

High throughput analysis of hemocompatibility

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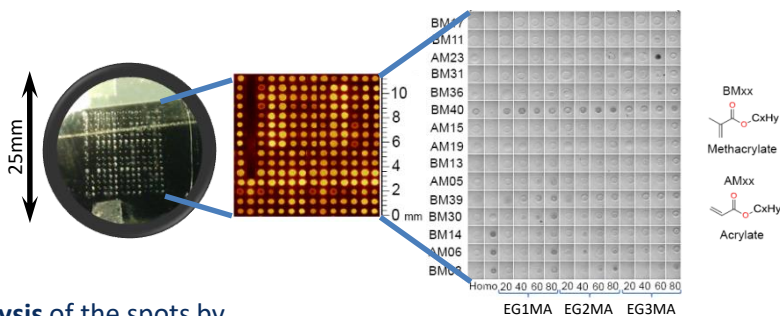
Motivation: HTS screening for hemocompatible surface modifications

- **Blood clotting** and **bacterial colonization** present ongoing problems of vascular catheters and other devices
 - Both effects are highly interrelated
- **Protein adsorption** is an early event which influences both coagulation and bacterial adhesion
- **Co-polymers** with PEG-like, linear and cyclic aliphatic and aromatic components at different ratios allow wide variation of surface properties with differences in their physical-chemical surface properties¹
 - Systematically varied co-polymers are provided as microarrays
 - Hemocompatibility assays need adjustment for microarray set-up

Method: Spotted polymer-microarrays for protein and cell adhesion

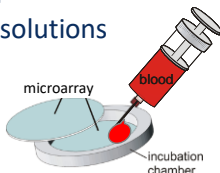
Microarrays printed on poly(hydroxyethyl methacrylate) (pHEMA) coated glass disks

- 15 monomers
- 3 ethylene glycol monomers
- various ratios of the components
- spot diameter 300 μm



Incubation with

- single protein solutions
- blood plasma
- whole blood

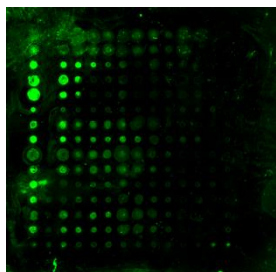


Analysis of the spots by

- direct fluorescence:
 - conjugated proteins
- immunofluorescence
 - proteins, blood platelets
- fluorescent nucleus stain
 - leukocytes
- colonization of fluorescent bacteria

First results: Polymer-microarrays in single protein solution and in whole blood

scan array Fibrinogen



density heat map Fibrinogen

homo		density heat map Fibrinogen											
		EG1AM				EG2AM				EG3AM			
		20%	40%	60%	80%	20%	40%	60%	80%	20%	40%	60%	80%
22,000	BM17	0.372	14.812	13.766	13.318	7.410	0.934	20.922	18.222	1.100	1.988	2.69	3.09
50,944	BM11	11.980	10.854	17.197	14.110	2.370	0.955	9.148	18.141	1.001	1.91	-1.11	4.77
207,398	AM23	181.619	109.136	89.977	12.813	0.408	4.44	2.761	7.57	2.63	4.75	1.79	1.11
30,748	BM31	31.276	31.933	8.47	3.52	1.411	3.19	4.46	1.368	4.69	3.95	-4.84	-1.007
129,229	BM36	138.096	49.900	6.09	3.60	2.104	3.75	7.71	9.86	2.76	4.26	-1.13	-1.410
59,901	BM40	10.985	1.081	3.57	4.92	8.88	2.76	3.08	4.17	3.51	4.76	5.54	4.18
39,922	AM15	64.174	31.312	14.876	15.119	19.219	2.041	0.318	9.14	7.18	7.5	1.2	2.28
197,641	AM19	86.574	21.205	28.238	29.702	29.888	11.977	1.343	6.47	5.16	8.85	6.25	6.73
10,181	BM13	4.885	13.797	20.011	9.981	10.750	0.180	2.375	1.723	1.55	2.43	-2.46	-2.67
81,433	AM05	51.016	12.041	12.199	14.081	12.867	8.931	8.748	9.76	4.86	1.21	1.42	3.65
80,442	BM39	1.495	1.297	4.422	0.380	0.080	8.32	1.123	8.57	6.0	-10.2	-1.15	-9.52
50,464	BM30	1.721	7.285	6.401	2.221	3.288	2.446	13.226	1.721	1.45	1.50	-4.78	-7.55
16,277	BM14	20.810	2.987	24.218	25.610	10.074	4.921	6.89	1.828	1.65	1.94	-2.06	2.48
30,709	AM06	20.927	36.246	17.213	6.446	1.009	2.943	4.943	1.729	1.42	1.80	8.1	13.60
1,809	BM08	13.366	1.883	4.86	2.701	0.240	1.713	1.316	1.130	3.09	7.33	8.63	14.996

density heat map Leukocytes

homo		density heat map Leukocytes											
		EG1AM				EG2AM				EG3AM			
		20%	40%	60%	80%	20%	40%	60%	80%	20%	40%	60%	80%
1,992	BM17	1.678	4.000	8.301	5.418	3.683	4.047	1.9121	8.728	1.362	9.439	9.677	13.033
760	BM11	1.994	1.775	4.862	11.044	1.991	4.776	10.762	25.245	2.261	17.457	22.614	21.380
13,940	AM23	17.989	27.945	37.148	15.702	15.921	14.998	10.980	9.515	5.038	1.276	13.980	9.540
13,613	BM31	13.247	10.024	17.446	5.304	11.362	10.764	1.907	6.607	10.106	11.920	8.228	17.933
14,671	BM36	14.366	47.808	39.875	20.388	16.851	18.193	10.616	20.412	14.400	4.356	10.535	9.421
58,422	BM40	16.465	59.532	52.710	62.118	50.717	40.103	11.884	11.107	10.187	12.916	26.054	19.980
40,023	AM15	10.988	4.628	7.085	4.394	6.910	9.201	7.822	23.850	10.011	107.332	121.403	62.705
52,818	AM19	13.780	50.333	19.547	81.159	12.478	112.074	29.980	60.233	41.976	5.946	12.942	12.248
5,343	BM13	5.400	18.125	14.443	11.910	11.769	4.141	3.210	6.744	7.385	6.453	27.146	11.453
16,818	AM05	14.783	7.818	3.990	1.919	2.919	4.964	4.851	6.905	8.351	17.340	19.637	13.620
11,027	BM39	17.204	30.444	9.208	8.044	13.275	7.406	17.574	12.246	14.749	17.801	10.256	10.040
10,961	BM30	14.971	19.846	19.139	13.618	11.041	5.439	8.805	6.180	16.910	7.649	9.085	13.640
7,189	BM14	10.018	2.230	2.991	3.743	11.679	2.144	1.818	4.012	5.213	9.435	21.210	41.832
8,648	AM06	27.709	2.481	3.918	2.514	1.362	5.212	1.976	4.220	4.507	7.128	5.065	20.740
2,385	BM08	8.214	3.094	2.833	3.614	2.645	2.007	2.103	2.120	3.339	3.190	3.981	13.921

- **Fibrinogen adsorption** is influenced by
 - chain length of the PEG-component EG_xAM in the co-polymer
 - concentration of the PEG-component

- **Leukocyte adhesion** shows different pattern
 - Trend for higher density at longer PEG

Conclusion: Hemocompatibility assays can be adapted for HTS screening

Hemocompatibility-relevant information can be obtained from single spots of a polymer microarray

¹: Hook A.L., ..., Alexander M.R. Nature Biotechnol 30: 868-875 (2012)