



In vitro cellular protective effect of FR Pro ophthalmic viscosurgical device (OVD) in free radical chemical and phaco energy damage simulations

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Experimental Procedure

1. 3t3 mouse fibroblasts cultured in Dulbecco's Minimal Essential Medium (MEM) +10 % Foetal Bovine Serum (FBS) were seeded at a density of 10,000 cell/well in 96 well plates (6 different treatment wells repeated 12 times) and incubated at 37 °C, 5% CO₂ 95% relative humidity for 24 hours.
2. Cell media was aspirated and cells were coated with 50 µl of an OVD: one each of FR Pro, Provisc, Healon, Ocucoat or no OVD control.
3. 200 µl of Buffered saline solution (BSS) was then added over the OVD treated and control test wells. For the lower phaco energy experiment a 7th well x 6 had 4% sorbitol added to the BSS, to assess free radical scavenger effect.
4. A Megatron phaco probe was carefully placed below the surface of the BSS to ensure OVDs were not disturbed.
5. Each well was then exposed to either 0, 1 (at 40% power) or 3 (at 60% power) seconds of ultrasound time on the linear chop mode setting.
6. Following exposure BSS and OVDs were aspirated using a vacuum pump and pasture pipette, and 100 µl of DMEM culture medium + 10% FBS added to each well.
7. Wells were incubated at 37 °C, 5% CO₂ , 95% relative humidity for 20 minutes
8. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and Calcein-AM staining.
9. The experiments were repeated up to n=3 (3 sec) & n=2 (1 sec), multiple t-test analysis was performed on experiments with n=3



Results 1

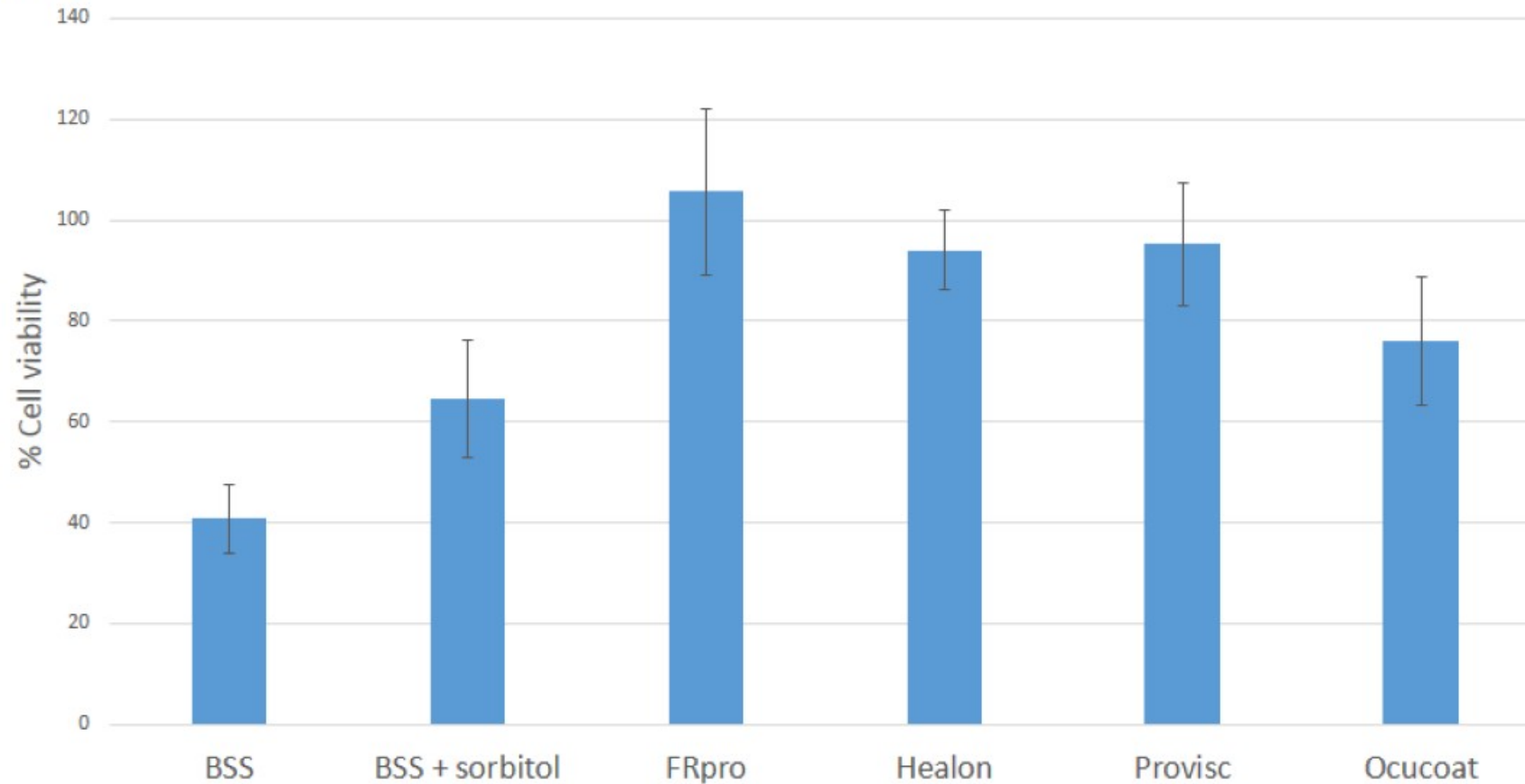


Figure 1. 3T3 Cellular viability post 1 sec ultrasound time: 3t3 Cells were exposed to 1 second ultrasound time at 40% power in linier chop mode. Cell viability was quantified using MTS assay. The cell viability for all OVD protected cell groups were significantly greater than BSS control. With BSS + 4% sorbitol also providing additional protection. (n=2)

Results 2

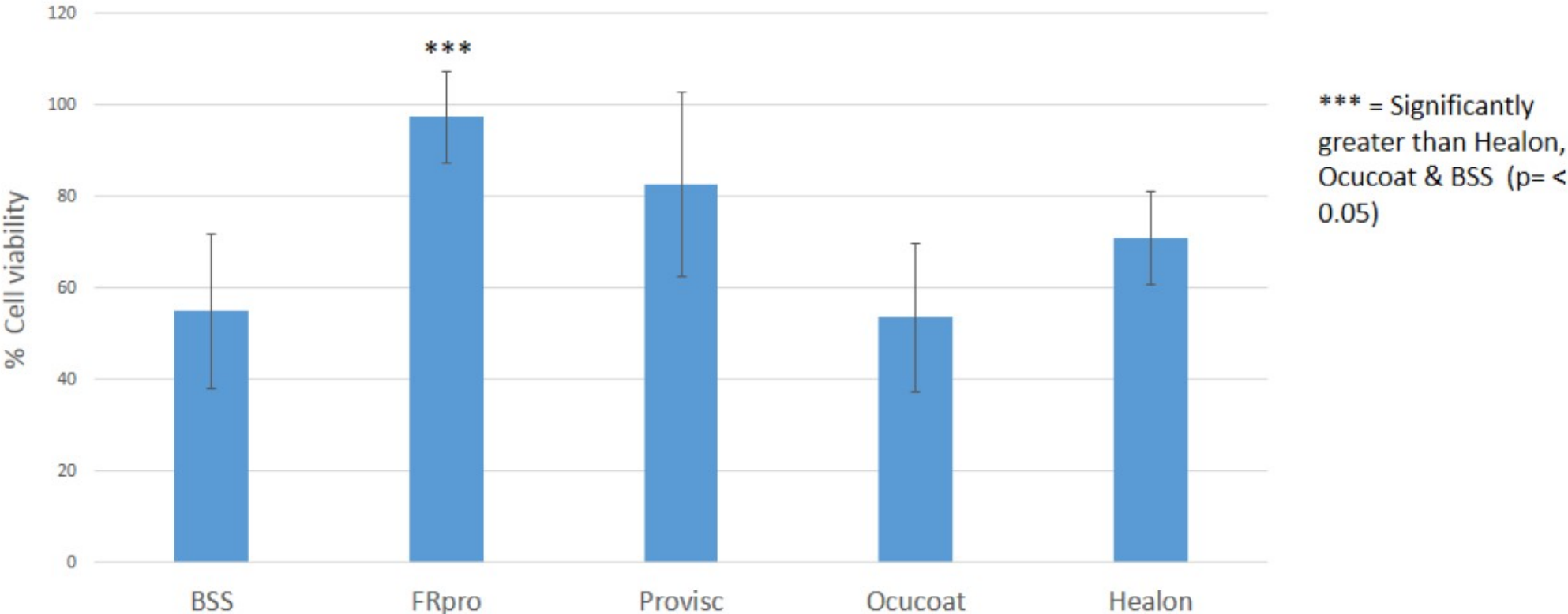


Figure 2. 3T3 Cellular viability post 3 sec ultrasound time: 3t3 Cells were exposed to 3 second total ultrasound time at 60% power in linier chop mode. Cell viability was quantified using MTS assay. FR Pro provided the greatest level of cell protection significantly greater than Healon, Ocucoat and BSS. (n=3)

Results: Cell viability staining (Calcein-AM)

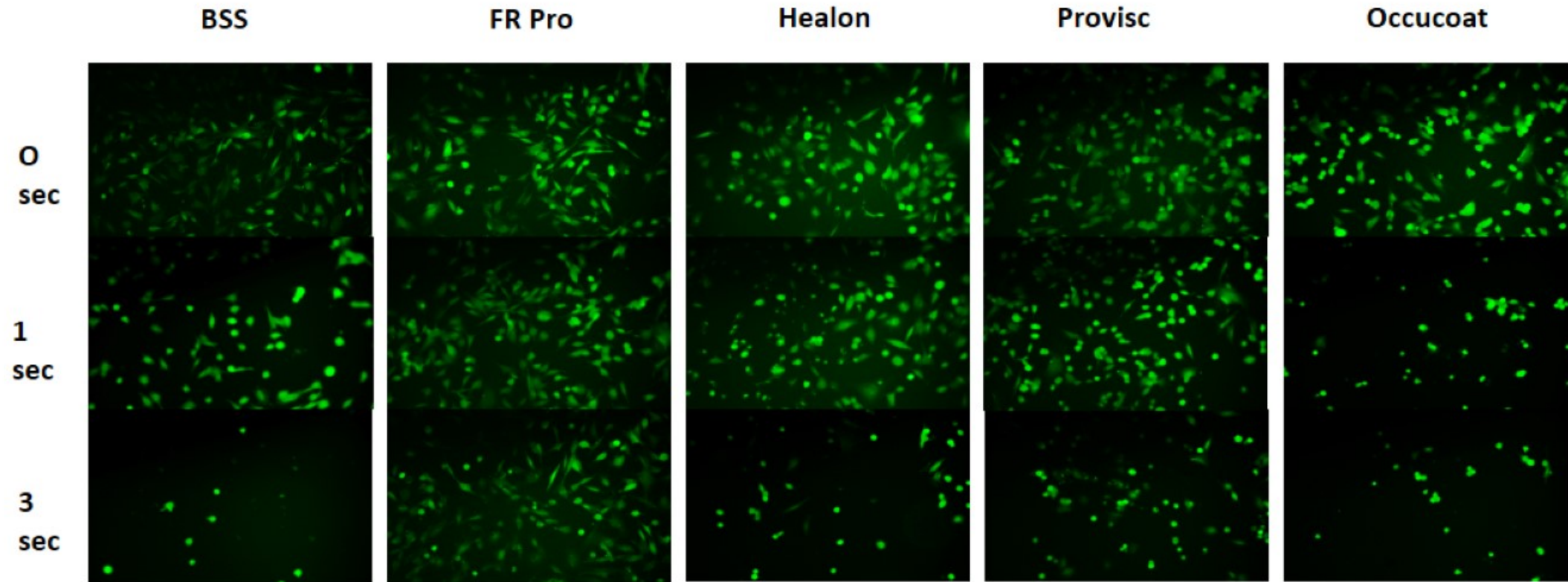


Figure 3. 3T3 Cellular viability post 0, 1 & 3 sec ultrasound time: Cell viability and morphology were visually assessed using Calcein-AM staining. The results closely match that of the MTS assay supporting the quantitative analysis findings. FR Pro provided the best level of cellular morphological protection following all exposures, with typical healthy spread fibroblastic form. While all other OVDs presented with spherical or reduced morphology.

Conclusions

1. 1 & 3 seconds of ultrasound time exposure was found to significantly damage cells without OVD protection.
2. FR Pro provided the best level of cell protection after 1 and 3 seconds of ultrasound exposure for cell viability and morphology preservation.
3. Provisc performed well at both exposure conditions in terms of cell viability being slightly lower than that of FR Pro but not statistically significantly so. However, at 3 seconds ultrasound exposure reduced cell morphology was observed for Provisc as well as the other OVDs, with the large majority of cells with a reduced spherical appearance.
4. Healon & Ocucoat provided poor protection at 3 seconds ultrasound time with significantly reduced cell viability compared to FR Pro.
5. BSS + 4% Sorbitol was shown to provide significant protection compared the BSS alone demonstrating that the free radical scavenger Sorbitol does provide added protection against Phaco free radical damage. This finding suggests additional protective effect of FR Pro which contains both 2% Hyaluronic acid and 4% Sorbitol.

What this study adds:

This study confirms the importance of ultrasound time and power on cellular damage and therefore their role in phaco-induced corneal endothelial complications. Furthermore, this study demonstrates the significant, but unequal, protective effect of different OVDs in cataract surgery, as well as the benefit of the free radical scavenger sorbitol.