Antibody-coupling and targeting of microbubbles to activated platelets allows molecular imaging of thrombosis and monitoring of success or failure of thrombolysis using non-invasive ultrasound.

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No disclosure
Ultrasound Imaging of Thrombi

• Long sought-after (e.g. diagnosis of myocardial infarction)
  – Early and rapid diagnosis
    • Fast invasive or non-invasive therapy
  – Monitoring of success or failure of fibrinolytic therapy
    • Early decision towards an interventional approach

Overall, such a technical advance could substantially reduce morbidity and mortality of myocardial infarction
Targeted Ultrasound Microbubbles (MB)

- Ultrasound contrast = Echo enhancers
- Increase the backscatter by > 300 fold
- Gas-filled bubbles that are encapsulated by a biocompatible lipid or protein shell
- Targeting of bubbles towards clots

➢ Coating of MB with antibodies directed against clot epitopes
Activated GPIIb/IIIa: The Ideal Target for Molecular Imaging of Thrombi

- Platelets are the central component of clots
- Each platelet expresses $\approx 80,000$ GPIIb/IIIa
- Absolutely specific for platelets
- Change in confirmation when activated
- Recombinant antibodies for specific targeting of activated GPIIb/IIIa receptors only
Single-Chain Antibody (scFv)

- Small recombinant antibody fragment
- Non-immunogenic
- Easy to modify

- \( \text{scFv}_{\text{anti-LIBS}} \) (targets activated platelets)
  - Ligand Induced Binding Site on activated GPIIb/IIIa
- \( \text{scFv}_{\text{mut}} \) (non-targeting)
Aims

• Coupling of scFvs to microbubbles and targeting to activated platelets and thrombi
Generation of biotinylated scFvs

- Molecular Biology
  - Genetic fusion of the scFv with avidin
  - Production of biotin-labelled scFv in bacteria
  - Purification of biotinylated scFv
Functionality of scFv Constructs

- Successful biotinylation and purification of scFv

Western blot

- Specificity for activated platelets

Flow cytometry
Targeting of Microbubbles to Microthrombi

Microthrombi

Microbubbles

Non-conjugated MB

Conjugated with scFv

Conjugated with scFv

Conjugated with scFv

Conjugated with scFv

LIBS-MB with Platelet Staining

Fluorescence staining
Strong Adhesion on Microthrombi as well as on a Platelet Monolayer

- Platelet microthrombi
- Monolayer of activated platelets

![Graph showing adhesion of microthrombosis and platelet monolayer](image)

- MB only
- LIBS-MB
- Control-MB

** P < 0.01
*** P < 0.001
Aims

• Targeting microbubbles to activated platelets and thrombi by conjugation to scFv

• Identifying thrombi \textit{in vivo} via non-invasive ultrasound based molecular imaging using targeted microbubbles
High Resolution Ultrasound Imaging

- High frequency small animal ultrasound scanner
- 40 MHz transducer
- Carotid artery of a mouse
  - Small diameter of 300 to 400 µm
  - Ferric chloride injury
  - Platelet-rich but non-occlusive thrombus
Injection of Microbubbles in Carotid Artery
Minimal Attachment of MB

Ultrasound imaging: vaguely distinguishable thrombus
Post digital subtraction: only non-specific movement artifacts
Minimal Attachment of Control-MB

Ultrasound imaging: vaguely distinguishable thrombus
Post digital subtraction: only non-specific movement artifacts
Strong Attachment with LIBS-MB

Ultrasound imaging: bright thrombus area
Post digital subtraction: strong green thrombus area
Detection of Thrombi

MB

LIBS-MB

Control-MB

Grayscale Area (TU)

Decibel

MB (n = 8)  LIBS-MB (n = 10)  Control-MB (n = 8)

Before injection

20 minutes  *** p < 0.001 after injection
Aims

• Targeting microbubbles to activated platelets and thrombi by conjugation to scFv
• Identifying thrombi \textit{in vivo} via non-invasive ultrasound based molecular imaging using targeted microbubbles
• Monitoring success or failure of thrombolysis with targeted microbubbles
  – Treatment (UPA) or Control (Saline)
Monitoring of Thrombolysis

- **LIBS-MB**
- **No microbubbles**

<table>
<thead>
<tr>
<th>Time</th>
<th>UPA Thrombolysis</th>
<th>Saline Control</th>
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<td>0 min</td>
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Graph showing Thrombus (%) over time from injection.
Conclusions

• Targeted microbubbles bind specifically to activated platelets *in vitro* and *in vivo* thereby facilitating ultrasound molecular imaging of thrombi.

• Thrombus size and its reduction following pharmacological thrombolysis can be monitored in real-time, offering early diagnosis and monitoring of therapeutic thrombolysis.
Future directions

- Development into a promising diagnostic tool for clinical use for early detection of thrombi
  - Carotid artery (prevention of stroke)
  - Cardiac chambers (therapeutic implications)
  - Coronary artery (early and rapid diagnosis of MI)
Acknowledgements

• Xiaowei Wang, BS
• Ingo Ahrens, MD
• Christoph Hagemeyer, PhD
• Jia Fu, MD
• Ephraem Leitner, BS
• Jan David Hohmann, BS
• Paul Armstrong, PhD

• Funding
  – Baker IDI Heart and Diabetes Institute
  – Monash University
  – National Health & Medical Research Council of Australia
  – German Research Foundation
Biocompatible conjugation

- Sortase A + GGG-malamide + MB$_{SH}$

![Graph showing the number of adherent microbubbles at different shear rates.](image-url)

Ta et al., Circ Res. 2011
Timeline post MBs injection

![Graph showing Greyscale area (IU) difference from baseline over time from injection of microbubbles (minutes). The graph compares LIBS MB, Control MB, and MB groups.](image)
A range of single chain antibodies to target different markers during the development of an atherosclerotic plaque.
Platelet Aggregation

Jackson & Schoenwaelder, Nat Rev Drug Discov 2003