

Cytotoxicity of Clinical Irrigants in Primary Human Knee Fibroblasts

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INTRODUCTION: In the context of total knee arthroplasties (TKA), prevention of periprosthetic joint infection (PJI) using irrigation solutions is a standard procedure in the operating room. However, little is known about the effects of different irrigants on the resident cell populations in the knee. The purpose of this study was to evaluate the toxicity of common clinically-used irrigants in primary human knee fibroblasts *in vitro*.

METHODS: Following Institutional Review Board approval, patients undergoing primary total knee arthroplasty (PTKA 1-3) or revision total knee arthroplasty for arthrofibrosis (RTKA-A) were consented, and their knee tissue collected. Knee fibroblasts from these tissues were isolated by collagenase I digestion and subsequently cultured in Advanced MEM media supplemented with 5% human platelet lysate, heparin, GlutaMAX™, and antibiotic/antimycotic. At confluence (Day 0), cells were treated with either normal saline (NS, 0.9%) for 3 minutes or one of the following clinical irrigants for 1 and 3 minutes: chlorhexidine (CH, 2%), acetic acid (AA, 3%), hydrogen peroxide (HP, 3%), Dakin's (DK, 0.0125%), or povidone-iodine (PI, 0.35%). These are commonly used concentrations of these irrigants in clinical practice. After treatment, cells were thoroughly washed with phosphate buffered saline and fresh media as administered. The following day (Day 1), two cell viability assays were performed: MTS (metabolic activity) and Hoechst (DNA staining as a proxy for cell number) (Table 1). Additionally, images were taken at 24 hours to characterize cell morphology and cell loss (not shown).

RESULTS: Substantial cell toxicity was observed with all irrigant treatments and durations compared to NS (Table 1 and Figure 1). There were no significant differences in individual irrigants when comparing treatment duration (1 vs 3 minutes). In the primary TKA cell lines (PTKA 1-3), AA and HP were most effective in reducing metabolic activity (MTS) and cell number (Hoechst). CH was consistently the least cytotoxic, followed by DK. For the arthrofibrosis cell line (RTKA-A), the above trend partially reversed, with CH causing the most reduction in metabolic activity, while DK caused the most reduction in cell number. HP reduced metabolic activity and AA reduced cell number the least in RTKA-A cells. PI remained intermediate amongst the irrigants but trended toward a milder reduction in both metabolic activity and cell number across PTKA and RTKA-A cells. Microscopic images confirmed extensive cell loss in the cultures (data not shown).

DISCUSSION: This study has established the cytotoxicity of irrigation solutions on resident fibroblasts obtained in the setting of TKA. These irrigants are cytotoxic to these resident cells in culture, in concentrations commonly used clinically, an effect to be considered when these solutions are used for their intended infection target in PJI.

SIGNIFICANCE/CLINICAL RELEVANCE: This study offers information regarding the cellular toxicity of various irrigant solutions used to prevent infection in the setting of TKA.

IMAGES AND TABLES:

Cell Line	Assay	Time	% Reduction Relative to Saline				
			CH	AA	HP	DK	PI
PTKA 1	MTS	1 Min	80.3 ± 2.5	98 ± 0.9	96.8 ± 0.6	78 ± 1.2	95.9 ± 2.0
		3 Min	83.9 ± 2.0	97.5 ± 0.3	95.2 ± 1.5	94.7 ± 0.7	97.3 ± 0.04
	Hoechst	1 Min	69.3 ± 1.5	77.1 ± 1.9	84.9 ± 0.7	76.6 ± 0.9	76.9 ± 0.2
		3 Min	70.8 ± 1.9	78.1 ± 1.3	86.4 ± 0.2	80.4 ± 0.9	83.4 ± 0.3
PTKA 2	MTS	1 Min	93.3 ± 1.0	97.9 ± 1.1	94.1 ± 1.6	87.5 ± 0.4	87.4 ± 0.4
		3 Min	88.8 ± 1.6	97.5 ± 0.9	93.7 ± 0.9	95.7 ± 0.8	94.2 ± 0.9
	Hoechst	1 Min	73.4 ± 0.4	88.4 ± 0.4	76.9 ± 0.2	74.6 ± 0.1	77.8 ± 0.6
		3 Min	79.6 ± 0.4	89.2 ± 0.2	79.9 ± 0.9	74.7 ± 0.1	89.5 ± 0.1
PTKA 3	MTS	1 Min	75.2 ± 2.4	98.9 ± 0.5	98.9 ± 0.8	97.7 ± 0.1	89.6 ± 0.8
		3 Min	80.7 ± 1.5	99.0 ± 3.0	99.2 ± 0.2	97.6 ± 0.3	92.1 ± 0.5
	Hoechst	1 Min	72.5 ± 0.5	76.2 ± 0.6	77.1 ± 1.7	79.1 ± 1.8	84.8 ± 1.1
		3 Min	77.2 ± 0.5	80.1 ± 0.6	79.2 ± 0.6	73.5 ± 1.5	79.9 ± 0.9
RTKA-A	MTS	1 Min	88.2 ± 0.2	84.5 ± 0.2	67.2 ± 0.5	86.3 ± 0.2	76.9 ± 1.6
		3 Min	88.6 ± 0.2	84.6 ± 0.2	72.5 ± 0.5	88.8 ± 0.4	84.8 ± 0.3
	Hoechst	1 Min	74.8 ± 4.0	73.1 ± 2.8	80 ± 2.2	92.5 ± 2.0	68.7 ± 5.6
		3 Min	80.5 ± 6.3	71.3 ± 2.0	85.4 ± 1.0	93.8 ± 0.4	79.2 ± 1.9

Table 1. MTS and Hoechst assays indicate extensive cell death. Metabolic activity (MTS activity) and cell number (Hoechst staining) were assessed 24 hours after irrigation treatment for 1 or 3 minutes. Data is shown as percent reduction from normal saline (n = 3, mean ± SD).

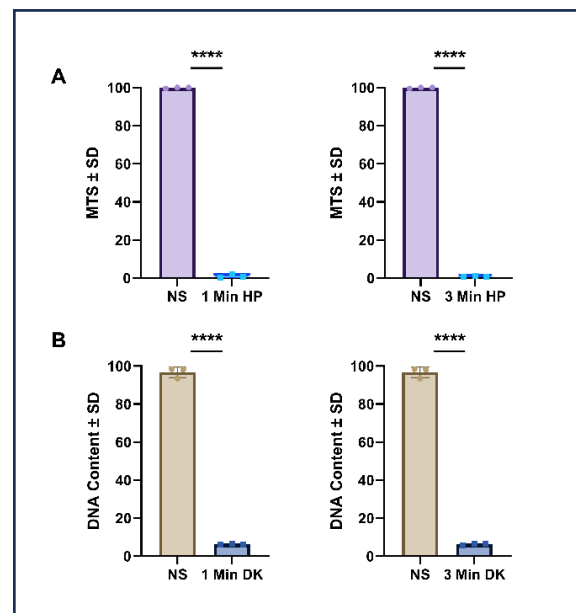


Figure 2. Hydrogen Peroxide and Dakin's Solution reduces metabolic activity and cell number.

Examples of data presented in Figure 1. Metabolic activity following 1 and 3 minutes of hydrogen peroxide (HP) treatment in PTKA 3 (A) (n = 3, mean ± SD). Hoechst staining following 1 and 3 minutes of Dakin's solution in RTKA-A (n = 3, mean ± SD). p = <0.0001).