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Nitroxides as Free Radical Scavengers in UHMWPE

[1]Marina K. Chumakov, [1]Alicia Zack, [2]Joseph Silverman, [2]Mohamad Al-Sheikhly

[1]Fischell Department of Bioengineering, [2]Department of Materials Science & Engineering
University of Maryland, College Park, MD 20742 U.S.A.

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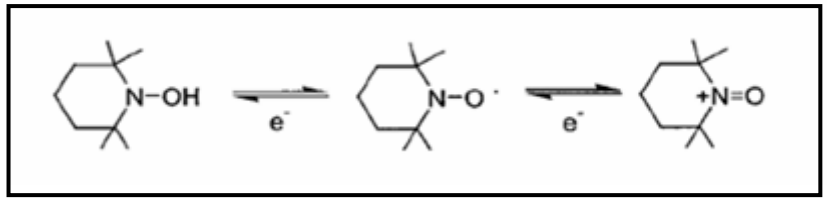
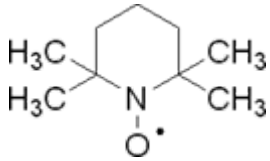
Outline

- Nitroxides
  - Objectives: investigate radical reactions, while using a lower infiltration temperature
  - Clinical applications
  - Possible reaction mechanisms
- Infiltration Prior to Irradiation
  - Radical interaction with dose
- Post-irradiation Infiltration
  - Residual Nitroxide Concentration
- Future Work

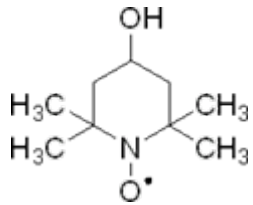
Nitroxides, a class of antioxidants

- Electron transfer mechanism
- Long radical lifetime, stable
- MRI contrast agent
- Spin labeling reagents, EPR probes
- Polymerization
  - Nitroxide Mediated Radical Polymerization (NMRP)
- Radioprotectants *in vivo*
  - Reactive oxygen species: O_2^-, H_2O_2
  - C-centered radical trapping
  - Lipid peroxidation prevention
- Induces apoptosis in hypoxic cancer cells
- Infiltration through solution or thermal treatment





2,2,6,6-Tetramethylpiperidine-1-oxyl
(TEMPO)



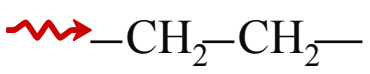
4-Hydroxy-2,2,6,6-Tetramethylpiperidine-1-oxyl
(TEMPOL)

http://www.physics.utoronto.ca/~key/PHY138/

Krishna, M.C., et al. *J. Med. Chem.* **41** 347 (1998).
Soule, B. P., et al. *Free Radical Biology & Medicine* **42** 1632-1650 (2007)
Goldstein, S. and A. Samuni. *J. Phys. Chem. A* **111** 1066-1072 (2007).

Proposed Mechanisms of Oxidative Degradation Protection

Free Radicals Produced in UHMWPE



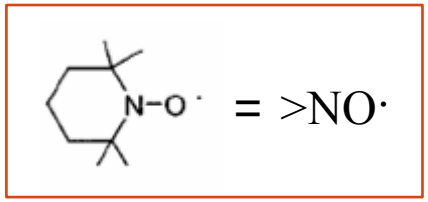
$-CH_2-CH_2-$ $R\cdot$ Alkyl $R\cdot + O_2 \rightarrow RO_2\cdot$ Peroxyl

$-RH=RH-\dot{R}H-$ Allyl $ROOH \rightarrow RO\cdot + OH$ Alkoxyl

Nitroxides-

TEMPO
(2,2,6,6-Tetramethylpiperidine-1-oxyl)

TEMPOL
(4-Hydroxy-TEMPO)



Addition

$R\cdot + >NO\cdot \rightarrow >NOR$

Electron Transfer Mechanism

$RO_2\cdot + >NO\cdot \leftrightarrow$ intermediate $\rightarrow >N^+=O + RO_2^-$

Hydrogen Transfer Mechanism

$R\cdot + >NOH \rightarrow >NO\cdot + RH$

$RO\cdot + >NOH \rightarrow >NO\cdot + ROH$

$RO_2\cdot + >NOH \rightarrow ROOH + >NO\cdot$

Krishna, M.C., et al. *J. Med. Chem.* **41** 347 (1998).
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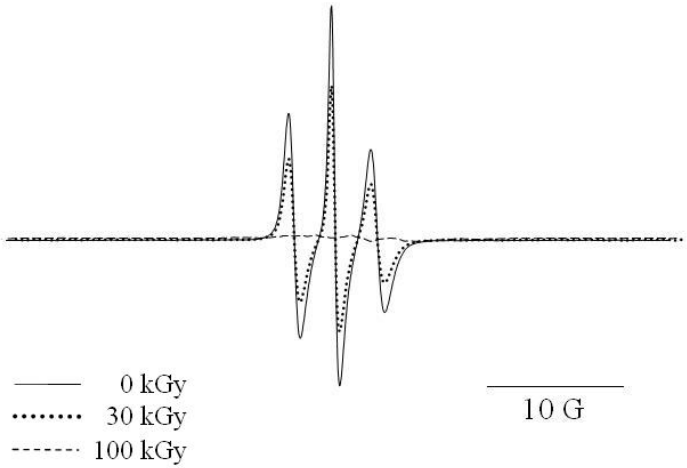
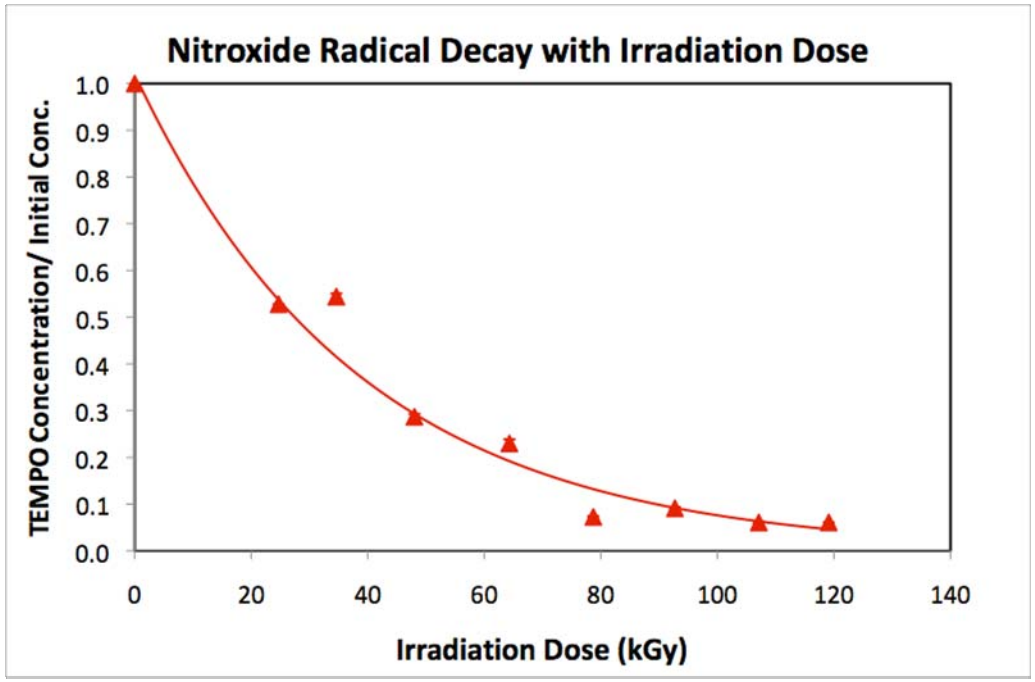
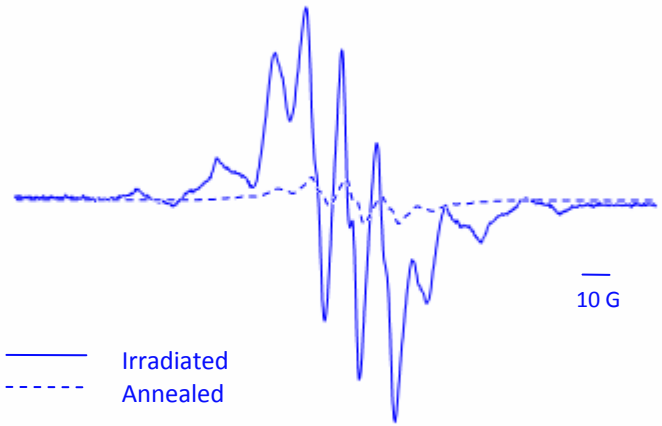
Infiltration Prior to Irradiation

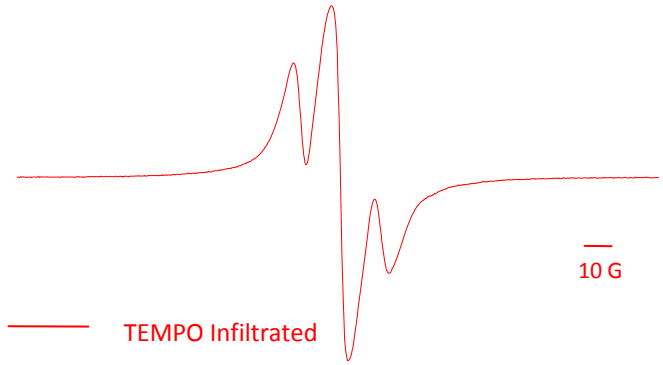


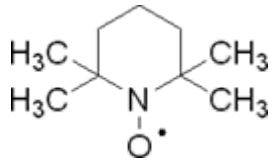


- UHMWPE Infiltrated with TEMPO
- Carbon-centered free radicals formed in UHMWPE interact with TEMPO, reducing its paramagnetic concentration

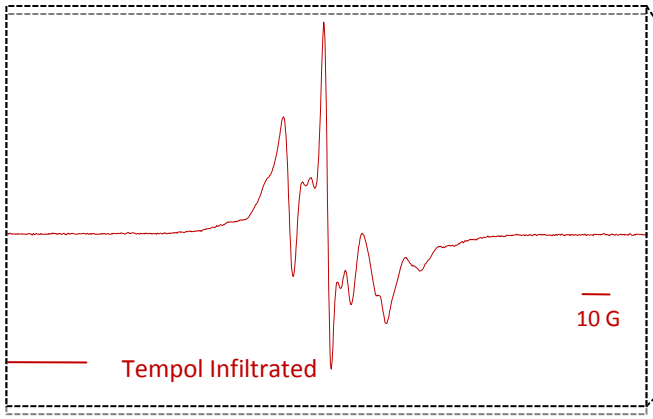
Post-Irradiation Scavenging



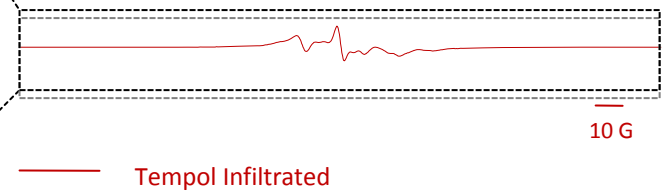




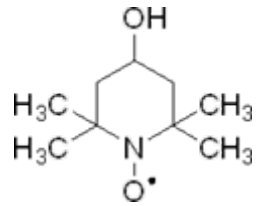
Reduced 40x



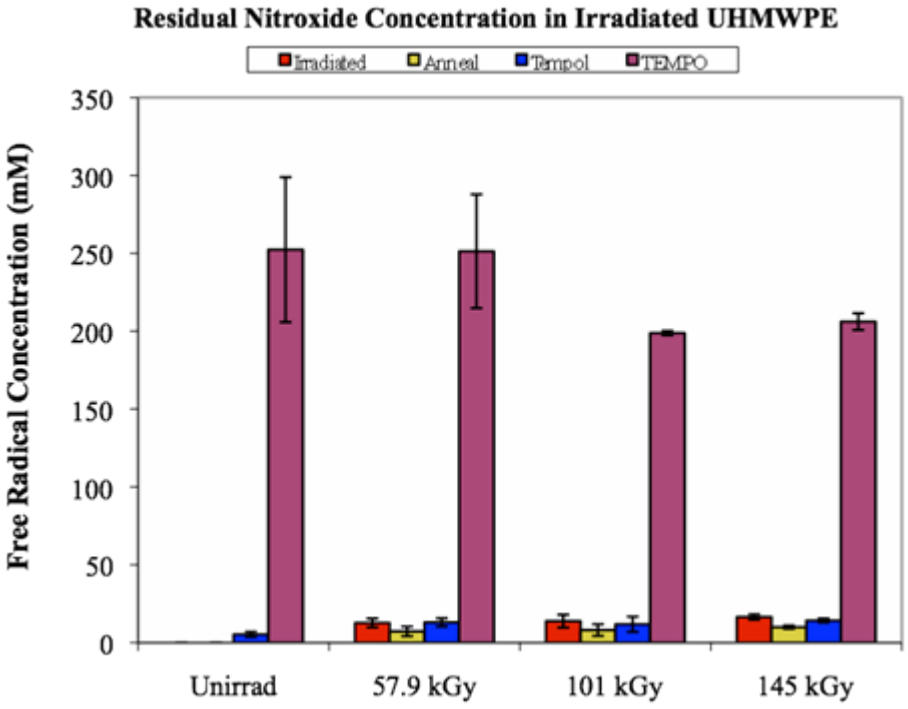
Reduced 4x

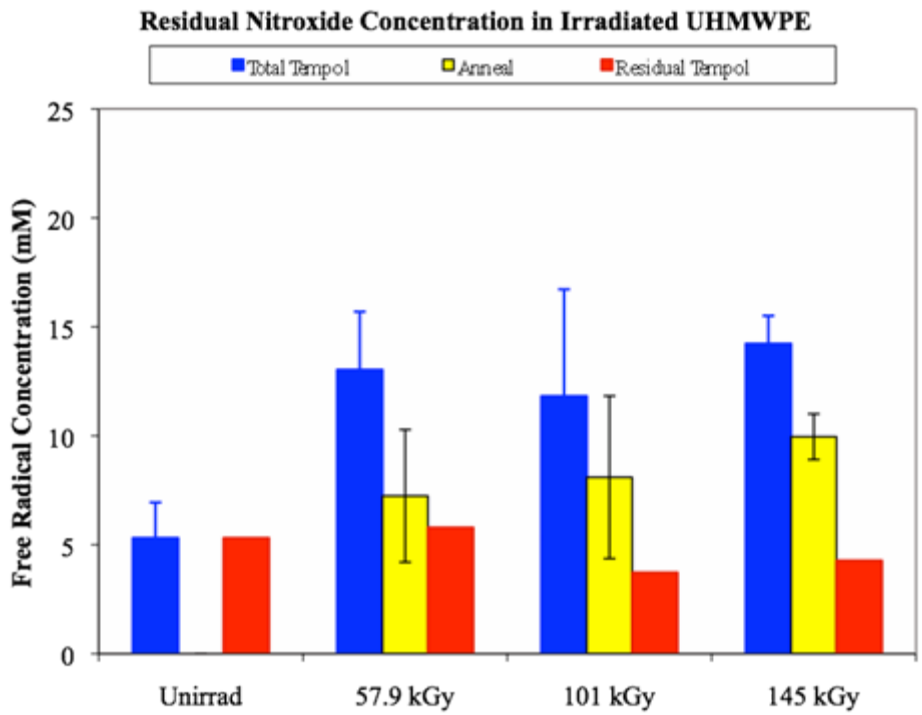


Reduced 40x



Residual Nitroxide Concentration





- 5 minutes 80ºC annealing and infiltration
- Tempol "resistant" to penetration, requires higher temperatures
  - Infiltration at lower temperatures→ safe concentrations, maintained properties

Conclusions and Future Questions…

- Nitroxide infiltration is a useful method of investigating reactions of carbon-centered free radicals
- Post-irradiation controlled diffusion of nitroxides may provide for optimal concentration in a practical process

- Spectral subtractions & simulations to resolve remaining carbon-centered radical concentration
- Cross-link density
- Oxidative stability
- Diffusion analysis
- Pin-on-disk wear testing

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Thank you for your attention!

- Questions?

Toxicity of Nitroxide Compounds

- Paradox: cytoprotective & cytotoxic in different cell types
  - Sensitize hypoxic cells to radiation (cancer cells)
  - Super oxide dismutase mimics (prevent oxidative damage in some cells)
- Higher toxicity tendency for lipophilic compounds
  - Tempol 200x more hydrophilic than Tempol
- TEMPO: $IC_{50} = 0.72 \pm 0.05$ mM in endothelial cells
- Neurophysiological toxicity
  - 1 mM Tempol minimal effect
  - 1 – 5 mM TEMPO significant neurophysiological effect

Table 1. Antiproliferative Effect of TEMPOL on Different Human and Rodent Neoplastic and Nonneoplastic Cell Lines

Cell Line	Tumorigenic Potential	MDR Phenotype	$IC_{50} \pm SE$ (mM)
Breast			
HBL-100	−	−	0.944 ± 0.082
MCF-7/WT	+	−	0.208 ± 0.023*
MCF-7/ADRR	+	+	0.410 ± 0.048*
MDA-MB-231	+	−	0.464 ± 0.063*
Colon			
LoVo/WT	+	−	0.499 ± 0.039
LoVo/DX	+	+	0.303 ± 0.059
HCT 116	+	−	0.380 ± 0.060
Liver			
BRL-3A	−	−	1.073 ± 0.070
MH1-C1	+	−	0.773 ± 0.038†
Ovary			
CHO-K1	−	−	0.891 ± 0.227
NIH: OVCAR-3	+	−	0.222 ± 0.020‡

Mean ± SE of four to six experiments.
Statistically significant differences were assessed by the analysis of variance, followed by Duncan's test for multiple comparisons.
* $p < 0.05$ vs. HBL-100.
† $p < 0.05$ vs. BRL-3A.
‡ $p < 0.05$ vs. CHO-K1.

Gariboldi, M.B. et al. *Free Rad. Biol. & Med.* **24** (6) 913 (1998).

Exploit hydrophilicity of Tempol: low toxicity and controlled infiltration