# Biocompatibility Study of Novel Poly(ethylene oxide) Hydrogels

King, R; Ward, J*; Sekharipuram, V; Hanes, M
DePuy Orthopedics, Inc., Warsaw, Indiana
*Univ. of Toledo, Toledo, Ohio

**Introduction** Poly(ethylene oxide) and poly(ethylene glycol) have been used extensively in consumer and pharmaceutical applications. They are classified as non-toxic and bio-inert. A novel PEO hydrogel was developed for demanding biomedical applications[1]. Earlier study[2] showed that fibroblast cell does not adhere to the PEO hydrogel. The purpose of this study was to determine biocompatibility of the PEO hydrogel and to characterize modified PEO hydrogels, in composite form and in semi-interpenetrating network form.

**Materials and Process** The conventional PEO hydrogels were made by solution process. The novel PEO hydrogels were fabricated by a three-step process: (1) molding of a PEO disc or a PEO composite disc, (2) high-energy radiation treatment in a reduced oxygen environment, and (3) hydration of the crosslinked PEO or the crosslinked PEO composite. The following PEO hydrogels were prepared using the new fabrication process:

| Hydrogel | Second Component | % 2nd Comp., post-molding | Radiation Dose | Structure |
|---|---|---|---|---|
| PEO | ----- | ----- | 50 KGy Gamma | Network |
| CH-PEO | Chitosan | 9 % | 50 KGy Gamma | Semi-Interpenetrating Network |
| HA-PEO | Hydroxy-apatite | 33 % | 50 KGy Gamma | Composite Network |

Commercial grade of PEO, WSR-303 (Dow), was used for fabrication of all PEO hydrogels. Reagent-grade of hydroxyapatite (Aldrich) and 75%-deacetylated chitosan (1 million molecular weight, Aldrich) were used to fabricate modified PEO hydrogels.

**Methods** Gel fractions were calculated based on the formula: ($W_{Dried\ Gel} / W_{Solid\ PEO}$). Swell ratios were calculated based on the formula: ($W_{Hydrogel} / W_{Dried\ Gel}$). Gel strength at ambient temperature were measured by compression of disc samples (~0.60"D x 0.275"H) between parallel plates on an MTS tester until fracture at a crosshead speed of 0.4"/min.
Biocompatibility of the PEO hydrogel was assessed by: (1) physicochemical test for non-volatile residue, (2) in-vitro hemolysis study, (3) cytotoxicity study by ISO elution method, and (4) USP and ISO systemic toxicity study. Cell adhesion tests were performed on two modified PEO hydrogels, CH-PEO and HA-PEO, along with polystyrene (positive control) and silicone rubber (negative control) in triplicate. The L-929 mouse fibroblast cells were seeded on one side of each test material and incubated for 18 hours in growth media. Following incubation, the adhered cells were harvested by trpsinization and counted.

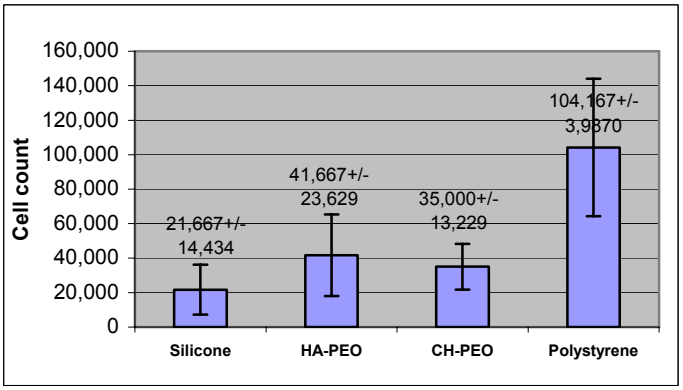
**Results** Table I shows that the PEO hydrogel and chitosan-PEO hydrogel have improved compressive strengths in comparison with the conventional PEO hydrogel (8% WSR-303 PEO solution, 50 KGy Gamma).

Table I Characteristics of PEO hydrogels

| | Conventional PEO | PEO | CH-PEO | HA-PEO |
|---|---|---|---|---|
| Gel Content | ----- | 67.8 % | 73.4 % | 69.9% |
| Swell Ratio | 12.5 | 16.1 | 13.2 | 12.0 |
| Compressive Strength | < 16 psi | 70.5 psi | 49.3 psi | < 20 psi |
| Strain at Fracture | < 35 % | 60.6 % | 55.2 % | < 30 % |

Unlike PEO, chitosan is not crosslinked by radiation treatment. Visual examination of chitosan-PEO hydrogel reveals existence of distinct second (chitosan) phase. Hydroxyapatite-PEO hydrogel has comparable compressive strength as the conventional PEO hydrogel. High loading of hydroxyapatite in the PEO matrix negatively impacts PEO hydrogel structural integrity. As expected, the PEO hydrogel passed all four biocompatibility tests. Table I shows cell adhesion test results. Incorporation of chitosan or hydroxyapatite in the PEO hydrogel has positive impacts on cell adhesion. However, statistically there are no differences between the modified PEO hydrogels and the negative control.

Figure 1 Cell counts on various substrates after 18-hour incubation




**Conclusions** The preliminary screening tests show that the PEO hydrogel is biocompatible. Further animal implantation study is required for determination of its in-vivo stability and suitability for long-term implant applications. Like PEO hydrogel, PEO hydrogels modified with chitosan or hydroxyapatite do not have sufficient cell affinity for use as cell scaffolds. Nevertheless, they are potential material candidates for anti-adhesion implant and drug delivery implant applications.

**References** 1. US patent pending. 2**.** R. King, et al Orthopedic Research Society 2006; 892