Correlating Akt Signaling Molecule Activation to Cytocompatibility of Photoinitiators

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Statement of Purpose: Photoinitiators are essential in triggering UV/visible light curing, a processwidely used to make a variety of drug formulations and tissue engineering scaffolds (Williams CG. Biomaterials. 2005;26:1211-8). AKT is a key signaling molecule, regulating cell proliferation,survival, invasion, and angiogenesis (Sheng S. J Cell Physiol. 2009;218:451-4). In the current study, we correlate the cellular toxicity ofthree UV sensitive photoinitiators including eosin Y-based photoinitiating system, 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2methyl-1-propanone (Irgacure 2959) and dimethoxyphenylacetophenone (DMPA) toAKT activation, providing a new insight into how to evaluate photoinitiator cytocompatibility. In addition, effects of free radicals generated by UV light were also examined.

Methods: Eosin Y photoinitiator stock solution was prepared by 0.1% eosin Y, 4%1-vinyl-2 pyrrolidinone(NVP) and 40%triethanolamine(TEOA) in PBS (Desai PN.Biomacromolecules. 2010;11:666-73). The prepared photoinitiator solutions were protected from light and stored at room temperature until use. HN4 cell was selected as our model due to its high p-AKT expression. Cell viability was determined by WST-1 assay and intracellular AKT activation was evaluated by Western blot analysis. In order to decompose the photoinitiator into free radicals, these photoinitiators were exposed to UV light at 365 nm with an intensity of 100 watts for 30 min. Pre-seeded HN4 cells on a 96-well plate were then subjected to these photoinitiators right after UV radiation.

Results: WST-1 assay indicated that HN4 cells can tolerate a wide range of concentrations of eosin Y and low concentrations of Irgacure 2959 (≤ 1 mg/ml) but not DMPA. Consistent with cytotoxicity, eosin Y had no effect on intracellular AKT inactivation but both Irgacure 2959 and DMPA concentration-dependently induced intracellular AKT deactivation (Figs. 1-3). However, HN4 cells failed to maintain cell viability (Fig. 4) and intracellular AKT activity (data now shown) in all three photoinitiating systems after UV exposure. To further demonstrate the stability of free radicals on cytocompatibility, the cell culture media were mixed with eosin Y to reach a final concentration of 25μl/ml, and they were exposed to the UV light for 30 min, kept for various lengths of time, and tested for toxicity effects. The results indicate that the presence of free radicals could persist up to 48 h given the fact that HN4 cells failed to maintain cell viability in eosin Y photoinitiating system after UV exposure (Fig. 5). However, toxicity of eosin Y was diminished following curing suggest most free radicals participated in photo-initiated polymerization in the presence of monomers.

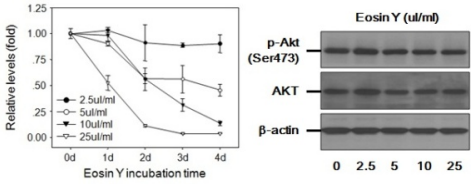

Figure 1. Effects of Eosin Y on HN4 cells.

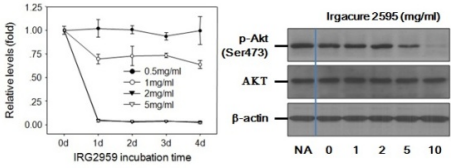

Figure 2. Effects of IRG2959 on HN4 cells.

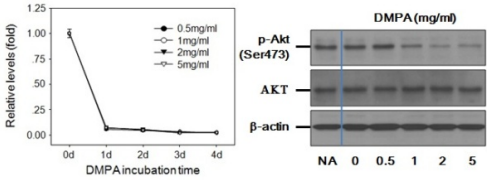

Figure 3. Effects of DMPA on HN4 cells.

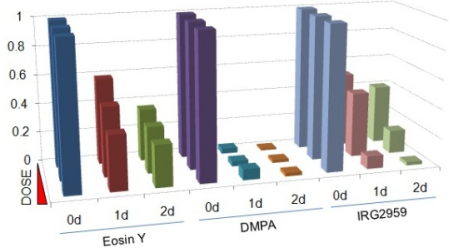

Figure 4. Effects of UV-exposed photoinitiators on cell viability of HN4 cells.

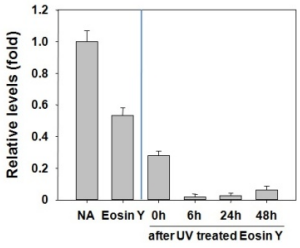

Figure 5. Effects of free radicals introduced by the UV exposure of Eosin Y on cell viability of HN4 cells.

Conclusions: AKT activity correlates well with cytocompatibility of photoinitiators, providing a new way to examine their potential toxic effects. Eosin Y-based photoinitiator is more cytocompatible than Irgacure 2959 and DMPA. Free radicals introduced by curing represent a significant source of cytotoxicity and warrants further examination for optimal doses for curing.

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