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Molecular Imaging: Immuno-targeting Probes for Magnetic Resonance and Optical Imaging

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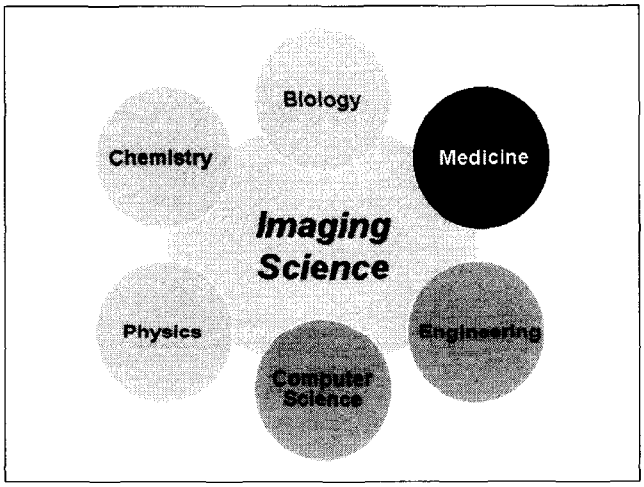


Image:

- –availability of high affinity probes with reasonable pharmacodyanmics
- – the ability of these probes to overcome biological delivery barriers (vascular, interstitial, cell membrane)
- – use of amplification strategies (chemical or biological)
- – availability of sensitive, fast, high resolution imaging techniques

Molecular Imaging Reviews

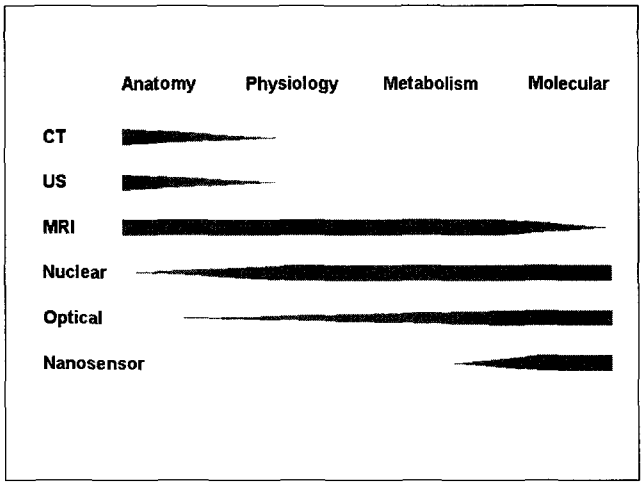
R. Weissleder, U. Mahmood 'Molecular imaging' *Radiology* 2001;219:316-333.

G. D. Luker, D. Piwnica-Worms 'Molecular imaging in vivo with PET and SPECT' *Acad. Radiol.* 2001;8:4-14.

S. Gambhir 'Molecular imaging of cancer with PET' *Nature Rev.* 2002;2:683-693.

S.D. Voss, J. B. Kruskal 'Gene therapy: a primer for radiologists' *Radio. Graphics.* 1998;18:1343-1372.

M. G. Pomper 'Molecular imaging: an overview' *Acad. Radiol.* 2001;8:1141-1153. C. Nichol, E. E. Kim 'Molecular imaging and gene therapy', *J. Nuclear Med.* 2001;42:1368-1374.



Optical Imaging:

– Fluorescence Imaging (e.g. Green Flouorescent Protein)

– Near Infrared Fluorescence (NIRF) Imaging

– Bioluminescence Imaging

Optical Techniques:

– Both colorimetric and fluorescent proteins require external source of light for excitation and emit light at a different wavelength for detection.

– Bioluminescent proteins (e.g. firefly luciferase) do not need external excitation light source and has lower inherent background noise.

Optical Imaging Advantages:

– Low cost

– Technically straight forward

– Reproducible

– Don't have to deal with radioactivity

Optical Imaging Disadvantages:

– depth dependent signal

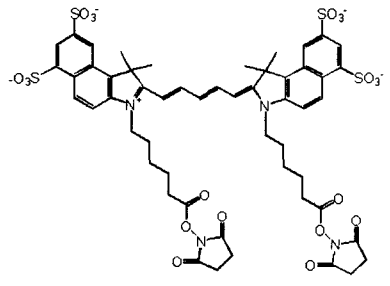
– Non-tomographic

– not usable with larger species

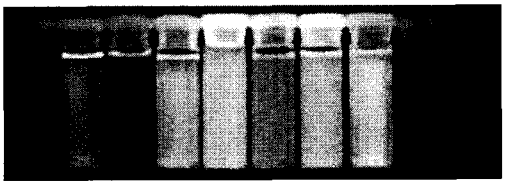
– No detailed anatomical information

– Not as generalizable as radiolabeled approaches.

– Because not any event can be monitored



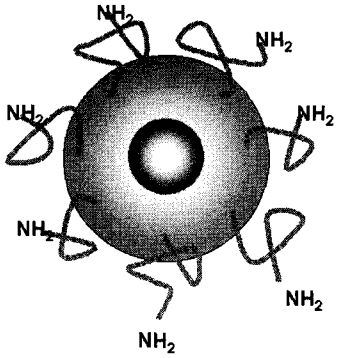
Cy 5.5: Absorption maximum 675 nm, Emission maximum 695 nm



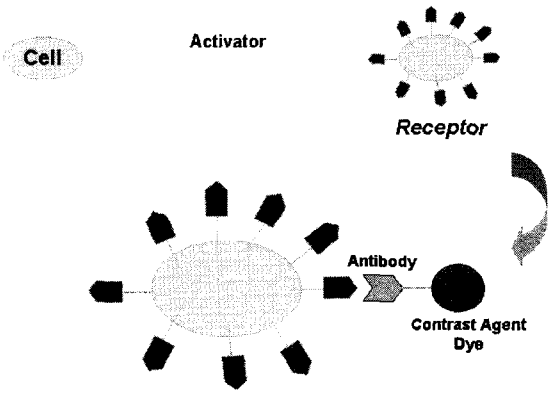
Quantum Dots (QDs)

MRI Contrast Agent:

- Gd-DTPA
- Iron Oxide Nanoparticles
- Chemical Shift



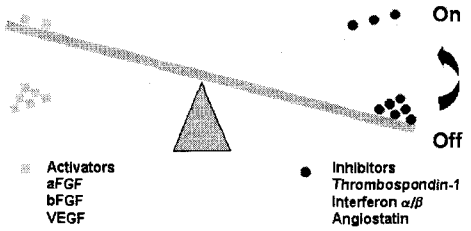
Cross-linked Iron Oxide Paramagnetic Nanoparticles



Angiogenesis:

- Formation of new blood vessels from an existing vascular network.
- Critical role in tumor progression.

Angiogenic Switch:



D. Hanahan, J. Folkman, Cell, 86, 353 (1996)

E-Selectin:

- Endothelium-specific protein participating in the rolling of leukocytes in inflammation.
- Affects endothelial cell migration and capillary tube formation in vivo.
- Expressed by proliferating endothelial cells in vitro.
- A potential target regulated by angiostatin.

→ E-selectin positive vessels are preferentially found in vascular-rich areas.

The Goal:

– To develop new E-selective specific diagnostic optical and magnetic resonance imaging probes using endothelial cell surface marker expression for identifying early signs of angiogenesis.

Immuno-targeting Probe

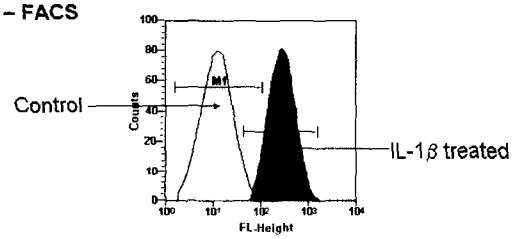
The Technical Issue :

– Limited utilization of biomolecules targeted against tumor-associated markers due to insufficient amount delivered and the high background ratio.

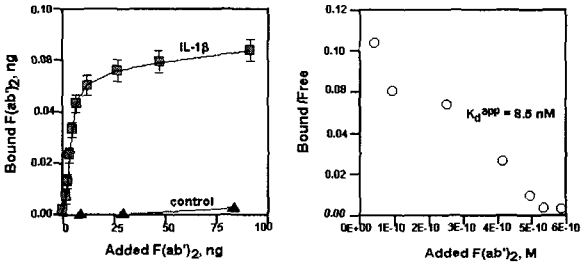
Primary model affinity molecules:

– 18/7 Fragment of monoclonal anti-human E-Selectin antibody, $F(ab')_2$.

– FACS



Affinity of 18/7 mAb fragment to HUVECs



Effective *Specific Binding* and *Apparent Dissociation Constant (K_d^{app}) Value* to HUVECs proves high affinity.

Suitable Carrier for Antibody-mediated targeted delivery: graft co-polymers protected by MPEG chains (protected graft co-polymers, PGC)

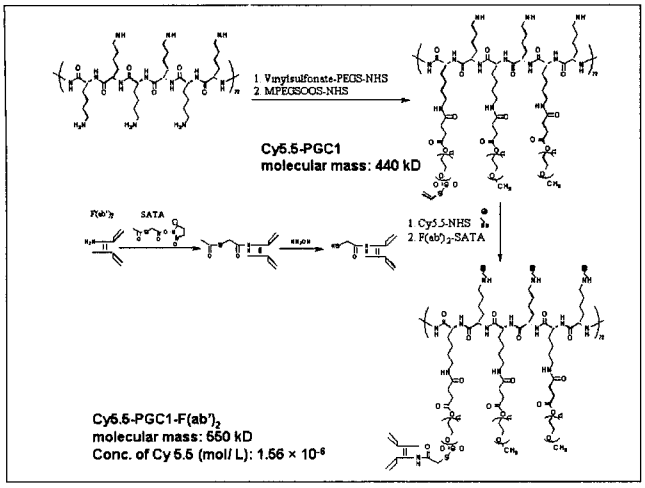
1) long circulation in the bloodstream
2) *high probe loading of graft co-polymer molecule*
3) the lack of immunogenicity and toxicity
4) *the ability to cross "leaky" endothelial barriers in tumors*

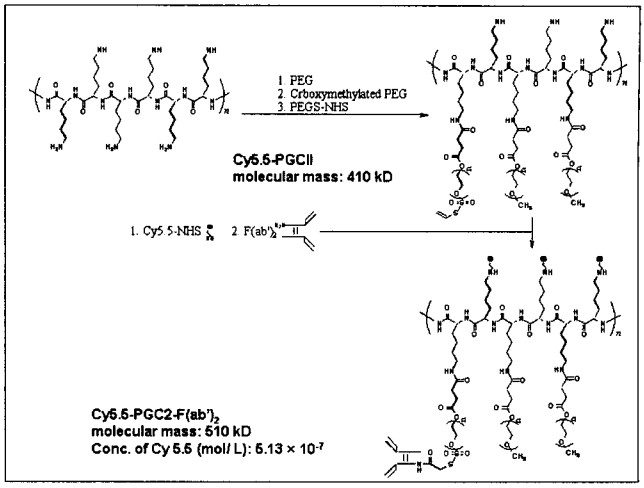
Syntheses of Protected Graft Copolymer (PGC):

Two types of graft copolymer of O-methoxy poly(ethylene glycol) and poly(L-lysine) in functionalized MPEG grafting:

I) containing sulfhydryl-reactive terminal vinylsulfonate groups (MPEG-PL-VS, PGCI)

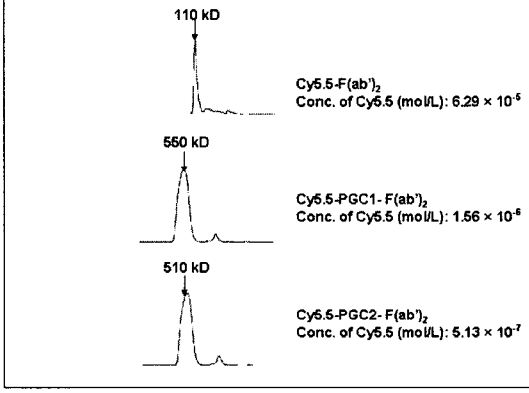
II) containing terminal carboxyl groups (MPEG-PL-COOH, PGCII).



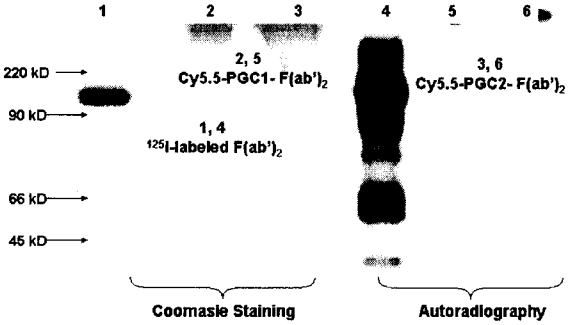


Analyses of PGC-based conjugates prepared

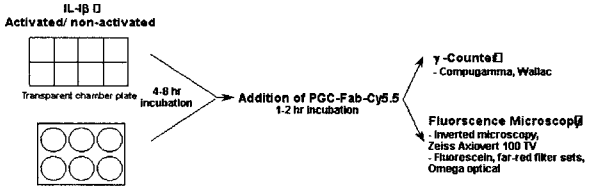
Size-exclusion HPLC

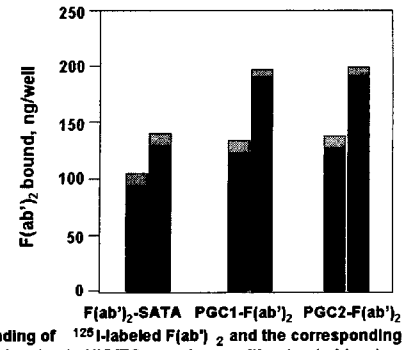


Electrophoresis

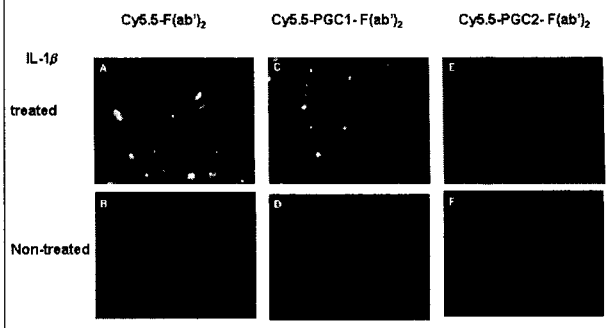


Sample Preparation





Binding of ^{125}I-labeled F(ab') $_2$ and the corresponding PGC based conjugates to HUVEC monolayers either treated (■, ■) or non-treated (■, ■) with 1L-1 β. Equivalent to 5 μg (■, ■) and 20 μg (■, ■) of F(ab')2 was added.



Conclusion I:

The binding of both conjugates allowed the delivery of near-infrared fluorophore molecules into the activated cells.

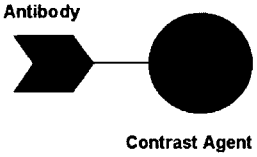
The intracellular NIR fluorescence was present only in endothelial cells only after the IL-1β stimulation demonstrating the feasibility of highly specific detection of cells expressing pro-inflammatory marker through endocytosis of the labeled co-polymer probe.

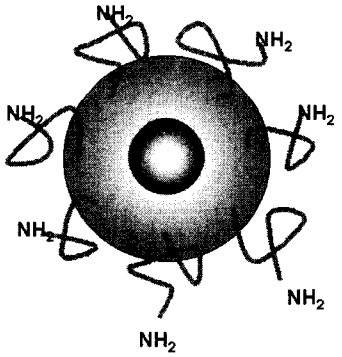
Conclusion II:

-These results suggest that targeting of optical imaging agents can be achieved in mixed cell culture populations (E-selectin-positive and negative) and that these studies can be potentially performed in vivo.

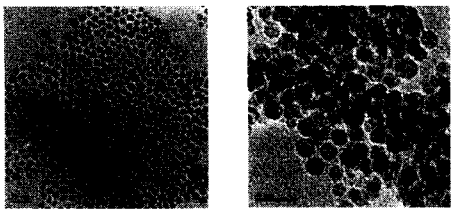
-Imaging of activated endothelium would be instrumental in detecting cells expressing pre-inflammatory phenotype linked to tumor angiogenesis and atherosclerosis.

Immuno-targeting MRI Probe

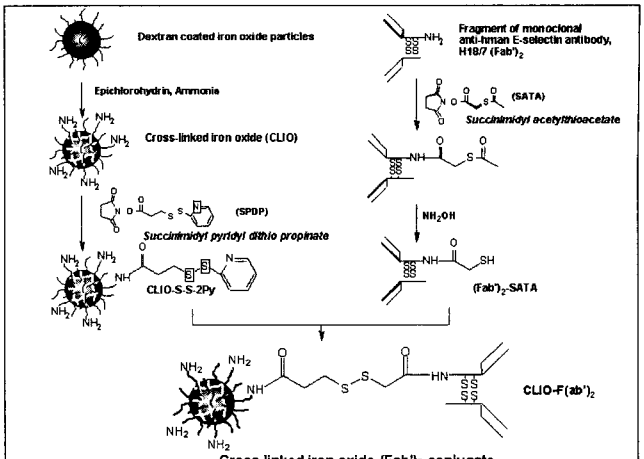




Cross-linked Iron Oxide Paramagnetic Nanoparticles

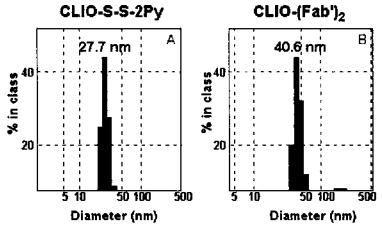


Iron Oxide Paramagnetic Nanoparticles



Cross-linked iron oxide-(Fab')$_2$ conjugate

Hydrodynamic Diameter of CLIO and CLIO Conjugates



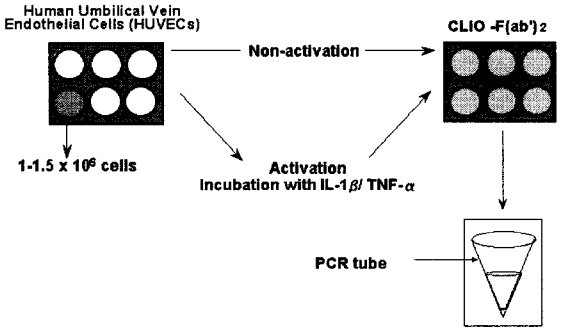
Laser Light Scattering (Zetasizer, Malvern Insts)

Properties of CLIO – F(ab')$_2$ Conjugates: Two Different Batches

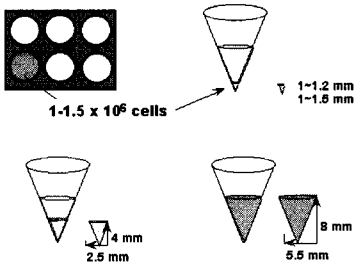
	CLIO-F(ab')$_2$ #1	CLIO-F(ab')$_2$ #2
Fe (μ g/mL)	600	310
F(ab')$_2$ (μ g/mL)	40.8	44.1
Fe/F(ab')$_2$ (molar ratio)	15	7

→ 1~2 molecules of F(ab')$_2$: 10 Fe nanoparticles

Sample Preparation for MRI



Sample Preparation for MRI



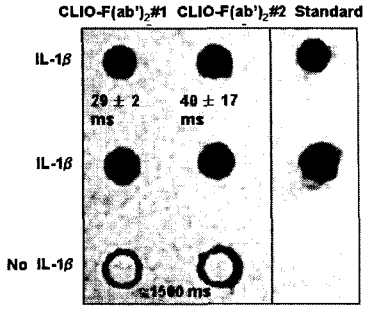
MRI Setup:

– T2 weighted spin echo sequences
TR 3000 ms, variable TE 16~100 ms

– Slice thickness 1 mm

– Relaxation Time (T2)
$SI = Ae^{(-TE/T2)} + B$

MR Imaging of HUVECs

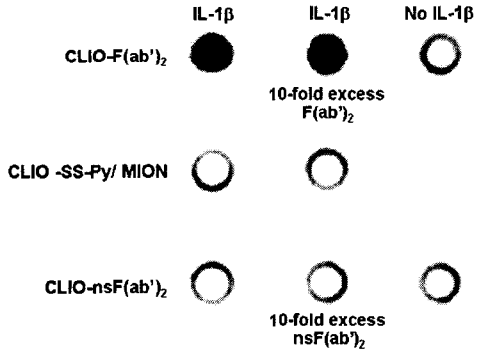


Is there additional cytokine medicated activation of adsorptive *endocytosis* or *pinocytosis* of conjugates by treatment of IL-1β to HUVEC cells?

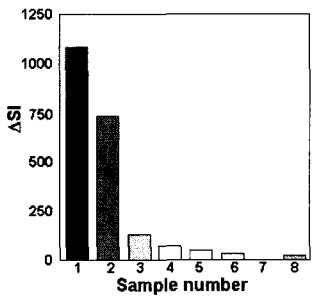
Experimental and Control HUVEC Samples for MR Imaging

	IL-1β	CLIO-F(ab')2	10-fold excess F(ab')2	CLIO -SS-Py	CLIO/ MION	CLIO -nsF(ab')2	10-fold excess nsF(ab')2
1	√	√					
2	√	√	√				
3		√					
4	√			√			
5	√				√		
6	√					√	
7	√					√	√
8						√	

MR Imaging of HUVECs



ROI T2 Weighted MR Signal Intensity Analysis



Conclusion III:

- H18/7 fragment of anti-human monoclonal antibody was covalently modified with SATA and attached to superparamagnetic nanoparticles using thiol-disulfide exchange reaction.

- Anti-E selectin nanoparticles were bound with high specificity to IL-1β stimulated HUVECs.

- Specific binding of CLIO-F(ab')$_2$ to activated HUVECs could be visualized using MR.