfectants. However, the potential oxidative stress of SOSs on eukaryotic cells has never been documented.

OBJECTIVE: To evaluate the potential cytotoxicity and oxidative damage of a novel, pH-neutral SOS (nSOS, Microcyn®) on primary diploid – human dermal fibroblasts (HDFs)

METHODS:

- HDFs were isolated from normal human neonatal foreskin
- Cell viability, necrosis & apoptosis was evaluated by flow cytometry
- Nucleic acids were isolated for gel and RT-PCR analysis
- DNA oxidation levelswere measured by 8-OHdG ELISA assay
- Chronic oxidative stress was evaluated by SA-ß-Gal staining
- Hydrogen peroxide (HP) was used as the positive control of oxidative damage for all experiments.

RESULTS:

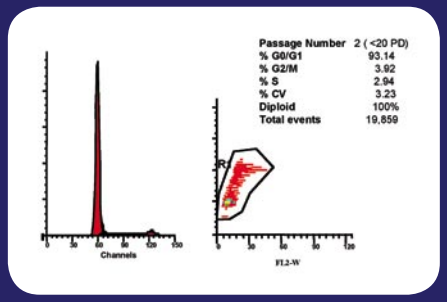

Figure 1. Cell cycle analysis of HDF cultures
This is a representative DNA histogram of a HDF culture at passage 2 (< 20PD) in which more than 93% of the population was on phase G_0-G_1 (n= 3).

When used at concentrations indicated for wound care, HP was significantly more toxic than nSOS. After 5 and 30 minutes of exposure, cell viability was 38% and 5% in 880 mM HP-treated cells versus 75% and 70% in nSOS-treated populations, respectively. HP induced both, apoptosis and necrosis, whereas nSOS induced only necrosis (Fig 2).

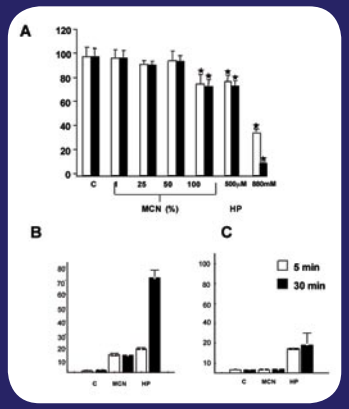

Figure 2. Cell Viability, Necrosis & Apoptosis

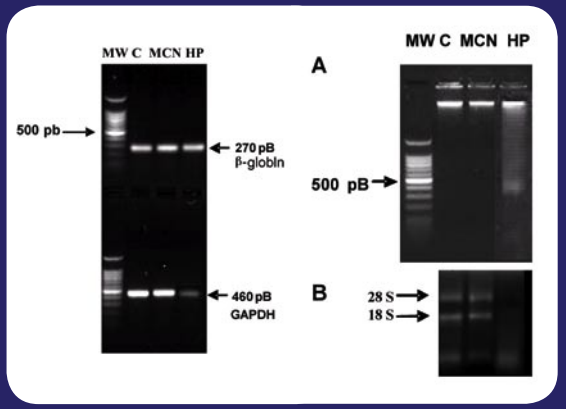

Figure 3. RNA & DNA integrity
These experiments show that only the acute exposure of HDFs to 500 mM HP induced gDNA and RNA degradation whereas undiluted MCN did not.

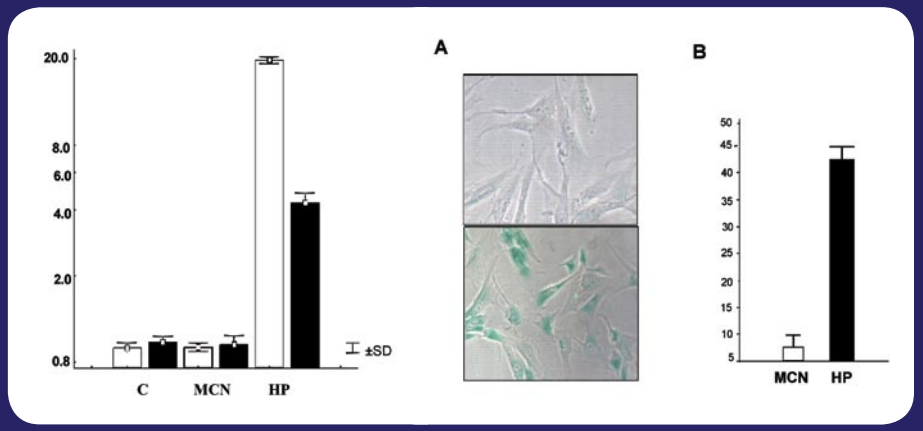

Figure 4. DNA Oxydation
500 µM HP induced the formation of 8-hydroxy-2'deoxiguanosine (8-OHdG) adducts in HDFs whereas these were not observed in saline- or nSOS-treated cells. Panel A & B. The expression of Senescence-Associated-ß-Galactosidase (SA- ß-Gal) was only elevated in cells exposed to 5µM HP -but not in those exposed to 10% nSOS- for 1 month.

CONCLUSION: Undiluted nSOS (Microcyn®) is significantly less cytotoxic than antiseptic-HP concentrations (i.e. 880 mM) *in vitro*. nSOS does neither induce genotoxicity or accelerated aging as sublethal-HP concentrations do it.