A CHALLENGE TO BLOOD COMPATIBLE MATERIALS

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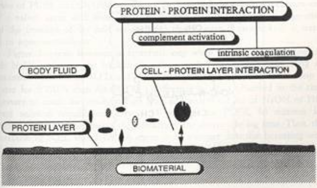
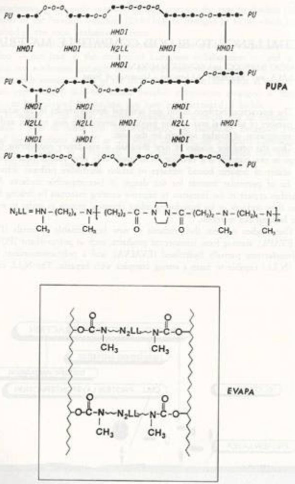
The interaction between blood and artificial devices results in the activation of a number of humoral and cellular processes involved in non specific and specific recognition of foreign surfaces by the host.

Thus the need for studies of new materials is mandatory considering that a change in raw material is a decision to be made only after careful consideration. The ability of heparin bound surfaces to inhibit alternative pathway activation may be of particular interest for the design of biocompatible surfaces. Many researches appear in the literature to improve existing materials by linking heparin to their surfaces or by introducing adapt chemical groups in order to render them heparin-like materials.

Our studies concern the synthesis of new heparinisable materials (PUPA and EVAPA), starting from commercial products, such as polyurethane (PU) and ethylvinylacetate partially hydrolised (EVALVA), and a poly(amidoamine) polymer (N_2LL) capable to form a strong complex with heparin. The N_2LL carries




PU
HMDI
N2LL
PUPA
N₂LL = HN – (CH₂)₄ – N –[(CH₂)₂ – C – N N – C – (CH₂)₂ – N – (CH₂)₄ – N]ₙ
CH₃
O
O-C-N~~N₂LL~~N-C-O
EVAPA

Schema 1

basic nitrogens that, once protonated, can electrostatically interact with the negatively charged molecules of heparin. The hypothesised structures for the two materials are reported in scheme 1. PUPA is a highly crosslinked material, due to the presence of a large excess of crosslinking agent (HMDI = hexamethylenediisocyanate) which forms bridges also between the PU chains. In the case of EVAPA instead, the EVALVA chains are crosslinked only by the N_2LL chains. The different degree of crosslinking is in some extent responsible for the different behaviour of the two materials. The physicochemical and biological characterisation put into light these differences.

PHYSICO-CHEMICAL CHARACTERISATION

Water up-take

Both PUPA and EVAPA swell in water because of the hydrophilic N_2LL component. Figure 1 shows the water up-take for both samples in 0.1 M NaCl and 1 M HCl solutions. The water up-take is almost the same for both samples and greater in acidic medium than in saline solution. The water up-take of PU and EVALVA is almost zero either in acidic or saline solution. PUPA and EVAPA differ only for the time necessary to reach the maximun degree of water up-take: less than one day in the case of PUPA, about a week in the case of EVAPA.

Basicity constants

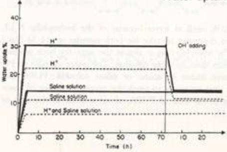
Only two protonation constants are sufficient to simulate the titration curves of PUPA and EVAPA (see Figure 2). Both the protonation constants and the n values agree with those reported for the free N_2LL polymer, suggesting that the freedom of the poly(amidoamine) chains, even if crosslinked, is restored in aqueous medium.

Figure 2 reveals however an hysteresis loop when titrating the two materials with NaOH solution and backtitrating them with HCl solution suggesting an interaction between the N_2LL and PU or EVALVA chains. The hysteresis loop is greater for EVAPA than for PUPA, a difference is infact observed in the time necessary to reach the steady voltage after addition of 0.1 ml of NaOH or HCl titrant solution (equilibrium curve): 15 minutes for PUPA, 60 minutes for EVAPA. Generally, the weaker the interaction, the shorter the lag time. Thus, the interaction of N_2LL with EVALVA seems to be stronger than that occurring with PU, according with the greater hydrophilicity of EVALVA with respect to PU.

Physico-chemical characterisation

Material	*Contact angle*		
	Θ_A	Θ_R	$\Theta_{R'}$
PUPA	75.4	65.1	49.3
Hep. PUPA	40.0	34.2	30.0
EVAPA	89.5	42.0	36.0
Hep. EVAPA	83.0	43.0	15.5

Water uptake



Water uptake of PUPA samples in 0.1 M NaCl (saline solution), acidic medium and in alkaline medium of: ——·——, PUPA I; ———, PUPA I bis; -○-○-○, PUPA I penta

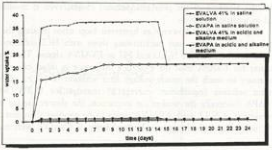
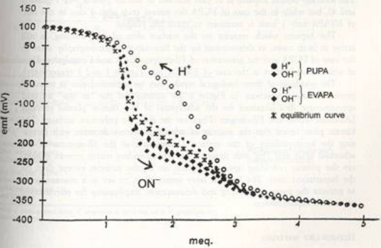


Fig. 1

Basicity constants of N_2LL (in 0.1M NaCl)

free polymer	$\log K_1 = 8.715$	$\log K_2 = 7.689$
in PUPA	$\log K_1 = 8.55$	$\log K_2 = 7.38$
in EVAPA	$\log K_1 = 8.66$	$\log K_2 = 7.30$



Titration curve

Fig. 2

Contact angle measurements

The top part of Figure 1 shows the advancing (Θ_a) and receding (Θ_r,Θ_r) contact angles for the dry and wet samples of PUPA, EVAPA and the heparinised surfaces. Comparison of the Θ_a values for the dry surfaces of PUPA and EVAPA emphasises the higher hydrophobicity of EVAPA with respect to PUPA. Both materials undergo to a surface restructuring once hydrated and the extent of surface reorientation is greater for EVAPA than for PUPA, as revealed by the receding contact angles of the dry and wet samples.

Once the surface of the two materials is heparinised, a different behaviour is still observed in terms of surface hydrophilicity e surface reorientation in aqueous media.

BIOLOGICAL CHARACTERISATION

In Figures 3-6 are summarised some of the biological data obtained for the two materials.

Both PUPA and EVAPA bind heparin through an electrostatic interaction. The ionically bound heparin is in part released in human plasma (see Figures 3 and 4), but while in the case of PUPA the release ends after 1 day, in the case of EVAPA only 1 hour is necessary to reach the plateau.

The heparin which remains on the surface after plasma washing, is still active in both cases, as demostrated by the Resonance Thrombography assay in the case of PUPA and the generation of Fibrinopeptide A and Coagulation Time of whole blood assays in the case of EVAPA (see Figures 3 and 5 respectively).

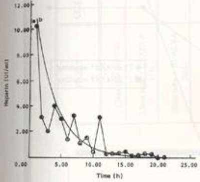
The goodness of these biological results may be explained also in terms of protein-surface interaction. In Figure 6 are summarised the "in situ" infrared spectroscopic data obtained for the adsorption of two human plasma proteins [Albumin (HSA) and Fibrinogen (Fbg)] on the different polymeric surfaces. The kinetic plots reveal that the amount of adsorbed protein decrease with increasing the hydrophilicity of the surface. Comparison of the IR spectra of the adsorbed HSA and Fbg with those of the corresponding native protein evidences the protein unfolding upon adsorption on all the surfaces except than on the heparinised ones. The heparin layer seems, thus, to act in a manner so as to prevent the protein unfolding and denaturation, emphasising the effectiveness of surface heparinisation.

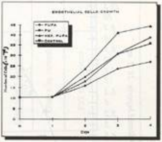
HEPARIN-LIKE MATERIALS

The anticoagulant activity of heparin is attributed to structural features, e.g. degree of sulfation, degree of dissociation, particular sequence of COO^- and

BIOLOGICAL TESTS ON HEPARINISED PUPA

Heparin release in human plasma:





Resonance trombography assay

Materials	r/r_0	F/F_0	P/P_0
Pellethane 2363-80AE after 4 days extraction	1.04 (±0.20)	0.90 (±0.20)	1.02 (±0.05)
PUPA	1.08 (±0.14)	1.09 (±0.16)	1.00 (±0.02)
Heparinized PUPA after washing with buffered solution	∞	—	—
Heparinized PUPA after washing with buffered solution and plasma	∞	—	—

*RTG: r, clotting time (min); F, amplitude of fibrin-leg (min); P, amplitude of platelet-leg (min).

PUPA material has not haemolytic activity

Fig. 3

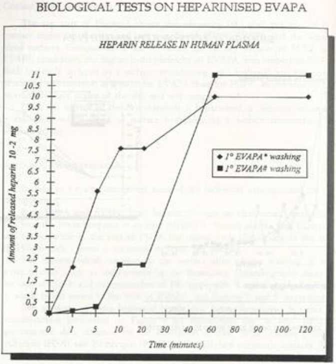
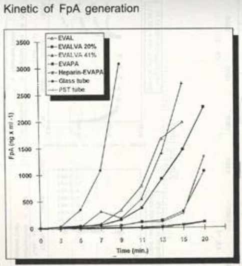


Fig. 4

SO_3^- groups, as well as to molecular shape and size. These factors appear to be related to the biological activity by virtue of their importance in the ion binding capacity of heparin.

Heparin by virtue of its high negative charges has a strong affinity for cations, and a pH dependence is observed.

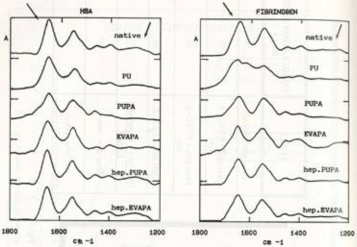
Most of the readily available natural polysaccharides have been sulfated in the attempt to obtain heparin analogs, and recently sulfate, carboxylic, and sulfonate groups were attached to some synthetic polymers such as polystyrene,



TUBES	*COAGULATION TIME (min.)*
Glass tube + EVAL	15'
Glass tube + EVALVA 41%	19'
Glass tube + EVALVA 20%	31'
Glass tube + EVAPA	22'
Glass tube + Heparinized EVAPA	67'
Glass tube control	10'
PST tube control	28'

Fig. 5

Adsorption of HSA and HFg



Kinetic plots

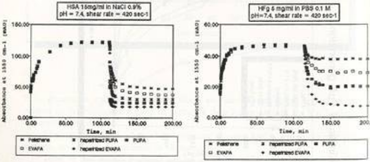


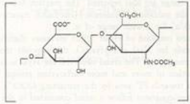
Fig. 6

and PU, too. The anticoagulant activities of these materials were much lower than heparin and were dependent on the type and binding of the substituents, the degree of substitution and sequences. Our approach for studying the structural properties associated with anticoagulant property was firstly to choose polymers possessing well-defined chemical groups consisting of regular repeating units and secondly to modify their chemical structure in order to render them more similar to the heparin molecule.

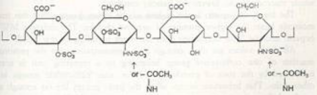
Therefore the macromolecule must satisfy these requirements:

1) to contain regular sequences of monomeric units
2) to be chemically modifiable without destroying its structure

Hyaluronic Acid (Hyal), the major component of mammalian extracellular matrix, consisting of alternating units of N-acetylglucosammine and glucoronic acid residues, seems a suitable macromolecule (see Figure 7).



Structural Unit of Hyaluronic Acid



Structural Unit of Heparin

Fig. 7

Hyaluronic acid has been modified by the introduction of sulfate groups, to create a potentially heparin-like material and the pH dependent behaviour of this macromolecule in aqueous solution, together with its antithrombotic activity has been evaluated.

PROTONATION EQUILIBRIA IN AQUEOUS SOLUTION

Potentiometric and viscosimetric titration

The potentiometric and viscosimetric data are summarised in Figure 8.

In the top part of the figure, the log K's relative to the protonation reaction $-COO^- + H^+ \rightleftharpoons -COOH$ of the carboxylate group present in the structural unit of HyalSO$_3$ are reported and compared to those of Hyal, heparin and Hyaff 11p25 (a sample of hyaluronic acid in which the 25% of carboxyl groups are esterified with benzyl alcohol). In the same figure the *n* value and the degree of protonation (α) range are also reported. The reaction follows a linear pattern corresponding to the modified Henderson-Hasselbalch equation [$\log K = \log K^\circ + (n - 1) \log (1 - \alpha)/\alpha$] for a wide range of α.

The protonation constant of Hyaff 11p25 increases slightly with the degree of protonation, while for Hyal and HyalSO$_3$ the opposite trend occurs (log K decreases with increasing α). The trend observed for Hyal is consistent with the fact that neutralisation of more and more carboxylate groups reduces the electrostatic attraction towards H^+ ions by the remaining COO^-'s.

The protonation constants follow a trend connected to the number of negative charges present in each repeating unit. In fact the sulfation of some $-OH$ groups of the structural unit of hyaluronic acid renders the $-COO^-$ of HyalSO$_3$ more basic than that of Hyal, allowing a stronger attraction towards H^+ ion. The importance of the negative charges on the basicity constant appears evident when observing that log K of Hyal is higher than that of Hyaff 11p25. The lower quantity of $-COO^-$ groups, even if they are random spread along the whole macromolecule, lowers the basicity constant.

The log K of heparin is the highest of the series showing that this macromolecule bears the highest number of negative charges. The different MW's of heparin and the Hyal derivatives does not seem to influence this trend.

All the *n* values are close enough to 1 demonstrating that the protonation reaction of one carboxylate group belonging to a repeating unit is scarcely influenced by the state of protonation of the other carboxylate groups in the other units. This behaviour occurs when the basic groups are far enough apart on the skeleton or some cumbersome chemical groups shield interactions among the groups to be protonated.

The bottom part of Figure 8 shows the viscosimetric titration of the above mentioned samples.

Protonation constants of polysaccharides in 0.1 M NaCl at 25°C

	log K_i	n	degree of protonation range
$HyalSO_3$	3.50 (2)	1.40 (2)	0.1 - 0.6
Hyal	3.04 (6)	1.18 (4)	0.1 - 0.6
Hyaff 11p25	2.92 (4)	0.88 (3)	0.1 - 0.7
Hep	~4.1	–	–

$$\log K = \log K^\circ + (n-1) \log [(1-2)/2]$$

Viscosimetric Titration

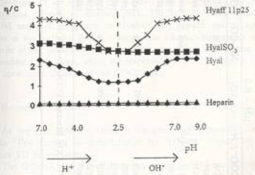


Fig. 8

MAIN WAVENUMBERS OBSERVED IN THE IR SPECTRA OF THE POLYSACCHARIDES SOLUTION AT DIFFERENT pHS

	pH ~ 6.5				pH ~ 4.5				pH ~ 2		
	COO^- / H		$O{-}SO_3^-$	NH-CO	COO^- / H		$O{-}SO_3^-$	NH-CO	COO^- / H	$O{-}SO_3^-$	NH-CO
Hyal	1618 1589 1400			1670	1615 1590 1400	1725*sh*		1660	1725 1700		1670 1648
Hyaff 11p25	1615 1580 1410	1743^b		1665	1610 1580 1410	1740^b 1730*sh*		1651	1745^c (*A*) 1725 (*A*)		1650 (*A*) 1628 (*A*)
$HyalSO_3$	1625 1585 1403		995	1670	1620 1585 1405	1735*sh*	996	1670	1735 1715*sh*	997	1665
Heparin	1607 1595 1405		1000	1675	1605 1595 1405	1735	1000	1670 1650 *sh*	1735 1710*sh*	1000	1670 1650 *sh*

(*A*) dried gel; (^{b}) COOR bands; (^{c}) COOR + COOH bands; *sh* shoulder

Fig. 9

The η/c decreases by adding H^+ to hyaluronate reaching a minimum when the macromolecule is fully neutralised. Further, by adding OH^- to this solution η/c rapidily increases reaching the same value of the starting solution of ialuronate. This behaviour is typical of a macromolecule which contracts or expands as a result of electrostatic repulsion of the dissociating groups. In this case, the unique groups bearing charges in this polymer are the carboxylate ions.

When some of the ionisable groups are esterified like in Hyaff 11p25 a trend similar to Hyal is observed, but more marked. The decreased number of charges in the partially esterified material and the presence of phenyl groups, which can interact each other intramolecularly, renders the macromolecule more sensitive to pH variations (i.e. to the uncoiling-coiling process).

On the contrary, by inserting other, not easily protonable groups like sulfate groups ($HyalSO_3$), in the Hyal backbone, a different trend is observed. A slight decrease of η/c occurs by adding H^+, then it remains constant. The presence of non neutralisable negative charges (SO_3^- groups) hinders the coiling of the macromolecule when the COO^-'s are neutralised, rendering the $HyalSO_3$ chains more rigid and stretched.

The viscosity of heparin by neutralising the COO^- groups shows a smoother trend than that of $HyalSO_3$, according to the greater number of negative charges per structural unit.

Infrared studies

In Figure 9 are reported the infrared data obtained for the polysaccharides in aqueous solution at different pH's. The lack of lower frequencies for the COOH and NHCO groups, in $HyalSO_3$ and heparin spectra upon the protonation of the carboxyl group, confirms the viscosimetric results and emphasises the role of the negatively charged sulfate group in hindering the intermolecular H-bonding interactions, which are responsible for the coiling of the macromolecule upon neutralisation of the COO^- group.

Cu(II)-COMPLEX FORMATION

As heparin, $HyalSO_3$ forms a complex with copper(II) ions in aqueous media. This finding is demonstrated by ^{1}HNMR (Figure 10) and infrared (Figure 11) experiments.

The ^{1}HNMR and ^{13}CNMR spectra of the Cu(II)-heparin system reveals the involvement of the COO^- and acetyl groups of heparin in the complex formation with Cu(II) ions.

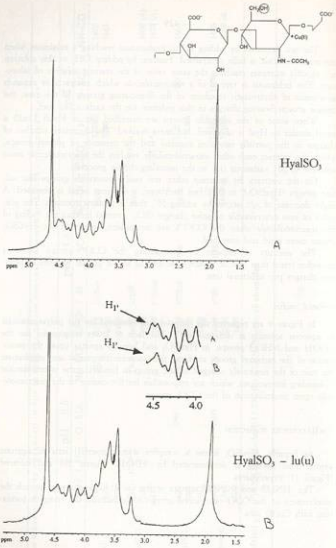

COO⁻
CH₂OH
COO⁻
OH
OH
OH
HN – COCH₃
Cu(II)
HyalSO₃
A
ppm 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
H₁'
H₁'
A
B
4.5 4.0
HyalSO₃ – lu(u)
B
ppm 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

Fig. 10

MAIN WAVENUMBERS OBSERVED IN THE IR SPECTRA OF THE Cu^{2+} POLYSACCHARIDE SYSTEMS AT DIFFERENT pHs

	pH ~ 6.5				pH ~ 4.5				pH ~ 2			
	COO⁻ /	H	O–SO_3^-	NH-CO	COO⁻ /	H	O–SO_3^-	NH-CO	COO⁻ /	H	O–SO_3^-	NH-CO
Heparin:Cu	1620 1570 1410		1003	1675*sh* 1650	1622 1574 1410		1005	1670*sh* 1650	1620 1575 1410	1729*w*	1008	1670*sh* 1650
HyalSO_3:Cu	1630 1575 1410		1015 1000	1674 1649	1620 1586 1410	1721*w*	1009	1675 1652	1615*w* 1570*w* 1400*w*	1725	1003	1675 1650
Hyal:Cu	GEL		GEL	GEL	1615 1588 1403	1725*w*		1668 1650		1730*s* 1695*s*		1668 1650

sh shoulder; *w* weak; *s* strong

Fig. 11

NMR

The main effect, induced by the Cu(II) ion on $HyalSO_3$, is the disappearance of the H_1-GlcNAc resonance (see Figure 10). As the chemical shift of the molecule does not change in the presence of Cu(II) the signal disappearance can be interpreted considering a strong Cu(II) paramagnetic contribution to the H_1-GlcNAc proton as a consequence of the short distance from the metal coordination site. A further effect is a linewidth enhancement induced by Cu(II) on the methyl proton of the acetyl group. This effect, in a lower extent, is also observed for the natural hyaluronic acid.

From these results the following conclusion can be drawn:

i) Cu(II) ion has a preferential binding site on heparin, hyaluronic acid and sulfated hyaluronic acid.

ii) The coordination site involves two different polymeric subunits, the carboxylic group of a residue and the amidic group of a previous D-glucosamine moiety.

iii) The sulfate group apparently is not directly involved in the coordination site, but play an important cooperative role in metal-complex formation. These effects include the increase of the negative charge on the polymeric surface responsible for the preliminary electrostatic interaction with Cu(II) ions. Moreover the sulfate groups may reduce segmental motion creating a more favourable conditions for metal complexing.

IR

The main frequencies observed in the spectra of the heparin-Cu(II), $HyalSO_3$-Cu(II) and Hyal-Cu(II) systems at three different pHs are summarised in Figure 11.

The presence of the COO^- absorption frequencies at $pH = 2.0$ in the case of Cu(II) complex with heparin and $HyalSO_3$ means that the carboxylic group is deprotonated by the metal-ion. This finding, together with the shift of the carboxyl absorption frequencies, with respect to those of the free ligands, confirm the involvement of such group in the complex formation.

The Cu-$HyalSO_3$ interaction through COO^- groups is however weaker than that occurring in the Cu(II)-heparin system. The drop of the carboxyl bands, with respect to those of the free $HyalSO_3$ at the same pH, is lower than in the case of heparin, indicating a weaker interaction of this group with the metal ion.

As for heparin, the presence of two amide $C=O$ absorptions in the spectra of $HyalSO_3$-Cu(II) system confirms that this group is involved in the interaction with the metal ion. Only one absorption is present in the spectra of the free $HyalSO_3$.

No evident variations are instead observed for the frequencies of sulfate group, in the case of both heparin and $HyalSO_3$.

In the case of Hyal-Cu(II) system, only the data relative to the lowest two pH's are reported. Infact at pH = 6.5 it was not possible to record the infrared spectrum of the system because of the formation of a gel.

Hyal seems not to significantly interact with the copper(II) ions, since the absorption frequencies of both carboxyl and amide groups remain mainly unchanged. The presence of the COOH bands only, at pH = 2.0 clearly demonstrates that the interaction of the Hyal carboxyl group with the metal ion, if any, is very weak. On the other hand, the shift observed in the amide C = O frequencies is very low too, and this proves the absence of any strong interaction with Cu^{2+} ion at this pH.

BIOLOGICAL CHARACTERISATION

Thrombin Time (TT) and human whole blood clotting time tests

The antithrombotic activity has been determined by measuring thrombin times of $HyalSO_3$ in plasma. For the sake of comparison, results obtained with Hyal and Hyaff 11p25 are reported in Figure 12. It was shown that TT times are not lengthened in the presence of Hyal or Hyaff 11p25. On the contrary, $HyalSO_3$ exhibits a lengthening of TT corresponding to 0.4 U.I. or 0.0026 mg/ml of heparin per mg of product.

Furthermore the clotting time of human non-anticoagulated blood was performed to evaluate the enhancing effect of hemostatic agents on both plasmatic and cellular activation of the coagulation cascade. The coagulation time was longer than 2 h in the presence of $HyalSO_3$, while for the whole blood control was 15 minutes. Moreover, after 45 minutes, blood in presence of Hyal was completely coagulated, and a network of small coagules was observed on the wall of the tube containing blood and Hyaff 11p25. These data show that $HyalSO_3$ exhibits an anticoagulant activity.

Interaction with erythrocytes (Hemolysis test)

The hemolysis assay measures the direct interaction of substances with the plasma membrane of erythrocytes.

The results obtained with sulfated hyaluronic acid (Figure 12) shows that this material does not have any hemolitic activity. In fact the control curve and the $HyalSO_3$ curve are superimposed.

Cultured human endothelial cells

In Fig. 12 the human umbilical vein endothelial cells (HUVEC) growth curves are shown.

BIOLOGICAL TESTS ON SULFATED HYALURONIC ACID

MATERIALS	*THROMBIN TIME (sec)*
Control	10.5
Hyaff 11p25	9.5
Hyal	9.4
$HyalSO_3$	30.0 ± 1

MATERIALS	*COAGULATION TIME (minutes)*
Control	15
$HyalSO_3$	> 120

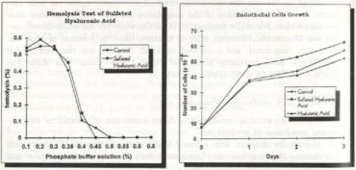


Fig. 12

The number of endothelial cells in medium containing HyalSO$_3$ increased with time and a better growth is revealed than in medium containing Hyal or in a pure medium control.

The morphology of endothelial cells was examined using inverted microscopy.

Endothelial cells in medium containing HyalSO$_3$ were well spread with no morphological alteration and without structural changes in cell organisation.

The same morphology was noted for the endothelial cells in presence of Hyal and for the control. The only remarkable difference was in the cell proliferation. In fact, after 1 day the cells in medium containing HyalSO$_3$ were almost a confluent monolayer, while the cells in medium containing Hyal or only medium reached a confluency only after 3 days.

BIBLIOGRAPHY

Barbucci R, Magnani A., *Physico-chemical characterization and coating of polyurethane with a new heparin adsorbing materia*, «Biomaterials», *10*, 429-432, 1989.

Barbucci R., Benvenuti M., Dal Maso G., Nocentini M., Tempesti F., Losi M., Russo R., *Synthesis and physico-chemical characterization of a new material (PUPA) based on polyurethane and poly(amido-amine) components, capable of strongly adsorbing quantities of heparin*, «Biomaterials», *10*, 299-308, 1989.

Barbucci R., Tempesti F., Magnani A., *Polyurethane based materials for applications in biomedicine*, First Mediterranean School of Science and Technology of Advanced Polymer Base Materials, Ed. by E. Martuscelli, G. Ragosta and C. Silvestre, 735-748, 1989.

Barbucci R. et al., *Workshop on the characterization of biomaterials in contact with blood*, «Biomaterials», *11*, 138-151, 1990.

Barbucci R., Casolaro M., Magnani A., Roncolini C., *FT-IR and potentiometric study of basic polymer behaviour in the free form, in different bulks, both in solid state and aqueous solution*, «Polymer», *323* (5), 897-903, 1991.

Barbucci R., Magnani A., Roncolini C., *Advantages and problems using FT-IR spectroscopy to study blood-surface interactions by monitoring the protein adsorption process*, in: «Modern Apects of Protein Adsorption on Biomaterials», Ed. by Y.F. Missirlis and W.G. Lemm, Kluwer Academic Publisher, The Netherlands, 91-96, 1991.

Barbucci R., Magnani A., Albanese A., Tempesti F., *Heparinized polyurethane surface through ionicbonding of heparin*, «ASAIO», *14* (8), 499-507, 1991.

Barbucci R., Albanese A., Magnani A., Tempesti F., *Coating of commercial devices with a new heparinizable material*, «J. Biomed. Mater. Res.», *25*, 1259-1274, 1991.

Barbucci R., Magnani A., Roncolini C., *Thermodynamicand FT-IRspectroscopic studies on heparin-polycation interaction*, «Clinical Materials», *8*, 17-23, 1991.

Barbucci R., Magnani A., Tempesti F., Benvenuti M., *Synthesis of two novel heparinizable polymeric materials starting from an ethylene vinylalcohol vinylacetate (EVALVA) terpolymer*, «Die Makromolekulare Chemie», *193* 12, 2979-2988, 1992.

Barbucci R., Tempesti F., Benvenuti M., Magnani A., Albanese A., *Preparation, physico-chemical and biological characterization of heparinizable materials*, in: «Advances in Biomaterials: Biomaterial-Tissue Interfaces», P.J. Doherty, R.L. Williams, D.F. Williams, A.J.C. Lee (Eds.), Elsevier Vol. 10, p. 217, 1992.

Barbucci R., Casolaro M., Magnani A., *Characterization of biomaterial surfaces: ATR/FT-IR and potentiometric/calorimetric analysis*, «Clinical Materials», *11*, 37-51, 1992.

Barbucci R., Magnani A., Albanese A., Benvenuti M., Casolaro M., *Biomaterials and artificial organs*, in: «First Kyoto-Siena Symposium: The development of science for the improvement of human life», R. Barbucci, F. Casprini (Eds.), pp. 19-37, 1992.

Barbucci R., Benvenuti M., Magnani A., Albanese A., *New trends in blood compatible materials*, in: «Innovative Materials - Prospects and Problems in a Competitive Industrial Contest», Advances in Science and Technology 2, P. Vincenzini Ed., Techna Publishers 1993.

Cimmino S., Martuscelli E., Silvestre C., Barbucci R., Magnani A., Tempesti F., *Structure-property relationships of polyurethane-based materials (PUPA) for application in biomedicine*, «Journal of Polymers Science», *47* (4), 631, 1993.

Magnani A., Barbucci R., *Fourier Transform Attenuated Total Reflection Infrared Spectroscopy: application to proteins adsorption studies*, in: «Test Procedures for the Blood Compatibility of Biomaterials», S. Dawids (Ed.), Kluwer Academic Publishers (NL), 171-184, 1993.

Barbucci R., Benvenuti M., Tempesti F., *New perspectives in the hemocompatible polymeric materials*, in: «The development of Science for the improvement of human life - Siena-Kyoto Symposium», Y. Imanishi (Ed.), Kyoto 1993.

Albanese A., Barbucci R., Belleville J., Bowry S., Eloy R., Lemke H.D., Sabatini L., *In vitro biocompatibility evaluation of a heparinizable material (PUPA) based on polyurethane and poly(amido-amine) components*, «Biomaterials», *15* (2), 129-136, 1994.

Barbucci R., Busi E., Magnani A., Ferruti P., Ranucci E., Taraviras S., *Biomaterials*, «La Chimica & l'Industria», *1*, 17-23, 1994.

Barbucci R., Benvenuti M., Tempesti F., *Modifications to polymer surfaces to improve blood compatibility*, in: «Polymeric Materials», Severian Dumitriu (Ed.), Marcel Dekker Publisher, 199-230, 1994.

Barbucci R., Magnani A., *Haemocompatible Materials: Surface and Interface Aspects*, in: «Handbook of Biomaterials and Applications», Marcel Dekker Publisher, in press.

Barbucci R., Magnani A., *Conformation of human plasma proteins at polymer surfaces, the effectiveness of surface heparinisation*, «Biomaterials» 15 (12), 955-962, 1994.

Barbucci R., Magnani A., Baszkin A., Da Costa M.L., Bauser H., Hellwig G., Martuscelli E., Cimmino S., *Physico-chemical surface characterization of hyaluronic acid derivatives as a new class of biomaterials*, «J. of Biomaterials Science, Polymer Edn.», *4* (3), 245-273, 1993.

Barbucci R., Magnani A., Casolaro M., Rossi C., *Modification of hyaluronic acid by sulfate groups insertion to obfain a heparin-like molecule. Part I. Characterisation and behaviour in aqueous solution towards H^+ and Cu^{2+} ions*, Gazzetta Chimica Italiana (in press).

ACTIVITY OF C.R.I.S.M.A.

C.R.I.S.M.A. is an Interuniversity Research Center for Advanced Medical Systems in which the Universities of Bologna, Brescia, Milano and Siena are involved.

The studies carried out in the Center are essentially focused on the obtaining of materials with an improved blood compatibility to be utilised for cardiovascular prostheses, oxygenators, extracorporeal circulation circuits, dialysis membranes, catheters and any other apparatus coming in contact with blood, included a whole artificial heart.

In particular, the research performed in Prof. Barbucci's laboratory regards the study of some polymers (polyamidoamines) capable to stably complex heparin at physiological pH. These polyamidoamines were synthesised and both bound to the surface of some commercial materials and crosslinked with other polymers originating new materials.

Recently, the study involved insulin controlled release systems modulated by glucose and thus simulating the functions of an artificial kidney.

GOD (glucose oxidase) was immobilised on polymeric chains which were previously linked on a cellulose membrane surface; successively, insulin permeability was studied by these functionalised membranes in reply to the variation of glucose concentration.

The scientific activity can be summarised in the following main points:

— *Surface modification (by chemical or plasma treatment) of commercial polymeric materials and synthesis of new materials with improved haemocompatibility*
  a) surface modification and subsequent heparinisation of substrates of: glass, Dacron, PVC, Silastic, polyurethane,
  b) synthesis of heparinisable materials,
  c) coating of medical devices with heparinisable materials,
  d) surface polymer grafting of cellulose membranes via chemical routes and glow discharge.

— *Physico-chemical characterisation of biomaterials*
  a) FTIR/ATR, FTIR/DR,
  b) contact angle measurements,
  c) potentiometry,
  d) calorimetry,
  e) SEM.

— *Study of the adsorption of plasma proteins by in situ infrared spectroscopy*

— *Biological characterisation (in vitro tests)*
  a) citotossicity tests,
  b) thrombocenicity tests,
  c) haemolysis test,
  d) study of heparin release in plasma.