Table 1: Summary of Biocompatibility testing performed on Replication Medical HPAN Implants.

Test Type	Test Title	Results
Cytotoxicity	L929 MEM Elution Test	No biological activity (Grade 0) was observed in the L929 mammalian cells at 48 hrs. post exposure to the test article abstract. Thus, the HPAN 80/HPAN 90 materials were shown to be non-cytotoxic.
	L929 MEM Elution Test	No biological reactivity (Grade 0) was observed in the L929 mammalian cells at 48 hours post exposure to the test article extract. Based on the criteria of the protocol, the Vessel Guard material is considered non-cytotoxic and meets the requirements of the Elution test ISO 10993-5 guidelines.
	L929 MEM Elution Test	Grade 4 biological was observed in the L929 mammalian cells at 48 hours, but no signs of reactivity (Grade 0) were observed for the 1:2, 1:4 and 1:8 dilutions of the test article. Based on the results of this test, the material is considered non-cytotoxic for reasons outlined in the Cytotoxicity memo dated 24Mar11.
	L929 MTT Cytotoxicity Test	75% viability was observed in the L929 mammalian cells at 24 hours. Cell viability percentages for the negative and positive control were greater and lower than 70.0%, respectively, confirming the validity of the assay. Based on the results of this test, the material is considered non-cytotoxic.
Sensitization	Skin Sensitization Kligman Maximization Test	Extracts of the test article elicited no reaction at the challenge (0% sensitization) following induction phase. This was defined as a Grade 1 reaction and the test article is classified as having weak allergenic potential. Grade 1 sensitization is not considered significant.
	Skin Sensitization Kligman Maximization Test	The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the Vessel Guard material, elicited no reaction at the challenge (0% sensitization), following an induction phase. Therefore, as defined by the scoring system of Kligman, this is a Grade 1 reaction and the test article is classified as having weak allergenic potential. Based on the criteria of the protocol, a Grade 1 sensitization rate is not considered significant and the test article meets the requirements of the ISO 10993-10 guidelines.
	Kligman Maximization Test	Extracts of the test article elicited no reaction at the challenge (0% sensitization) following induction phase. This was defined as a Grade 1 reaction and the test article is classified as having weak allergenic potential. Grade 1 sensitization is not considered significant.
Intracutaneous reactivity	Intracutaneous Injection Test	The results from this study indicated that the test sites did not show a significantly greater biological reaction than the control article site. The Primary Irritation Index for both the NaCl and CSO extracts of the test article was 0.0 and the extracts were characterized as negligible irritants.
	Intracutaneous Injection Test	The test article sites did not show a significantly greater biological reaction than the sites injected with the control article. Based on the criteria of the protocol, the test article is considered a negligible irritant and meets the requirements of the ISO 10993-10 guidelines.
	Intracutaneous Injection Test	The results from this study indicated that the test sites did not show a significantly greater biological reaction than the control article site. The Primary Irritation Index for both the NaCl and CSO extracts of the test article was 0.0 and the extracts were characterized as negligible irritants.
Systemic toxicity (acute toxicity)	Systemic Injection Test	None of the test or control animals exhibited overt signs of toxicity at any of the observation points. The test was considered negative for acute toxicity.
	Systemic Injection Test	The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the HPAN 80 and PET material did not induce a significantly greater biological reaction than the control extracts, when tested in Swiss Albino mice. This test was considered negative based on standards set by ISO 10993-11.

Test Type	Test Title	Results
	Systemic Injection Test	The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the HPAN 80 material did not induce a significantly greater biological reaction than the control extracts, when tested in Swiss Albino mice. This test was considered negative based on standards set by ISO 10993-11.
	Rabbit Pyrogen Test (Material Mediated)	The test article is considered non-pyrogenic.
	Rabbit Pyrogen Test (Material Mediated)	Based on the criteria of the protocol, the test article is considered non-pyrogenic and meets the requirements of the Pyrogen Test, ISO 10993-11 guidelines.
	Short Term Intramuscular Implantation Test (Rabbit)	The results indicated that the test article was non-toxic when implanted for 14 days.
	Short Term Intramuscular Implantation Test (Rabbit)	The results indicated that the test article as non-toxic when implanted for 4 weeks. (Toxicity Rating -0.03)
	Short Term Intramuscular Implantation Test (Rabbit)	The results indicated that the test article was non-toxic when implanted for 8 weeks. (Toxicity Rating 0.77)
Genotoxicity	Mouse Lymphoma Mutagenesis Assay	Based on the criteria of the study, the test article is considered non-mutagenic.
	Rodent Bone Marrow Micronucleus Assay (38 Animals)	The 0.9% USP Sodium Chloride for injection extract of the test article did not induce a statistically significant increase in micronucleated cells as compared to the negative control at 24 and 48 hrs. after dosing. The test article is considered non-mutagenic.
	Salmonella Typhimurium & Escherichia Coli Reverse Mutation Assay	Results of both the activated and non-activated assays indicated that the test article did not produce a statistically significant increase. Thus, the test article is considered non-mutagenic under the experimental conditions utilized.
	Salmonella Typhimurium & Escherichia Coli Reverse Mutation Assay	A statically significant increase in the number of revertant colonies was not observed with either of the test article extracts as compared to negative controls in non-activated and activated conditions. All positive controls exhibited a statistically significant increase in the number revertant colonies as compared to the corresponding negative controls in both non-activated and activated conditions, validating the functioning of the assay. Based on the criteria of the study protocol, the test article, Vessel Guard, is not considered to be mutagenic in the test species under the experimental conditions utilized.

Test Type	Test Title	Results
	Salmonella Typhimurium and Escherichia Coli Reverse	A statically significant increase in the number of revertant colonies was not observed with either of the test article extracts as compared to negative controls in non-activated and activated conditions. All positive controls exhibited a statistically significant increase in the number revertant colonies as compared to the corresponding negative controls in both non-activated and activated conditions, validating the functioning of the assay. Based on the criteria of the study protocol, the GelFix™ material is not considered to be mutagenic in the test species under the experimental conditions utilized.
Implantation	Intramuscular Implantation Test	The HPAN 80 test material was considered non-reactive in rabbit muscle after thirteen weeks. There were no significant differences at the interface between the control and test sites.
	Long Term Intramuscular Implantation Test (Rabbit)	The results indicated that the test article was non-toxic when implanted in muscle for 6 months.
	A Study to Determine the Safety of an Artificial Disc Device in the Bovine Spine Model	The host tissues, intervertebral or spinal, showed no adverse reaction to the presence of the device material evaluated at multiple time points up to one year. The device material was well tolerated by the host tissues. Blood chemistry and organ pathology were normal.
	Histological and Qualitative Evaluation of a Hydrogel Cloth in the Sheep Lumbar Spine Model	HPAN 80 vessel guard was implanted on the anterior lateral aspect of the spinal column in a sheep model. A total of 9 sheep were evaluated at 3 times points (3wk, 6wk, and 13wk) for implant mobility, resistance and histology. Implant was easily mobilized from its surgical site in 7 of 9 cases evaluated. Histology showed normal healing response with minimal foreign body reaction which subsided over time between 3 weeks and 13 weeks. A thin synovial like layer of tissue was observed separating the implant from the surrounding muscle and bone.
	Histological and Qualitative Evaluation of a Hydrogel Cloth in a Sheep Lumbar Spine Model Phase II	Purpose of the study was to evaluate adhesion barriers comprised of sheets made up of hydrogel surrounding a PET weave cloth. HPAN 80 on PET and HPAN 80 alone was compared to a commercially available product Gore Preclude for use in spine surgery procedures to prevent tissues from adhering to one another and allow ease of access. Results of study show that cell adherence to HPAN implants was similar to cell adherence to Gore however HPAN implants showed reduced cell attachment at 30 days compared to Gore Preclude and showed reduced tissue attachment based upon retrieval force. Bone found near the implant appeared normal with some perisosteal surfaces closest to the implants undergoing bone formation.
	Intramuscular Implantation	The HPAN 80 material, when implanted in rabbit paravertebral muscle tissue, demonstrated no difference from the control implant site (Bioreactivity Rating of 1.5) after 13 weeks.
Neurotoxicity	Particulate Biocompatibility and Safety Study in Rabbit Epidural and Intradiscal Space	HPAN 90 hydrogel particles were injected into New Zealand White rabbit epidural and intradiscal space. Histological analysis of the spinal specimens at injected levels was performed at 30 and 90 days post-injection. Hydrogel particles elicited minimal inflammatory response which declined with time. Hematology, serum chemistries and clinical observations were conducted at the two time points and the results were normal.

Test Type	Test Title	Results
Chronic Toxicity	Long Term Muscle Implantation & Chronic Toxicity in Rats	The results indicate that there were no adverse histopathological changes associated with the test article.
Carcinogenicity	Not required.	
Hemocompatibility	*In Vitro* Platelet Aggregation Assay - ISO Indirect Contact	The results indicated that the Vessel Guard material, when compared to controls, did not possess an intrinsic platelet aggregation effect in the absence of ADP, nor did it modulate ADP induced platelet aggregation.
	In Vitro Hemocompatibility Assay - ISO Indirect Contact	The Vessel Guard material did not have any adverse effects on any of the hematological parameters tested. Based on the evaluation criteria of the study protocol, the test article passes the test for *in vitro* hemocompatibility.
	Hemolysis – Human Blood – ISO Indirect Contact	The HPAN 80 material exhibited 0.4% hemolysis. Therefore, the material is considered non-hemolytic under the experimental conditions employed.
	Unactivated Partial Thromboplastin Time Assay – ISO Indirect Contact	The UPTT of the plasma exposed to test article extract was not significantly decreased when compared plasma. Therefore, the HPAN80 material meets the requirements of the UPTT test.
	Prothrombin Time Assay – ISO Indirect Contact	The results indicated that the average Prothrombin Time fell within the normal range for human plasma. Based on the results, the Vessel Guard material did not have an adverse effect on the prothrombin coagulation time of human plasma.