Covalent heparin attachment – one step:

Proprietary commercial application Clotting times

ID of a polysulfone tube was coated with a covalently bound water-based heparin coating. Modified Lee-White clotting time test from NAMSA on Steam-sterilized parts.

Clotting time changes were significant. This information combined with un-coated data suggests that the Surface Solution samples did reduce clotting time.

J.Strony PhD CWRU Thrombin times- human plasma – one step

Two samples of coating resins were prepared. In one, heparin was bound covalently to the supporting resin through a linker and in the other the heparin was not bound. Films 3 mils thick were cast on PE sheets and removed. A control of benzalkonium heparin was prepared by casting the coating resin on PE and post-dipping in the ionic heparin solution. Films were soaked in saline for 24 hours and then removed and placed in fresh saline. Thrombin times for soak solutions, based on a pooled human plasma, yielded IU data shown below.

Lost effectiveness was defined as <1 IU/24 Hour release. Benzalkonium control and unbound heparin were all ineffective at 24 hours or less. The bound heparin is sustained and released over atleast 14 days.

C) Biocascade- Covalent attachment and sustained release measured by Anti Xa

Two separate sections (~1-2 cm 2 each) of films (6-65D) were incubated in isotonic Anti-Xa buffer at 37 o C. At various times (e.g. 1 hr, 24 hr, 48 hr, 1 wk, 2 wk,1,3 and 6 months), the incubation buffer was removed from the surface film and assayed for heparin Anti-Xa activity by the USP Anti-Xa method. Fresh buffer solution was then used to replace the removed buffer and the incubation continued until the next assay point, where the procedure was repeated. The film released heparin, at a relatively slow rate, resulting in about 20% of the theoretical heparin content being eluted as biologically active heparin in the first month.The film did not change physical shape during the incubation in buffer, but appeared to be hydrophilic and retain its original shape and texture.

The remaining heparin is biologically active and accessible at the surface of the film as measured with the USP Anti-Xa method. This would appear to be an interesting, and stable anti-thrombotic film, as it appears that only small amounts of heparin are eluting from the film after one month, and that the remaining heparin is tightly bound and all heparin is biologically active.

Anti-Xa Activity of Heparin-containing Surface Films

Summary:

Two separate sections (~1-2 cm 2 each) of two distinct surface films (6-65B and 6-65D, respectively) were incubated in isotonic Anti-Xa buffer at 37 o C. At various times (e.g. 1 hr, 24 hr, 48 hr, 1 wk, 2 wk, etc.), the incubation buffer was removed from the surface film and assayed for heparin Anti-Xa activity by the USP Anti-Xa method. Fresh buffer solution was then used to replace the removed buffer and the incubation continued until the next assay point, where the procedure was repeated.

The results with the two films were quite distinct. The 6-65B films appeared to be quite hydrophobic, with the film “scrolling” into a small tube in the incubation buffer. Also, within 1 week of extraction, over 2 mg (~450-500 Units) of heparin was extracted from each of the duplicate, 6-65B films. After two weeks, the 6-65B films had released a quantity of heparin equivalent to ~12-13% of the original film mass (see Table 1, attached). Film 6-65D, on the other hand, only eluted or released heparin at a relatively slow rate, resulting in about ~2-3% of the original film mass being eluted as biologically active heparin in the first two weeks (see Table 1). The 6-65D film also did not change physical shape during the incubation in buffer, but appeared to be hydrophilic and retain its original shape and texture.

Experimental Design and Details

- Cut 5-6 separate pieces of each of the 6-65 films and placed these in tared 12 X 75 mm Falcon tubes.
- By weight, closely matched duplicate films for each of the two film types (4 samples total) were then treated with 1 ml each of USP Anti-Xa buffer for 1 hour at 37 o C.
- The incubation buffer was removed to a separate tube, and then assayed for Anti-Xa activity, after appropriate serial dilution with additonal Anti-Xa buffer.
- After removal of the first 1 ml of buffer from each film, the buffer was replaced in each tube with 5 mls of buffer for continued incubation. For all subsequent assays and incubations (beyond the 1 hour point), 5 mls of incubation buffer was used in each case.
- Anti-Xa assay buffer consists of 0.05 M Tris, 0.0075 M EDTA, 0.175 M NaCl, and 0.1% PEG-8000, pH = 8.4
- Anti-Xa assays were perfomed according to the USP Heparin Sodium monograph procedure on a STA Compact hemostasis instrument (Parsippany, NJ).

Conclusions:

Based upon the quantities of heparin released from each of the film types, the 6-65D appears to be retaining a significant portion of the starting heparin mass. If the remaining heparin is biologically active and accessible at the surface of the film, we will be able to measure this with the USP Anti-Xa method. This experiment is planned for the 1 month time period. If the latter experiment shows biologically active heparin on the film surface, this would appear to be an interesting, and stable anti-thrombotic film, as it appears that no further heparin is eluting from the 6-65D films at two weeks and that the remaining heparin is tightly bound.

By contrast, the 6-65B films appear to have “released” all of the starting heparin within the the first two weeks, leaving little chance that there remains biologically active heparin at the film surface. My suggestion is to not assay or incubate the 6-65B films any further.

Heparin release/retention in urethane 6-65D

Time	Ave eluted, mg	Ave cum % eluted	Surface Active
0 Hr	0	0	by factor Xa
1 Hr	0.0349	3.43	
1 Day	0.1609	17.415	
2 Day	0.0081	18.215	
3 Day	0.0063	18.835	
7 Day	0.0068	19.5	
14 Day	0.0004	19.58	
30 Day	0.0007	19.64	2.2mU/cm2
60 Day	0.00005	20.66	2.5mU/cm2
90 day	below detection		1.5mU/cm2
6 month	below detection		2.0mU/cm2
Film load,mg	1.02	100	

Test Facility: Biocascade

Notes:

The films were tested for bioavailable heparin via an antifactor Xa test. Surface heparin can bind AT III and inhibit anti Xa. Functional testing is more relevant than other styles of reporting heparin activity.