Surface-grafted heparinizable materials

R. Barbucci, G. Casini, P. Ferruti and F. Tempesti
C.R.I.S.M.A., University of Siena, Le Scotte-Nuovo Policlinico, 53100-Siena, Italy
(Received 18 October 1984)

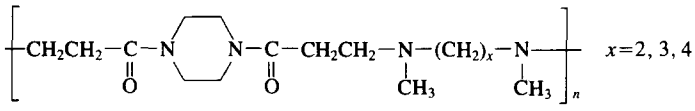
Poly(amido-amine) chains have been grafted on the surface of different materials including glass, silastic, PVC, Dacron, and polyurethane. The poly(amido-amine) is able to complex with heparin by electrostatic interaction. The heparin-adsorbing capacity of the materials so obtained has been tested by biological tests. Heparin is only released at pH > 10, confirming the strong interaction between poly(amido-amine) and heparin. Preliminary theoretical studies have been made to construct a tentative model of the arrangement in the space of poly(amido-amine).

(Keywords: heparin; poly(amido-amine)-grafted materials; glass; silastic; PVC; Dacron; polyurethane; heparinizable surfaces; biological tests)

INTRODUCTION

Thrombus formation induced by non-physiological materials is a great hindrance in the use of plastic devices for biomedical applications. An interesting approach in the development of non-thrombogenic materials is surface-heparinization.

We have synthesized[1] a class of tertiary amino polymers of poly(amido-amine) structure:



these have shown many interesting properties:

- heparin-complexing capacity both in a linear and in a cross-linked form
- no undesirable effect on blood composition or function
- possibility of grafting on different materials.

The capacity to retain heparin seems to increase with the number of methylene groups between the two amine nitrogens and, consequently with their increased basicities.

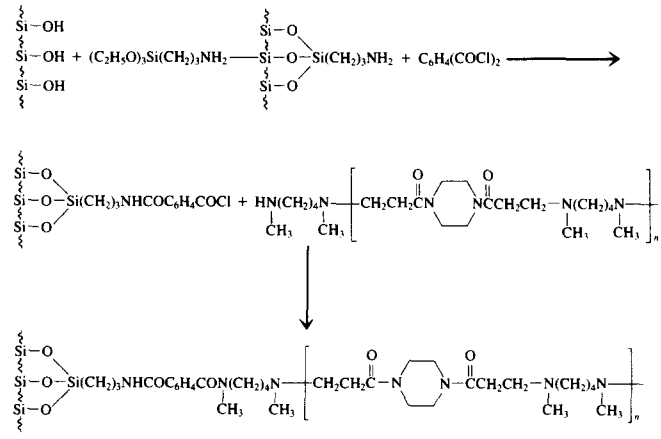
At pH = 7.4 these nitrogens are partially protonated and the percentage of the monoprotonated (HL^+) and diprotonated (H_2L^{2+}) forms is higher for the polymer containing four methylene groups ($x = 4$, N_2LL). A further increase of the number of $-CH_2-$ groups does not provoke a subsequent increase of the basicity[2], but only a larger distance between the two positive charged nitrogen groups.

These groups are capable of interacting electrostatistically with the negative charged groups present in the heparin molecule. It is worthy of mention that the heparin adsorbing capacity of the polymer-grafted surface depends on the average positive net charges present in the repeating unit of the polymer.

Surface-grafting of this heparin-complexing poly(amido-amine) on a given material should result in a heparinizable surface. We have studied poly(amido-amine) grafting on the following materials: glass; silastic, PVC [poly(vinylchloride)]; Dacron [poly(ethyleneterephthalate)]; and polyurethane.

GLASS

The chemical grafting of poly(amido-amine) is possible with all types of glass-devices. The grafting is performed starting from a secondary amino-end-capped poly(amido-amine) obtained by using a slight excess of diamine during the reaction of polyaddition. This polymer[3] was reacted with a glass surface on which acylchloride groups had been introduced, by treating glass with γ-aminopropyltriethoxysilane followed by isophthaloyl chloride according to the following scheme:



E.s.c.a. measurements confirm this scheme of grafting. The heparin adsorbing capacity of these heparinized materials has been evaluated by studying the behaviour of glass microspheres grafted with the poly(amido-amine) in different media.

As is shown in *Table 1* most of the adsorbed heparin appears to be stably retained in saline or plasma, and its release is possible only on treating the microspheres with a 0.1 M NaOH solution. The amount of heparin adsorbed on the microspheres correspond to about 16.4 $\mu g\,cm^{-2}$.

0032-3861/85/091349-04$03.00

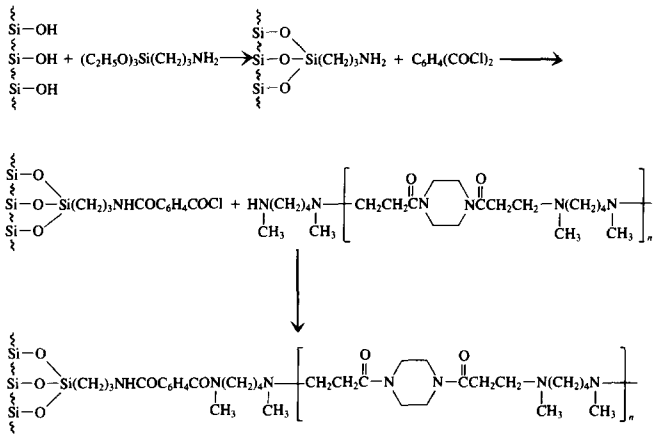
Table 1 Desorption of heparin from heparinized poly(amido-amine)-grafted glass microspheres[a]

Medium[b]	Heparin conc. ($\mu g\ ml^{-1}$)
Saline	7
Plasma	12.5
NaOH 0.1 M	1030.0

[a] Microspheres were treated with $100\ \mu g\ ml^{-1}$ heparin solution
[b] Heparinized microspheres (1 g) were stirred 30 min at 25°C with 1 ml of either saline, plasma or 0.1 M NaOH

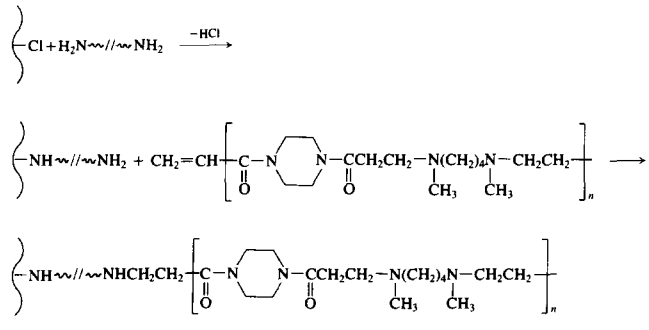
SILASTIC

The chemical scheme for poly(amido-amine) grafting is similar to that performed on glass surfaces[4]. The low amount of hydroxyl groups in silastic and its fragility in organic solvents requires a skilful choice of both appropriate solvents and reaction (T, time) conditions.



PVC

The grafting of poly(amido-amine) on poly(vinyl-chloride) was attempted by reacting an acrylamido end-capped poly(amido-amine) with a PVC surface to which primary and secondary amino groups had been attached. This was in turn performed by treating PVC with a concentrated aqueous solution of triethylen-tetramine[5]. The process is summarized in the following scheme:



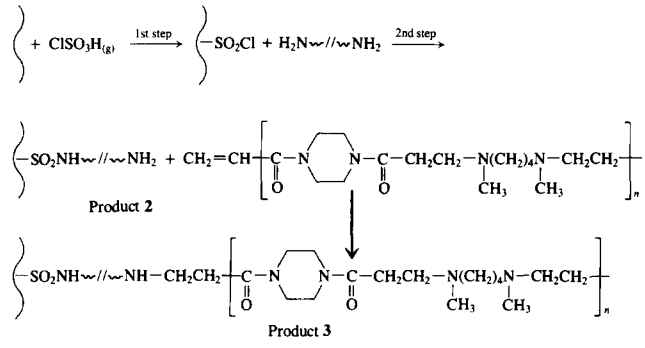
E.s.c.a. analysis of the devices was carried out at each step of the above described treatment. The stability of heparin interaction with polymer surface-grafted PVC and the amount of adsorbed heparin were evaluated by extracting the heparinized PVC with saline, citrated plasma and aqueous 0.1 M NaOH. The heparin was detected in the different elutes by biological tests. Heparin can be completely released only by treating the heparinized PVC devices with NaOH solution[6]. All these results are shown in *Table 2*.

The amount of heparin stably adsorbed on PVC surface correspond to about $1.2\ \mu g\ cm^{-2}$.

DACRON

The chemical process of grafting starts with an heterogeneous reaction between Dacron surfaces and the chlorosulphonic acid vapours; the grafted chloro-sulphonic groups are reacted with an excess of a poly-amine (i.e. triethylentetramine), in order to obtain terminal amino groups on the surface, which in turn react with vinyl-terminated poly(amido-amine)[7].

The whole process is the following:



Titrimetric analyses show the presence of the poly-amine (2nd step) and poly(amido-amine) (3rd step) on the surface (*Table 3*). The heparin adsorbing capacity of the treated surface has been measured in products **II** and **III** (*Table 4*).

As in the previous cases a large quantity of heparin is released at physiological pH by saline, but even after several washings a considerable amount of heparin remains bonded to the poly(amido-amine) grafted on

Table 2 Desorption of heparin from heparinized poly(amido-amine) grafted PVC tubes

		Contact time NaOH 0.1 M[a] (h)			
	Standard time	1 h	2 h	12 h	24 h
APTT[b]	32.1	33.3	47.7	58.1	55.9
TT[c]	16.0	17.2	24.0	34.4	30.2

[a] No heparin desorption was observed after contact times up to 3 h with either saline or plasma
[b] APTT = Activated Partial Thromboplastin Time (s)
[c] TT = Thrombin Time (s)

Table 3 Analytical data of the titration tests performed on treated Dacron surfaces

	Cl^- ions found (μmol)	Basic nitrogens ($\mu mol\ g^{-1}$ Dacron)
Product 1	3.4	–
Product 2 ξ—SO_2—$NH(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$	–	110.6
Product 3 ξ—SO_2—$NH(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH$—PAA	–	234.8

Table 4 Desorption of heparin from heparinized polyamine grafted surfaces after exhaustive rinsing with saline performed by biological tests

			Contact time NaOH 0.1 M		
		Standard time	30 (min)	30 (min)	30 (min)
Product **II** heparinized	APTT[a]	31.4	134.7	49.6	35.8
	TT[b]	11.6	116.3	14.6	12.2
Product **III** heparinized	APTT[a]	33.5	>360	124.0	35.7
	TT[b]	13.2	>360	60.3	13.2

[a] APTT = Activated Partial Thromboplastin Time (s)
[b] TT = Thrombin Time (s)

Table 5 Analytical data of the titration tests performed on treated polyurethane films

Time[a] (days)	Cl^- ions found (μ mol cm^{-2})	Ave.
1	0.58 0.79	0.69
2	1.25 1.16	1.21
3	1.47 1.33	1.4
6	1.39 1.33	1.36

[a] Reaction time between polyurethane films and esamethylene diisocyanates

Table 6 Desorption of heparin from heparinized poly(amido-amine) grafted polyurethane films after exhaustive rinsing with saline

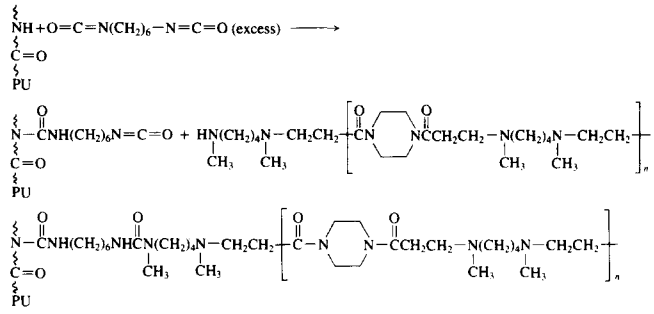
		Contact time[c] NaOH 0.1 M		
	Standard time	30 (min)	30 (min)	30 (min)
APTT[a]	37.0	>360	166.6	40.1
TT[b]	10.8	>360	90.6	11.4

[a] APTT = Activated Partial Thromboplastin Time (s)
[b] TT = Thrombin Time (s)
[c] 5 ml of fresh solution added after each period of contact time

the surface, and this last portion can be removed only by NaOH.

POLYURETHANE

The grafting of diamines or polyamines on polyurethane surfaces is proceeded by the reaction between polyurethane and diisocyanates as shown in the following scheme:



The amount of diamines or polyamines grafted, as evaluated by a titrimetric method, increases with increasing contact times (between the polyurethane film and the diisocyanate), for the first three days, then it remains constant. These results are shown in *Table 5*.

Heparinized treated polyurethane surfaces were carefully rinsed with saline until no heparin was found in the elutes, then surfaces were eluted with NaOH 0.1 M. The amount of heparin stably retained at physiological pH and released only after a treatment with NaOH, corresponds to about 47 μg cm^{-2} (*Table 6*).

EXPERIMENTAL

Materials

Polymer N_2LL was synthesized as previously described[1b]

Silastic devices were purchased from Dow-Corning Co.

PVC tubes were purchased from Verneret (France).

Dacron devices were medical Dacron patch fabrics purchased from USCI (USA).

Polyurethane was synthesized by polyaddition of polypropylene glycol P 425 to diphenylmethane diisocyanate in DMSO solution followed by polyaddition of ethylenediamine.

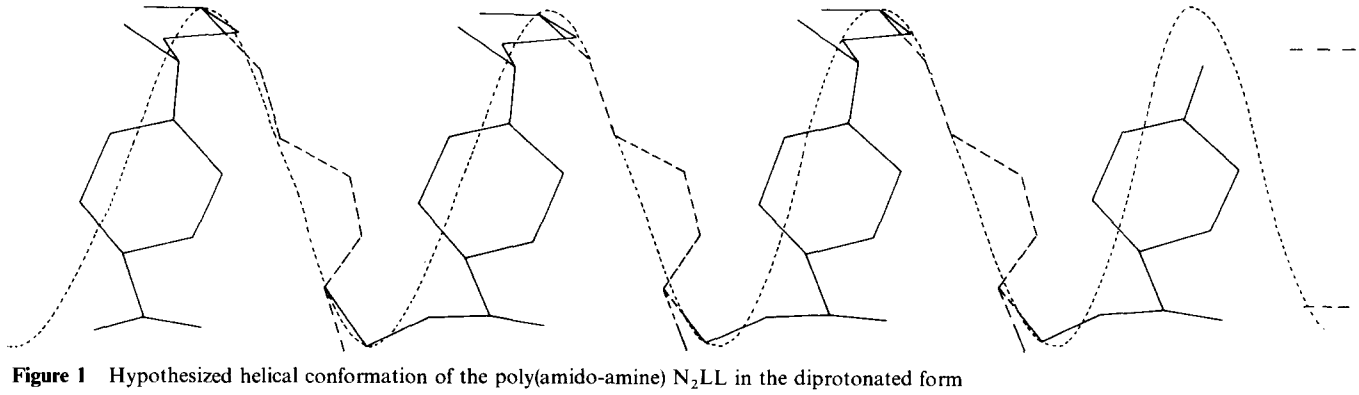


Figure 1 Hypothesized helical conformation of the poly(amido-amine) N_2LL in the diprotonated form

Synthesis

N_2LL grafting on glass surface was performed as described[3].

N_2LL grafting on silastic was performed as described[4].

N_2LL grafting on PVC tubes was performed as described[5].

N_2LL grafting on Dacron surfaces was performed as described[7].

N_2LL grafting on polyurethane films was performed by dipping the films in a esamethylenediisocyanate/ methylene chloride solution, followed by treatment with a methylene chloride solution of the amino-terminated poly(amido-amine) N_2LL (in preparation).

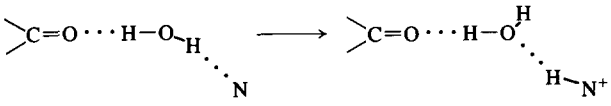
Titrimetric analyses of basic nitrogens were performed as previously described[7].

CONCLUSIONS

Poly(amido-amine)s have been fairly easily grafted on all the different materials without loss of the mechanical performances of the starting articles, and the treated surfaces are able to adsorb heparin. Most of the adsorbed heparin is stably retained in saline and can be removed only by eluting at pH > 10 with 0.1 M NaOH solution.

The strength of this bond depends on an electrostatic interaction between the protonated aminic nitrogens of poly(amido-amine)s and the negative charged groups of heparin.

In acidic solutions the protonation of the two basic nitrogens and/or bridging by a water molecule will provide the longitudinal hydrogen bonds needed to stabilize a helical conformation for the N_2LL poly(amido-amine)[8].



In fact such a pair of hydrogen bonds will give an N···O= distance of approximately 4.5 to 5 Å. Considering that the C=O bond can lie almost on a plane parallel to the axis of a possible helix, then the latter must have a step of ≈6 to 7.5 Å. We have found that this is roughly correct: a helix involving a single monomer per step having a radius of ≈2.5 Å and a step of ≈7.3 Å, measured between homologous nitrogens of rings (Fig. 1).

It is important to see that all the atoms lie outside the ideal cylinder on which the polymer is wound without steric hindrances, so the aminic nitrogens of the repeating unit are in the most favourable position for their interaction with heparin.

We can hypothesize that heparin chains, formed by a sequence of hexose units carrying sulphate and carboxyl groups, wrap around the poly(amido-amine) following its helicoidal structure.

REFERENCES

1 (a) Danusso, F. and Ferruti, P. *Polymer* 1970, **11**, 88; (b) Barbucci, R., Casolaro, M., Ferruti, P., Barone, V., Lelj, F. and Oliva, L. *Macromolecules* 1981, **14**, 1203

2 Barbucci, R., Paoletti, P. and Vacca, A. *J. Chem. Soc. (A)* 1970, 2202; Barbucci, R., Casolaro, M., Barone, V. and Ferruti, P. *Macromolecules* 1983, **16**, 1159

3 Ferruti, P., Domini, I., Barbucci, R., Beni, M. C., Dispensa, E., Sancasciani, S., Marchisio, M. A. and Tanzi, M. C. *Biomaterials* 1983, **4**, 218

4 Barbucci, R., Ferruti, P. and Provenzale, L. It. Pat. Appl. 23610 A/80

5 Ferruti, P., Barbucci, R., Danzo, N., Torrisi, A., Puglisi, O., Pignataro, S. and Spartano, P. *Biomaterials* 1982, **3**, 33

6 Ferruti, P., Casini, G., Barbucci, R., Tempesti, F., Mastacchi, R. and Sarret, M. *Biomaterials* 1984, **5**, 234

7 Barbucci, R., Benvenuti, M., Casini, G., Ferruti, P. and Tempesti, F. *Biomaterials* 1985, **6**, 102

8 Barbucci, R., Ferruti, P. and Del Re, G. preliminary study