

**MEMO TO THE RECORD
PMA P030032**

DATE: August 1, 2003; revised November 3, 2003
FROM: David Krause, Biologist/Expert Reviewer
OFFICE/DIVISION/BRANCH: ODE/DGRD/PRSB, HFZ-410
DEVICE NAME: Hylaform® (Hylan B Gel)
APPLICANT: Genzyme Corporation; One Kendall Square; Cambridge, MA 02139

INDICATIONS FOR USE:

Hylaform is indicated for the correction of nasolabial fold wrinkles.

DEVICE DESCRIPTION:

Hylaform Description:

Hylaform (Hylan B) is a sterile, nonpyrogenic, viscoelastic, clear, colorless, transparent gel composed of cross-linked molecules of hyaluronan. Hylan is a derivative of hyaluronan (sodium hyaluronate) and consists of repeating disaccharide units of N-acetylglucosamine and sodium glucuronate. Hylan B is produced by chemically cross-linking hylan molecules to form an infinite molecular network. It is water-insoluble, viscoelastic and highly hydrated. The hydration fluid is isotonic physiological sodium chloride solution.

Hylan B gel slurry contains hylan B polymer at a concentration of 4.5 to 6.5 mg/ml, in a hydration fluid of 0.15 M NaCl. The osmolality of hylan gel is approximately 290 to 330 mOsm. The average size of particles in hylan gel slurry is approximately 200 to 700 microns. The level of heavy metals is less than 2 ppm. Hylan B is susceptible to degradation by mammalian hyaluronidase, with production of low molecular weight oligosaccharides. Hylan B is also degraded by oxygen-derive free radicals.

Hylan B gel is derived from hyaluronan, present in all intercellular matrices of human connective tissue, where it acts as a tissue stabilizer and elastoviscous shock absorber. Hyaluronan in the dermis, sub dermis and subcutaneous tissue contributes to space filling between the collagen and elastin fibers and cells, and stabilizes the collagen fibrous network. To prevent the rapid turnover of native hyaluronan, the cross-linking processes used in Hylaform manufacture produce an infinite molecular network of hyaluronan that forms a water-insoluble gel.

Chemical Properties:

Hylan is a modified form of the naturally occurring hyaluronan, a glycosaminoglycan. The sodium salt of hyaluronan contains disaccharide units made of sodium D-glucuronate and N-acetyl-D-glucosamine linked together with beta-1,4 glycosidic bonds. These disaccharides are linked by beta-1,4 glycosidic bonds to form long unbranched polysaccharide chains. Hylan B is a polymer resulting from cross-linking reaction of hyaluronan with vinyl sulfone. Vinyl sulfone is a bifunctional molecule in which 2 vinyl groups are attached to a sulfonyl group. Each vinyl group can react with any chemical group containing an active hydrogen atom. The reaction with a hydroxyl group proceeds as follows, with the formation

of an ether bond:



Hylan gel is a hydrogel of cross-linked insoluble hylan B hydrated in 0.15 M aqueous NaCl. The hylan B concentration in the gel is expressed in terms of concentration of the polysaccharide chains of hylan, and is found to be 4.5 to 6.5 mg/ml. The pH range is 6.0 to 7.5.

Device Properties and Formulation:

The hyaluronan in Hylaform is the cross-linked biological polysaccharide hylan B (also called hylan gel). Hylan B is a hydrated gel with the same polysaccharide chain and polyanionic characteristics as native hyaluronan; the viscoelastic properties of hylan B are enhanced as compared to those of native hyaluronan. The hyaluronan in hylan B is derived from the combs of domestic fowl and is chemically cross-linked and hydrated with a hydration fluid composed of water and a physiological concentration of sodium chloride. Hylan B remains in the dermal tissue for a considerably longer period of time compared to native hyaluronan, which diffuses away from the site of injection.

The most important characteristics of the Hylaform formulation, which form the basis of its benefit in tissue augmentation are: 1) Deformable irregularly shaped gel particles of about 500 microns in size; 2) extremely high water content (~ 95.5%), which is maintained in the tissue; 3) very high elasticity; 4) biocompatibility; and 5) the cross-linked gel resists degradation and migration in the extracellular matrix of the skin.

How Supplied:

Hylaform is supplied as a 0.75 ml volume in a single-use 0.9 ml glass syringe with a protective sleeve, a needle-locking device and 2 sterile needles. Contents of the syringe are sterile and nonpyrogenic. Each 0.75 ml of Hylaform contains 4.1 mg of hylan B gel, 6.4 mg of sodium chloride, and USP water for injection to comprise a total volume per syringe of 0.75 ml. The units are to be stored at 2°C - 30°C and are not to be frozen. The syringe is a Hypak® glass syringe manufactured by Becton Dickinson and is a legally marketed device. The 30 gauge x 1/2" needles provided are also legally marketed medical devices. The syringe with the Hylaform and needles are provided in a polyethylene terephthalate glycol tray with a blister lid. These packages are placed into cardboard boxes.

PRODUCT HISTORY:

Hylaform® (under the name of Hylan B Gel) was studied (not controlled or masked) in the US under IDE G900060 (Protocol BXCP 515) at 6 sites (216 patients) and in Sweden (Protocol 151:107) at 8 sites (63 patients). In these two studies, a total of 279 patients with depressed cutaneous scars and/or wrinkles/folds were treated at 843 individual facial sites by 1,466 individual Hylaform® injections. The original PMA application for Hylan B Gel (P950028) was submitted on August 1, 1995. FDA had issues with the study design, and based on the information in the PMA, FDA did not approve the product.

Biomatrix Inc., submitted a new study protocol for Hylan B Gel (now called Hylaform®) in IDE G000315. The study was conditionally approved in a letter dated January 5, 2001. Biomatrix Inc. subsequently was purchased by Genzyme Biosurgery of Ridgefield, New Jersey, a subsidiary of Genzyme Corporation. Representatives of Genzyme Biosurgery met with members of the Agency staff on January 23, 2002 to address several key issues specific to the protocol and assessment of safety and effectiveness. The IDE was unconditionally approved in a letter dated May 21, 2002.

On October 10, 2002, the Agency sent Genzyme Biosurgery an agreement letter in regards to the endpoints of the study. The agreements listed in that letter were as follows:

1. The following identified features of the study:

- ?? Control (Zyplast);
- ?? Inclusion/exclusion criteria;
- ?? Validated photographic assessment as primary effectiveness endpoint;
- ?? Performing an initial superiority analysis where superiority is determined by a difference of greater than 1 on the Blinded Independent Panel Review (IPR) Photographic Wrinkle Assessment Score (See Agreement #2);
- ?? If superiority is not confirmed, performing a subsequent non-inferiority analysis (Non-inferiority is pending the standard deviation, delta and sample size obtained from the Zyplast data collection study.); and
- ?? Duration of the study (12 weeks);

are appropriate for determining non-inferiority (or superiority as per fourth bullet), with respect to effectiveness, of Hylaform as compared to the Control in the following indication for use: Hylaform is indicated for the correction of nasolabial fold wrinkles. The Zyplast data collection is to be provided to confirm the estimate of the standard deviation and sample size.

2. The primary effectiveness analysis will be an analysis of the blinded Independent Panel Review (IPR) wrinkle assessment scores at 12 weeks after the last implantation of the device. Baseline score will be included as a covariate.
3. Based on an appropriate training procedure and demonstration of intra-rater and inter-rater reliability (0.75 kappa score) and the Lemperle et al article, the Genzyme photo scale is an appropriate tool for the scoring of nasolabial folds. Please note that the Agency has not yet seen this training procedure to determine whether or not it is appropriate.
4. The results of the Zyplast data collection, looking at before (time 0) and after (at 12 weeks) photos of 30 patients using the same methodology as described in the approved IDE clinical protocol and with the same follow-up time period and blinded Independent Panel photo assessment scores, will be appropriate for estimating the standard deviation of the control group using the Genzyme six-point grading scale.

Genzyme Biosurgery decided to submit a Modular PMA and submitted a Shell Proposal. The Shell Proposal was accepted on January 23, 2002. Module 1 was submitted on October 1, 2002 and received on October 2, 2002. Module 1 contained Volume 1 of the PMA while this module, Module 2, contains Volume 2 and 3 of the PMA. Module 2 contains the Preclinical Testing and Chemical Analysis of Hylaform.

MODULE M020015/02 REVIEW:

BIOLOGICAL TESTING:

Short Term Biological Tests:

Irritation:

The objective of these tests was to evaluate the potential of hylan B Gel to cause irritation in rabbits. Two studies were conducted for this purpose, an intracutaneous toxicity study and a subcutaneous implantation study. Under the conditions of these studies, hylan B did not produce any evidence of inducing local irritation.

Intracutaneous Toxicity Study in the Rabbit (Study BXR-25204-F-I):

Hylan B was injected into each of five intradermal sites in two rabbits. Injection sites were examined 24, 48, and 72 hours post injection for erythema and edema. No evidence of significant irritation or of other signs of local toxicity were evident. It was concluded that hylan B causes no local short term irritation in the dermis.

Subcutaneous Implantation Study (with Histopathology) in the Rabbit (2 day) (Study BXR-23004-I):

Rabbits were injected with hylan B, four sites in each rabbit. Rabbits were euthanized after 2 days, and macroscopic and microscopic evaluations reactions were not significant as compared to reactions caused by the control article (saline). Hylan B was classified as a nonirritant.

Sensitization and Immunogenicity:

The objective of these studies was to test the potential irritation, sensitization and immunogenicity of hylan B. Under the conditions of these tests, hylan B gel did not elicit a humoral or cellular response nor did it show potential to cause dermal sensitization.

Immunization and Subchronic Intramuscular Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits (Study BXR-23006-I):

This study was performed in order to assess the humoral and cellular immunity of hylan B, Collagen I (Zyplast®) or Collagen II (Zyderm®) in the presence and absence of adjuvant following repeated administrations of the test articles. Rabbits were injected 6 times intramuscularly on days 1, 29, 36,

43, 50, and 57. If adjuvant was used, Freund's Complete Adjuvant was used with the first injection and Freund's Incomplete Adjuvant was used with all the rest. At 43 and 85 days, each rabbit received an intradermal injection of the corresponding test article as a challenge dose. Injection sites were monitored for erythema and edema 1-3, 24 and 48 hours post injection. Clinical observations were made weekly and animals were checked twice daily for mortality and moribundity. Body weights were recorded weekly and at necropsy. Blood was collected within 7 days prior to the first injection, then at 1 day prior to day 28, 56, and day 91-94 at necropsy. At the end of the study, a complete gross necropsy was performed on all rabbits. Complete histopathological examination included the test article injection site and the challenge injection site. Two identical sets of serum samples from all study groups and all time points were analyzed.

No animals died during the study and there were no differences in mean body weights. Signs of local irritation were minimal and the maximal response observed in hylan gel treated animals was Grade 1 erythema or Grade 1 edema. Collagen treated rabbits produced Grade 2 erythema and Grade to edema. Animals injected repeatedly with hylan B gel without adjuvant did not develop significant serum immune reactivity to the hylan B gel. In 7 of 20 of these animals a titer was detected to avian proteins and/or endotoxin. These study animals exhibited no general health reactions or significant local reactions, nor did they produce positive reaction to skin testing. Both hylan B gel and collagen elicited a greater humoral immune response in the presence of adjuvant than in the absence of adjuvant. The results indicate that the antibody response to repeated injections of hylan B gel was adjuvant mediated because in the absence of adjuvant there was no significant titer.

Guinea Pig Dermal Sensitization Test (Maximization Study Using Degraded Hylan Gel) (Study BXR-20501-I):

This study was conducted to determine the skin sensitization potential for hylan B following hyaluronidase digestion. The test material was administered intradermally in two rigorous induction phases, first intradermally, then topically. Following the induction phase, each treated animal was challenged with the test article using the nonwoven cotton disk contained in a Hill Top Chamber. At 24, 48, 72 and 96 hours the dermal reaction was evaluated. Under the conditions of this test, the hylan B did not exhibit any potential to cause dermal sensitization in the guinea pig.

Delayed Contact Sensitization Study (A Maximization Method) in the Guinea Pig (Study BXR-20008-I):

The objective of this study was to evaluate the potential for hylan B gel to cause delayed dermal contact sensitization. Undegraded hylan B gel was administered intradermally as before. Following induction, each treated animal was challenged with undiluted hylan B using a Hill Top Chamber. At 24, 48, 72, and 96 hours the dermal reaction was evaluated. The same animals were rechallenged at a different topical site 4 days after the 96 hour score. Observations for any reactions were conducted at 24, 48, 72, and 96 hours after rechallenge patch removal. Under the conditions of this study, the hylan B gel test article showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

Cytotoxicity:

In vitro testing was conducted to assess the potential of hylan B to produce cytotoxicity in the fibroblast cell culture model. Two *in vitro* studies were conducted using the L929 mouse cell line: the *In vitro* Cytotoxicity Study (MEM Elution Method) in the L929 Mouse Fibroblast Cell Line (Study BXR-25203-F-I) and the *In vitro* Cytotoxicity Study (Agarose Overlay Method) in the L929 Mouse Fibroblast Cell Line (Study BXR 23005-I). Under the conditions of these tests, hylan B did not produce any evidence of causing cell lysis or cytotoxicity.

Acute Systemic Toxicity:

Acute systemic toxicity was studied to evaluate the ability of hylan B to cause systemic toxicity following intraperitoneal injection into the mouse. The Systemic Toxicity Test in Mice (Study BXR 25200-F-I) gave no evidence of systemic toxicity when the mice were observed after a large dose of hylan B gel was injected at observation times of 4, 24, 48, and 72 hours. There was no mortality or evidence of toxicity in any animals. Based on the results of this study, it was concluded that the injected hylan B was not systemically toxic to mice.

Hemocompatibility:

Hemocompatibility was evaluated using oxalated rabbit blood in an *In vitro* Hemolysis Study (Direct Contact Method) (Study BXR 25202-F-I). In this procedure, hylan B suspended in 0.9% saline solution is added to rabbit blood and incubated. The resulting suspension is compared spectrophotometrically against a negative and positive control sample. Hylan B gel was not hemolytic under these test conditions.

Pyrogenicity:

In order to test the pyrogenic potential of hylan B, three rabbit model studies were employed: USP Rabbit Pyrogen Study (Studies BXR 20001-I, BXR-20002-I, and BXR 20003-I). Reduced viscosity hylan B was injected intravenously into rabbits in a single dose of 10 ml/kg of diluted test article (1/50). The total rise in body temperature during the three-hour observation period was observed. Under the conditions of these tests, hylan B was determined to be nonpyrogenic.

Implantation:

Implantation studies were performed to study the potential of hylan B to cause irritation or toxicity when implanted into living tissue.

Muscle Implantation Study (with Histopathology) in the Rabbit (Study BXR 23003-I):

Hylan B was implanted into 4 test sites per rabbit. The rabbits were sacrificed 7 days after implantation. Macroscopically, all hylan B implants were found to produce a response comparable to the negative control. Microscopically, hylan B was classified as a nonirritant when compared to the USP negative control; the score for hylan B was 0.

Muscle Implantation Study with Histopathology) in the Rabbit (7 Days) (Study BXR 25201-F-I):

Hylan B was implanted into 4 sites in the muscle tissue of rabbits. Rabbits were sacrificed 7 days after implantation. Macroscopically, hylan B implants were found to produce a response similar to the USP negative control. Microscopically, hylan B was classified as a slight irritant when compared to the USP negative control; the score for hylan B was 2.

Reviewer Comment: It is important to note that in this test 0 = nonirritant, 1-15 = slight irritant, 16-30 = moderate irritant, > 30 = severe irritant. Therefore, in this case, hylan B is at the bottom of the slight irritant scale. This is considered to be acceptable.

Muscle Implantation Study with Histopathology in the Rabbit (30 Days) (Study BXR 25205-F-I):

Hylan B was injected into each of four muscle test sites per rabbit. Study animals were sacrificed 30 days after implantation. Macroscopically, hylan B material was found to produce a response similar to the USP negative control. Microscopically, hylan B was classified as a nonirritant with a score of 0.

Mutagenicity:

Hylan B was evaluated for mutagenic and clastogenic potential in a series of test procedures designed for this purpose. No mutagenic or clastogenic potential was detected.

Ames Mutagenicity Test of Degraded Hylan Gel (Study BXR 20202-F-I):

Mutagenic potential of ultrasound degraded hylan B was studied in the Ames *Salmonella typhimurium* model. There was no mutagenic activity detected in this assay at any concentration of hylan B (range 0 – 100%).

Ames Mutagenicity Test of Hylan Gel (Study BXR 20201-F-I):

The same model system as above showed no mutagenic potential of non degraded hylan B solution in similar concentrations as above.

Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation (Study BXR 23000-I):

This study was conducted in order to assess the potential for hylan B to induce a gene mutation at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in CHO cells with and without exogenous metabolic activation. The concentrations of hylan B tested included the standard dose range and a concentration range five times greater than the highest recommended calculation. Six doses were tested in the first assay, four doses were tested in the confirmatory assay. There was no indication of mutagenic activity of hylan B at any of the concentrations tested.

Test for Chemical Induction of Chromosome Aberations in Cultured CHO Cells with and without Metabolic Activation (Study BXR 23001-I):

This study was conducted in order to evaluate the potential of hylan B to induce chromosome aberrations in cultured CHO cells with and without metabolic activation. There was no evidence that hylan B at any of the concentrations tested (up to 500 µl/ml) was able to induce chromosome aberrations in the test cultures, with or without metabolic activation.

Test for Chemical Induction of Morphological Cell Transformation in Cultured BALB/C-3T3 Cells with and without Metabolic Activation (Study BXR 23002-I):

Potential carcinogenic effects of hylan B were assessed using the *in vitro* Cell Transformation Assay in cultured BALB/C-3T3 cells with and without metabolic activation. Toxicity was assessed by measuring the reduction in Relative Cloning Efficiency (RCE). Concentrations of hylan B tested were from 0 to 100% cytotoxicity. Hylan B did not induce cell transformation in this test system in any concentrations studied.

Long Term and Repeated Application Biological Tests:

Subchronic Toxicity:

The objective of the subchronic toxicity tests was to evaluate the potential toxicity of hylan B in guinea pig and rabbit models.

Subchronic Two-week Intraperitoneal Toxicity Study on Hylan Gel in Male Guinea Pigs [Effect of Repeated Intraperitoneal Injections of Hylan Gel or Degraded Hylan Gel (2 mg/ml) on Blood Parameters and Histology of Selected Tissues in male Guinea Pigs] (Study BXR-25305-F-I):

Guinea pigs were injected twice (one/week) intraperitoneally with hylan B, degraded hylan B, buffer alone or with nothing (untreated animals). One week after the second injection, blood was drawn and the animals were sacrificed and necropsied. Hylan B, when given in two intraperitoneal injections of 20 mg/kg, produced no significant changes in body weight, weight gain or organ weights. Hematology and clinical chemistry comparisons were equivalent in hylan B and buffer treated groups. No treatment-related gross or histomorphological changes were observed.

Immunization and Subchronic Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits (Study BXR 23006-I):

This study was conducted to evaluate the potential of hylan B and collagen to produce a toxicological response after repeated injections in the rabbit model. Rabbits received intramuscular injections of 1 mg/kg of the test items with and without Freund's adjuvant at 1, 4, 5, 6, 7, and 8 weeks. Rabbits were euthanized 12 weeks after the first injection. Microscopic evaluation of injection site tissue revealed minimal to minimal-mild inflammation in animals given hylan B without adjuvant and mild to mild-moderate inflammation in animals given hylan B with adjuvant. Animals given hylan B without adjuvant had no other remarkable tissue lesions, with the exception of one rabbit out of 21, which had a single microgranuloma in the lung. Most animals given hylan B with adjuvant had microgranulomas in the lungs, and one had microgranulomas in the liver and spleen.

Histological analysis of intradermal injection sites from rabbits treated with Zyplast and Zyderm, revealed a minimal to moderate inflammatory tissue reaction characterized either by the presence of numerous eosinophils and macrophages or by a minimal encapsulation and minimal macrophage infiltration. Based on this study, it was concluded that repeated injection of hylan B at a total concentration > 30 times that proposed for clinical use, is not locally or systemically toxic to rabbits.

Reviewer Comment: This study has an unusual result. The sponsor seems to imply that the microgranulomas observed in the lungs, liver and spleen of the hylan B-treated animals were as a result of “systemic translocation of adjuvant moieties”. However, it appears that such translocations were not evident when Zyplast or Zyderm II were injected with or without adjuvant. This question was posed to Genzyme Biosurgery in minor deficiency letter. The company responded by pointing out that Complete Freund’s Adjuvant (CFA) has been shown in the literature to cause pulmonary granulomatous disease (Bergeron et. al. Eur. Resp. J. 2001; 18:357-61). Other published literature provide evidence that CFA can induce granuloma formation in the liver, kidneys, heart, lymph nodes and skeletal muscles as well. In light of this evidence, I believe that the sponsor has adequately addressed this concern.

Chronic Toxicity and Carcinogenicity:

These studies were performed in order to assess the chronic effects of continuous exposure to hylan B gel.

One Year Subcutaneous Toxicity Study of Hylan B in Female Rats (Study BXR-25405-F-I):

This study was performed in order to evaluate the potential of hylan B to cause toxicity or carcinogenicity in the rat. Test animals were exposed to a large volume of hylan B, administered subcutaneously, for a 12-month period, which represents approximately one-third of their anticipated life span. The study consisted of 10 groups of animals (5 hylan B treatment and 5 saline control). Animals were sacrificed at 1 week, 1, 3, 6, and 12 months post implantation. Selected organs and skin tissues were processed for histopathological evaluation.

There were no treatment-related clinical changes in animal health or behavior and there were no detectable clinical signs of local reaction to the injected material (absence of swelling, redness, heat, lesion, etc.). Histopathological evaluation revealed that there were no treatment-related microscopic changes in any organ; all other changes, neoplastic and nonneoplastic were incidental background lesions that were consistent in incidence and severity with the age of the rats. In skin sections from injection sites an initial inflammatory reaction (at one week) gradually resolved, and at one year post-treatment, inflammation was not observed in the treatment animals. Hylan B was still present in the injected tissue at one year.

Under the conditions of this study, subcutaneous administration of hylan B to rats, at a dose over 500 times the proposed clinical dose, was not associated with local or systemic toxicity, nor was it associated with carcinogenic potential or with development of neoplastic lesions.

Reproduction Studies:

Reproduction studies were undertaken to determine if there was any potential for hylan B Gel to induce teratogenic changes in mammalian offspring.

General Reproduction Study (Segment I) of the Effect of Intraocular/Intra-articular Injection of Hyaluronan, Hylan A, Hylan B, and Synvisc® (hylan G-F 20) in Owl Monkeys (Study BXR 12243-F-I):

A general reproduction study was conducted in owl monkeys. The study assessed male and female parameters and the viability, external morphology, growth, and general functional development of the offspring. Observations were compiled from 48 male and 57 female owl monkeys. In each study, the number of injections and the total material injected per animals represented a significant increase over the maximum number of injections and total amount of material intended to be used in clinical situations in humans. The concurrent control group consisted of seven reproductive pairs of monkeys.

The incidences of pregnancies, abortions, stillbirths, and early infant deaths in the test groups were not significantly different from those of the concurrent control pairs and the colony historical incidence. Based on the results of this study, intravitreal injection of hylan B did not affect mating or fertility of the animals or the viability, external morphology, or growth of the offspring. It did not appear that hylan B adversely affected reproduction or develop of these monkeys.

Pharmacokinetics:

Pharmacokinetic studies were undertaken in rats and guinea pigs to assess how and where hylan B gel was eliminated from the system. Radiolabeled hylan B was introduced into guinea pigs and rats so that intradermal residence time and blood elimination kinetics and tissue distribution could be assessed.

Intradermal Injection of [³H]-Hylan B ([³H]-Hylan gel) in Guinea Pigs (Study BXR 25407-F-I):

This study was conducted to evaluate the intradermal residence time and local tissue reaction to hylan B Gel. [³H]-hylan B was administered intradermally at 4 sites in 12 guinea pigs. The dose was more than 50x the clinical dose. Skin injection tissue was harvested and analyzed for total radioactivity and histopathology at time 0 (immediately after injection) and at 1, 2, and 4 weeks following injection. Histopathological analysis was performed to verify the presence of hylan B in the tissue and to assess the local tissue reaction while determination of radioactivity was used to estimate product residence time in the tissue.

After 4 weeks, approximately 90% of the injected radioactivity remained at the injection site. A minimal inflammatory response was observed at 4 weeks. The sponsor concludes that administration of [³H]-hylan B, at an amount that exceeds clinical use amounts, results in the formation of a stable biocompatible implant, which is not associated with local or systemic toxicity in the guinea pig.

Distribution of [³H]-Hylan Gel (Degraded) in Rats (Study BXR 25212-F-I):

This study was conducted to evaluate the kinetics and completeness of systemic elimination of hylan B. Hylan B, in degraded form, was administered via intravenous injection in rats. The gel particles were previously degraded by acid hydrolysis. More than 100-fold the proposed clinical dose was administered to the rats.

The radioactive hylan B was quickly eliminated from the blood (> 75% in the first hour) via urine, and very little (< 2.5%) was recovered from body organs. There was no evidence of accumulation in any tissues involved in hyaluronan metabolism (liver, spleen, kidney). The major site of tissue uptake at each time point evaluated was determined to be the liver. By the 24-hour time point, 90% of the radioactivity was detected in the urine and feces, whereas only 2% was detectable in the organs. It appears that hylan B follows the known blood elimination pathway for hyaluronan and does not exhibit hepatotoxic properties after direct intravenous injection.

Pharmacodynamics:

Pharmacodynamic studies were conducted to gain an understanding of how and where hylan B Gel was broken down and where adjuncts of the material migrated.

Six Month Study of Residence Time of [¹⁴C]-Hylan B Gel after Intradermal Administration in Female Guinea Pigs (Study BXR 25213-I):

This study was undertaken in order to evaluate the residence time, systematic distribution, and local tissue reaction to hylan B following intradermal administration. In this study, 18 female guinea pigs were injected intradermally with more than 50x the clinical dose of [¹⁴C]-hylan B into 8 sites on each animal. Injection sites were harvested from study animals at time zero (immediately following implantation), at 7 days, and at 1, 3, and 6 months following administration. Radioactivity was analyzed in 6 of 8 sites from each animal; the remaining two sites were evaluated for histopathology.

Dermal tissue analysis revealed substantial recovery of radioactivity at all time points through 6 months. A half-life residence time of 9.16 months was calculated for the hylan B Gel from the available data. At all time points, the appearance of the injection site skin was considered normal with no evidence of a treatment-related effect on the dermal tissue. There was no detectable inflammation or dermal fibrosis at any injection site. Analysis of major organs (lungs, liver, spleen, kidneys) from animals at the 3 and 6 month time points revealed no detectable radioactivity in these organs.

The sponsor concludes that [¹⁴C]-hylan B is biologically compatible, is relatively stable in the dermal tissue, and does not produce any remarkable local tissue reaction or toxicity. Though a small decrease in recoverable radioactivity was observed over the time period of the experiment, there was no evidence that radioactivity was accumulating in major organs. It appeared that the [¹⁴C]-hylan B was stable and biologically compatible in the dermis of guinea pigs.

Intradermal and Subcutaneous Injection of Hylan Gel in Guinea Pigs (Study BXR 25408-F-I):

This study was intended to assess the behavior and long-term tissue effects of intradermally and subdermally injected hylan B as a potential soft tissue augmentation material. Sixteen guinea pigs were injected with hylan B, collagen (Zyplast® and Zyderm®) or saline control at twelve injection sites (6 intradermal and 6 subdermal). Each implant site was palpated and examined for evidence of biocompatibility (erythema, edema, vesication, etc.). At 3 days, 1, 2, 4, 9, 13, 26, and 52 weeks, two animals were sacrificed and all injection sites were harvested, processed and evaluated histologically.

Both collagen and hylan B produced an inflammatory response shortly after injection (by 2 weeks). This response was more prominent when either material was localized in the fat. With time, the inflammatory reaction diminished with hylan B and had essentially resolved by Week 26. The inflammatory response to collagen was more protracted. The sponsor concludes that these results indicate that hylan B is stable and tolerated when implanted into the dermal and subdermal tissue of guinea pigs. Hylan B was present at week 52 with no inflammatory reaction associated with the implant.

CHEMICAL AND PHYSICAL TESTING:

Hylaform consists completely of hylan B gel. Hylan B gel is prepared by chemical cross-linking of hylan A (Hyaluronic acid) with vinyl sulfone. The rheological characteristics of hylan B gel are critical for the mode of action of Hylaform.

Vinyl sulfone is a bifunctional cross-linking agent where the two vinyl groups can react with a nucleophile or any chemical group containing an active hydrogen atom (hydroxyl, amino, sulfhydryl, etc.). The reaction with a hydroxyl group (such as contained in hylan A) results in the formation of an ether bond. A hydrogel is formed as a result of the reaction between hylan A in an aqueous alkaline solution and vinyl sulfone. This gel is extensively washed in physiological salt solution and then it is passed through a series of screens to produce a gel slurry.

Chemical Composition of Hylan B:

Elemental Analysis of Hylan B:

Hylan B gel is composed of carbon, hydrogen, nitrogen and sulfur. Each pendant or cross-linking group present in hylan B contains one sulfur atom. Each disaccharide residue of the polysaccharide chain contains one nitrogen atom. It was determined that there is one sulfonyl residue per approximately 3.3 – 3.6 disaccharide units of the polysaccharide chain. This ratio was confirmed via Infrared, Ultraviolet and Visible Spectroscopy methods.

Chemical Composition of Hylan A:

In order to gain a clearer understanding of Hylaform (hylan B gel), one must first analyze the hylan A units used to construct hylan B gel. Hylan A is a modified form of the naturally occurring

glycosaminoglycan, hyaluronan. The sodium salt of hyaluronan contains disaccharide units made of Na D-glucuronate and N-acetyl-D-glucosamine linked together with β -1,3 glycosidic bonds. These disaccharides are linked by β -1,4 glycosidic bonds to form long, unbranched polysaccharide chains. Hylan A is extracted from the combs of domestic fowl (*Gallus gallus*). Before extraction, combs are treated with a dilute formaldehyde solution. The chemical cross-linking process involves reaction between OH groups of hyaluronan, formaldehyde and one or several reactive groups of a specific protein, most probably, NH_2 groups of lysine or other basic amino acid residues. This reaction involves 2 hyaluronan molecules and two or more reactive groups of the same protein molecule. There are negligible amounts of free or unbound proteins in hylan after formaldehyde treatment since the proteins that are not bound to hylan A molecules will be cross-linked to the tissue structure and become insoluble and, hence, will not be extracted from tissue with water.

Elemental Analysis of Hylan A:

Although there is bound protein, formaldehyde and several impurities, these make up a minor component of hylan A as the elemental analysis of hylan A reveals that it closely corresponds to that of pure hyaluronan (carbon, 41.9; hydrogen, 5.0; and nitrogen, 3.5 wt %). It is found that the elemental content of hylan A for three separate batches is very similar to this: carbon, 43.7, 44.4, and 45.2 wt %; hydrogen, 5.0, 4.9, 3.5 wt %; and nitrogen 3.5, 3.5, 3.5 wt %).

Chemical Analysis of Hylan A:

Three aspects of hylan A composition were investigated by chemical analysis. Colorimetric assays were employed to quantitate hexuronic acid and hexosamine contents. Formaldehyde content was chromatographically analyzed in order to determine the amount bound within hylan A. Total protein and individual amino acid content were determined to characterize the protein moiety of hylan A.

The hexuronic acid content of hylan A was determined by an automated modification of the carbazole reaction method. Hexosamine content was determined by a modification of the Elson-Morgan reaction. An analysis of 42 batches of hylan A determined the molar ratio of hexuronic acid to hexosamine was in the range of 0.93 to 1.11, with an average value of 1.02 ± 0.04 , which is similar to the 1:1 ratio of the parent molecules (β -D-glucuronic acid and N-acetyl β -D-glucosamine) observed in hyaluronan.

Formaldehyde in hylan A exists in two forms, "free formaldehyde" and "bound formaldehyde", which must be liberated in order to be detected. An analysis of 42 batches of hylan A determined that the combined formaldehyde content ranged from 0.0052 to 0.32 weight %, with an average value of 0.016 ± 0.006 weight %. Free formaldehyde usually is found to contribute less than 0.01 weight %. Calculations determined that for each 1 molecule of formaldehyde there are approximately 750 disaccharide units of polysaccharide chain.

The total amount of protein in hylan A was determined using the Lowry colorimetric assay. In an analysis of 42 batches of hylan A, the protein content ranged from 0.12 to 0.85 weight %, and averaged $0.43 \pm 0.02\%$. Most of the amino acids were determined to be aspartic acid, glutamic acid and glycine.

Spectrophotometric Analyses of Hylan A:

Hylan A samples were analyzed by four spectroscopic methods: ¹³C-NMR spectroscopy, infrared absorption spectroscopy, circular dichroism spectroscopy and visible and ultraviolet light spectroscopy. These methods were used to compare the results with those obtained for hyaluronan.

The pattern and positions of the resonances obtained for hylan A via ¹³C-NMR spectroscopy were essentially identical to those of hyaluronan, indicating the major component of hylan A is indistinguishable from hyaluronan. Comparisons of the infrared spectrographs obtained for hylan A were indistinguishable from those obtained for hyaluronan implying that the basic structure of hyaluronan is retained in hylan A. Conformational structures of biological macromolecules are investigated using circular dichroism (CD) spectroscopy. Hylan A CD spectra are identical in shape to those obtained for hyaluronan under neutral aqueous conditions. The ultraviolet and visible light spectra for hylan A and hyaluronan are identical with no absorption in the visible light spectra and some absorption in the UV spectrum where nucleic acid contaminants would appear. These are more evident in hyaluronan preparations since nucleic acid contamination is characteristic of these preparations.

Enzymatic Analysis of Hylan A:

The degree to which the polysaccharide chain of hylan A is susceptible to enzymatic degradation provides an indication of the extent of chemical modification. Several samples of hylan A were treated with enzymes in parallel with hyaluronan samples in order to determine the relative degree of susceptibility. Hylan A is completely degradable to oligosaccharides by the three endoglycosidases known to degrade hyaluronan by different mechanisms. This indicates that hylan A and hyaluronan are identical with respect to susceptibility to enzymatic degradation.

Physical Testing of Hylan B Gel:

A number of physical tests have been performed on multiple samples of hylan B gel. The testing performed includes rheology, hydration level, pH, osmolality, cross-linking/swelling ration, particle size, heavy metals, and enzymatic and oxygen-derived free radical degradation.

PRODUCT SPECIFICATIONS:

The chemical, physical and biocompatibility testing performed and described in this module were used to assemble a number of product specifications. These are provided in the following table:

Hylaform Product Specifications

Diagnostic Test	Product Specification
Rheology	50-220 Pa @ 35°C
Hydration Level	4.5-6.5 mg/ml

pH	6.0-7.5
Osmolality	290-330 mOsm
Cytotoxicity	= 2
Sterility	No growth
Endotoxin Level	= 0.5 EU/ml

PRODUCT STABILITY:

The sponsor has provided 6 stability studies that support a 24 month shelf life for Hylaform at 2 to 30°C. These studies are summarized in Table 3-6 on Page 194 of Volume 2 (Module 2).

STERILIZATION:

The syringed Hylaform product is terminally sterilized using a validated autoclave cycle in accordance with EN554. All of the raw materials used in the manufacturing process; Water for Injection, hylan B and the saline diluent; meet established acceptance criteria for Bioburden or sterility prior to release. Also Quality Control measures are in place to assure product accuracy and reliability.

The sterilization process is validated with a steam/air cycle. Initial validation consists of three consecutive loaded chamber heat penetration/biological challenge trials. Revalidation is performed using a single trial on an annual basis. Temperature and pressure are monitored throughout the cycle. A minimum of 22 External (calibrated) Type “T” thermocouples are used to monitor the temperature of the chamber and load. A total of three biological Indicator (BI) spore strips (*Bacillus stearothermophilus*) loaded in Hylaform Gel within syringes are placed in minimum loads and twelve BIs are placed in each maximum load. The minimum size validated load is 100 syringes in one tub on one shelf while the maximum size validated load is 4,800 syringes (48 tubs) located on 4 shelves. The sterility assurance level is at least 10⁻⁶.

PACKAGE VALIDATION:

The packaging components that come into contact with the product are received sterile from the vendor. Package integrity testing was performed at an outside lab to evaluate the barrier properties of syringes containing Hylaform product. For the package integrity test, a microbial challenge test was performed on syringes from a media fill operation. All samples passed testing. All confirmatory testing is located in Appendices 3-9 and 3-10.

Recommendation: This Module is acceptable as modified in response to the issues raised in the original reviews.

Biologist/Expert Reviewer
Division of General, Restorative & Neurological Devices
Plastic and Reconstructive Surgery Branch