

Fate of Porous Starch Microbeads in Mammalian Tissues

Introduction:

This summary will describe the current literature and some experimental results related to the degradation of starch microbeads after implantation into human tissues. Starch microbeads, prepared by reaction of epichlorohydrin with soluble starch, are used by Medafor to prepare their hemostatic product, ARISTA_{AH}[™]. This material has been widely studied and used for a variety of medical purposes. Its chemistry and metabolism is well understood. The same chemical reactions and the same of soluble starch are used to produce similar starch micro particles currently available for medical use in Japan under the trade name Spherex[™]. These particles are injected parenterally as a saline suspension for blockage of the portal vessels as an adjunct to chemotherapy for hepatic tumors. The information on the degradation of Spherex particles is applicable to Medafor's ARISTA_{AH}. Since this information is already available in the abundant Spherex literature, it will not be repeated here. See for instance (Lindberg, B, Lote K, Teder H; Biodegradable Starch Microspheres – A new medical tool; in Davis SS, Illum L, McVie JG, et (eds); Microspheres and Drug Therapy. Amsterdam, The Netherlands, Elsevier, 1984 pp 153 - 188). The safety data for Spherex shows conclusively that starch micro particles are well tolerated and rapidly cleared from the circulation.

Structure and Chemistry:

To prepare the micro beads, an alkaline solution of highly purified potato starch, emulsified in an organic solvent, is cross-linked by the addition of a limited amount of epichlorohydrin. When the reaction is complete the particles are washed extensively, dried and separated.

The final product consists almost entirely of pure starch plus the addition of a small number of glycerol ether linkages which represent the cross linking groups. The chemistry of the cross-linking is described in US Patent 4,124,705. The reaction of epichlorohydrin with hydroxyl groups in the starch molecule produces a variety of glycerol ether linkages that serve to cross link the starch molecules into a three dimensional network. The degree of cross linking is controlled to produce the desired porosity. The manufacturing process produces spherical particles with diameters ranging from 10 to 200 micrometers with a controlled pore size.

Since the particles are composed almost entirely of starch, enzymes that can catalyze the hydrolysis of alpha-glycosidic bonds readily degrade them. Alpha amylases, which catalyze breakage at random positions on the starch molecule, are highly active in degrading the starch particles and are widely distributed in mammalian tissue. Other enzymes such as beta amylase and alpha glycosides can also contribute to the breakdown of the particles. A study of the kinetics of alpha amylase mediated dissolution of epichlorohydrin cross-linked starch particles is given by Hamdi and Ponchel (Enzymatic Degradation of Epichlorohydrin Crosslinked Starch Microspheres by alpha Amylase; Pharmaceutical Research 16:867-875 (1999)). The enzymatic hydrolysis occurs primarily on the surface of the particles since the pore size of the particle excludes entry of the large enzyme molecules. The rate of dissolution of the particles is dependent upon the level enzyme activity and proceeds until the entire mass of particles is converted to soluble material.

Studies by Medafor using ARISTA_{AH} starch particles have shown similar results. Similar studies have been reported for the Spherex particles (See Lindberg, et al above). All of these studies support the conclusion that the action of alpha amylase will degrade the starch particles to small water-soluble fragments. These fragments are then either excreted in the urine or bile or further metabolized in maltose and glucose by beta amylase and alpha glycosidase

Animal Studies:

Medafor has conducted studies in animals to measure the disappearance of starch particles after injection. We have used iodine – potassium iodide staining to locate the particles in tissue. This stain colors intact starch particles an intense purple due to the incorporation of iodine into the polymeric starch structure and can be used to detect single particles. As the particles degrade, the color reaction fades.

In the first study, a series of injections were made subcutaneously into mice. Animals were sacrificed at intervals and examined for the presence of intact particles. After 15 minutes, particles were easily seen in the tissue injection sites. After 30minutes, none of the animals showed any particles stainable with iodine. The laboratory report is attached in Appendix A. A copy of the data from this study is shown below as Table 1.

Table 1.
Number of Animals with Starch Detected after Subcutaneous Injection

Time	Treatment	Iodine Foci Present
15min	ARISTA _{AH} Beads, 1 ml	2/2 (100%)
30min	ARISTA _{AH} Beads, 1 ml	0/10 (0%)
1h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)
6h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)
24h	ARISTA _{AH} Beads, 0.5 ml	0/10 (0%)
24h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)

A second study, also attached in Appendix A was designed primarily to study the possible formation of adhesions following intraperitoneal injection of ARISTA_{AH} particles.

As a part of this study, the animals were examined for starch particles using the iodine stain described above. The results (shown below as Table 2.) are abstracted from the full study contained in Appendix A.

Table 2.
Animals with Starch Detected by Iodine after Intraperitoneal Injection

	Control	10mg/kg	50mg/kg	100mg/kg
1 day	0/4 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
1 week	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
2 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
4 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)

These results are consistent with the results seen following subcutaneous injection even though the time scale is longer. Again we see that after interval of six to 24 hours, starch micro particles are not detectable in tissue or body fluids.

These animal experiments provide direct evidence that Medafor’s ARISTA_{AH} particles are rapidly cleared from tissue and body fluids following application.

Summary:

The available information regarding the properties of the ARISTA_{AH} starch particles, such as their chemical structure, their sensitivity to enzymatic attack, and the available experimental data from a variety of sources all combine to show that particles exposed to serum or tissue will be completely converted to soluble fragments. These fragments are then excreted or further metabolized resulting in the complete disappearance of the particles from the initial site of application.



Final Results: Degradation Rates Following Subcutaneous Injections of ARISTA_{AH}TM Hemostatic Beads

Summary:

A mouse model was used to document and describe the time course of starch degradation after the subcutaneous injection of hemostatic starch microspheres (ARISTA_{AH}TM). When iodine was applied at the test site 30 minutes after subcutaneous injection, no starch foci were observed.

Conclusion: ARISTA_{AH} beads applied by subcutaneous injection in mouse model rapidly degrade after 30 minutes.

Purpose:

Serum α -amylase and other common enzymes readily break down starch-based microspheres. A mouse subcutaneous injection model was used to document and describe the time course of hemostatic starch microsphere degradation.

Sponsor:

Medafor, Inc., 5201 East River Road, # 312, Minneapolis, MN 55421

Test Facility:

Transfusion, Coagulation and Cardiopulmonary Bypass Research Group, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905

Veterinary Surgeon:

Michael C. Blanco, DVM

Investigators: Mark H. Ereth, M.D., Mark B. Dekutoski, M.D.

Animals:

Fifty-two mice (MF1 outbred strain from Harlan Inc.) of either sex weighing between 20-25g were used. All of them were housed and cared for under the AAALAC guidelines at Mayo Clinic Rochester.

Experimental Design:

The ARISTA_{AH} particles were suspended in normal saline at a concentration of 50 mg/ml. A small short needle was used to administer a single subcutaneous injection. Injections consisted of either 0.5 or 1 ml of the 50mg/ml

ARISTA_{AH} suspension. Injections were administered on the dorsal surface of the animal, just lateral to the midline, beneath the skin and above fascial muscular tissues at 15 min, 30 min, 1, 6 and 24 hours prior to planned sacrifice. At sacrifice, this area was dissected out and iodine (1% iodine, 1% potassium iodide in normal saline) in was used to stain the ARISTA_{AH} beads. This stain colors individual starch particles an intense purple and will easily show individual starch particles. The following scoring system was used:

Iodine Color Score

0 = no color

1 = less than 10 starch foci

2 = 10 to 20 starch foci

3 = greater than 20 starch foci

Table 1.

Number of Animals with Starch Detected after Subcutaneous Injection

Time	Treatment	Iodine Foci Present
15 min	ARISTA _{AH} Beads, 1 ml	2/2 (100%)
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1 h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)
6 h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)
24 h	ARISTA _{AH} Beads, 0.5 ml	0/10 (0%)
24 h	ARISTA _{AH} Beads, 1 ml	0/10 (0%)

Results and Conclusion:

No iodine-stainable foci were observed in any of the animals except the initial 15-minute post injection group (Table 1., column 3). The iodine-stainable foci represent starch particles that have an intact molecular structure. We therefore conclude that within 30 minutes post injection endogenous enzymes in the subcutaneous tissue have substantially begun to degrade ARISTA_{AH} starch micro particles.



Final Results: Intraperitoneal Injection of ARISTA_{AH}TM Beads Into Mice Results in No Residual Adhesion

Summary of Results:

A mouse model was used to evaluate the possibility of adhesion development following the use of hemostatic starch microspheres (ARISTA_{AH}). After injecting escalating doses of ARISTA_{AH} suspension into mice peritoneal cavities, we monitored the recovery of the mice for up to four weeks. No adhesions were found throughout the duration of the monitoring, and no post-op complications occurred.

Conclusion:

No residual starch adhesions occurred after intraperitoneal injection of ARISTA_{AH} beads in a mouse model.

Purpose:

Adhesion formation has historically been a concern when starch molecules are introduced into the peritoneal cavity.² The purpose of this study is to evaluate the possibility of adhesion development following the use of ARISTA_{AH} hemostatic starch microspheres.

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Investigators:

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Test System Justification: Mice were chosen as the experimental species because they closely resemble humans when monitoring and measuring adhesion response from abdominal surgical procedures.¹

Animals:

Eighty mice (MF1 outbred strain from Harlan Inc.) of either sex weighing between 20-25g were used. All of them were housed and cared for under the AAALAC guidelines at Mayo Clinic Rochester.

Experimental Design:

Each of 80 mice was given 1 ml ARISTA_{AH} suspension by intraperitoneal injection in escalating doses. The mice were divided into 4 groups with the following bead doses: a normal saline control (no beads); 10mg/kg; 50mg/kg; and 100mg/kg. On the second day following injection, 5 animals from each group were sacrificed for abdominal examination and adhesion scoring. This was repeated on the 7th, 14th and 28th days after injection. Any additional side effects were noted. After sacrifice, iodine solution 3 was used to enhance visualization of any remaining starch material in the areas of hemostatic bead injection.

Evaluation and Statistical Methods:

The following scoring system was used:

Adhesion Score

- 0 = no visible adhesion
- 1 = minimal visible adhesion
- 2 = moderate visible adhesion
- 3 = extensive visible adhesion

Iodine Color Score

- 0 = no color,
- 1 = less than 10 starch foci
- 2 = 10 to 20 starch foci
- 3 = greater than 20 starch foci

Data Collection and Observations:

Group Definitions

Dose	Saline Control	10mg/kg	50mg/kg	100mg/kg
1 day	5	5	5	5
1 week	5	5	5	5
2 weeks	5	5	5	5
4 weeks	5	5	5	5
Total	20	20	20	20
Equivalent Dose in 80kg Human	0	0.8g	4g	8g

Animals with Adhesions Detected after Intraperitoneal Injection

	Control	10mg/kg	50mg/kg	100mg/kg
1 day	0/4 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
1 week	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
2 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
4 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)

Animals with Starch Detected by Iodine after Intraperitoneal Injection

	Control	10mg/kg	50mg/kg	100mg/kg
1 day	0/4 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
1 week	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
2 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
4 weeks	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)

Sample Size	Observed Adhesion	Observed Percent	Exact 95% C.I
5	0	0.0%	0.0 to 52.2%
15	0	0.0%	0.0 to 21.8%
30	0	0.0%	0.0 to 11.6%
45	0	0.0%	0.0 to 7.9%
60	0	0.0%	0.0 to 6.0%

In effect, we can rule out with 95% certainty, that the proportion of subjects with starch present at 4 weeks would be 6% or greater. With only 5, we can only rule out 52% or greater...

References:

1. Falk K, Bjornquist P, Stromqvist M, Holmdahl L, Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1, British Journal of Surgery, 2001, 88: 286-0
2. McEntee GP, Stuart RC, Byrne PJ, Leen E, Hennessy TP, Experimental study of starch-induced intraoperative adhesions. British Journal of Surgery 1990, 77: 113-4
3. McNaught GH, Gloves, starch and povidone Iodine. Lancet 1973, 1: 887