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Establishing stable antibody-magnetic nanoparticle conjugates: A methodological study

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Abstract

Antibody-conjugated magnetic nanoparticles offer considerable potential for morphological and functional assessment of vascular pathologies using Magnetic Resonance Imaging or Magnetic Particle Imaging. However, reliable antibody-nanoparticle conjugation remains technically challenging and strongly depends on the applied coupling chemistry. In this work, different maleimide-based conjugation strategies were evaluated to establish stable antibody attachment to amine-functionalized magnetic nanoparticles. This study highlights the critical impact of linker chemistry on antibody-nanoparticle conjugation and provides a methodological basis for further functional and imaging-based investigations with magnetic particle imaging.

I. Introduction

Systemic sclerosis, a prototypic systemic autoimmune disease, is characterized by a triad of vasculopathy, inflammation, and fibrosis and is frequently associated with microvascular and cerebrovascular alterations. Beyond classical organ manifestations, a substantial proportion of affected patients develop fatigue, post-exertional malaise, and cognitive impairment, suggesting involvement of the cerebral microvasculature. Increasing evidence implicates circulating autoantibodies, particularly those targeting G protein-coupled receptors such as angiotensin-II receptor type I (AT1R), in endothelial dysfunction and immune-mediated vascular injury [1].

To investigate autoantibody-driven vascular mechanisms in a controlled and human-relevant manner, experimental approaches are required that enable both functional in vitro studies and imaging-based readouts. Antibody-conjugated magnetic nanoparticles (AcMP) represent a promising platform to bridge clinical observations with mechanistic models, including microfluidic

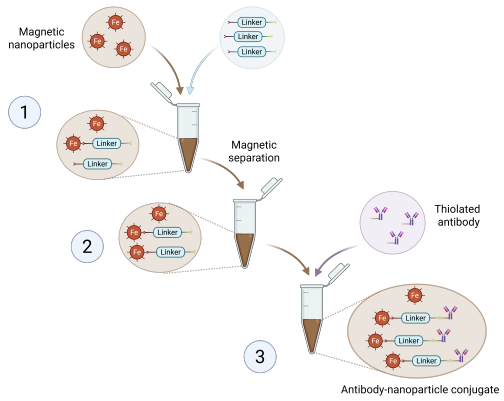

Figure 1: Antibody-nanoparticle coupling strategy. Magnetic nanoparticles are conjugated to linker (1), followed by magnetic separation to remove unbound linker (2). Subsequently, thiolated antibodies are added to obtain AcMP (3). Created in BioRender. Kerstein-Stähle, A. (2026) https://BioRender.com/8zwk98k

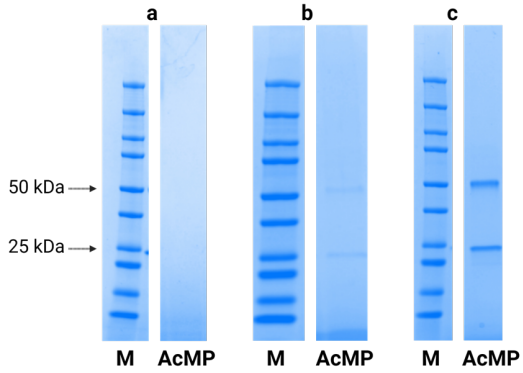

Figure 2: SDS–PAGE of AcMP after magnetic purification under reducing conditions showing antibody heavy (∼ 50 kDa) and light (∼ 25 kDa) chains. No bands were detected in the first coupling (a), weak bands in the second (b), and more pronounced bands after optimized coupling (c). M: molecular weight marker; AcMP: antibody-conjugated magnetic particles.

vessel-on-a-chip systems and magnetic particle imaging (MPI). However, the reliable covalent coupling of antibodies to nanoparticle surfaces remains challenging. Conjugation strategies must ensure chemical stability, compatibility with magnetic applications, and preservation of antibody integrity, making systematic optimization of coupling chemistries a critical prerequisite for vascular and imaging studies.

II. Methods and materials

Amine-functionalized superparamagnetic iron oxide nanoparticles (Synomag®-D NH_2, Micromod Partikeltechnologie GmbH) were used as carrier particles for antibody conjugation. Antibody-nanoparticle conjugation was performed using thiol-reactive chemistries (Figure 1). We tested conventional maleimide-based coupling approaches as well as the implementation of a self-hydrolyzing maleimide linker to improve chemical stability of conjugates. Monoclonal antibodies directed against the AT1R were thiolated to enable site-compatible coupling, followed by incubation with functionalized nanoparticles. Unreacted binding sites were chemically quenched, and conjugates were purified by magnetic separation followed by IgG affinity chromatography.

Coupling efficiency was assessed indirectly by quantifying unbound antibody fractions using Bradford protein quantification assay and SDS polyacrylamide gel electrophoresis (SDS-PAGE) to separate denatured proteins by molecular weight and to allow detection of antibody heavy and light chains. Magnetic properties, suitability for imaging-based applications and first investigations on potential multi-contrast applications were examined using magnetic particle spectroscopy (MPS) [2].

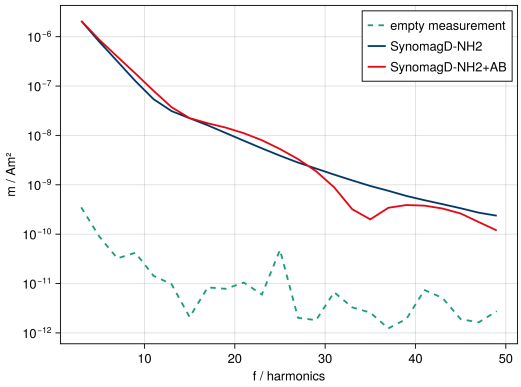

Figure 3: MPS spectrum of SynomagD-NH2 nanoparticles with antibodies (red) and without (blue) including background measurement (green). Sample volume: 100 µL; nanoparticle suspension diluted 1:5 with deionized water. AB: antibodies

III. Results and discussion

SDS-PAGE indicated that the initial antibody-nanoparticle coupling did not result in detectable antibody association with the particles, as no heavy or light chain bands were observed in the particle-conjugated fraction (Figure 2a).

Following optimization of the conjugation conditions, antibody-derived bands became detectable in the particle-associated fraction (Figure 2b), indicating particle attachment at low apparent levels. Further optimization led to more pronounced heavy and light chain bands in the particle fraction (Figure 2c), consistent with improved conjugate formation. These findings were supported by Bradford analysis of particles and flow-through fractions. MPS measurements (Figure 3) indicate that

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bound nanoparticles are suitable for imaging with MPI and change the MPI signal properties. Further investigations are necessary to evaluate to what extent these changes can be used in terms of multi-contrast MPI.

Importantly, thermal unfolding analysis of the conjugated samples revealed defined unfolding transitions, demonstrating the presence of higher-order protein structure after conjugation. Together, these results indicate that linker chemistry critically determines conjugation outcome. Furthermore, the use of a self-hydrolyzing maleimide linker could provide a suitable methodological basis for obtaining stable conjugates and for subsequent functional and imaging-based analyses.

IV. Conclusions

The presented work reflects an early stage of method development aimed at establishing reliable antibody-nanoparticle conjugation. Initial experiments highlight critical parameters influencing reproducibility and structural integrity of the conjugates, providing a foundation for further optimization. The approach is intended to support subsequent functional and imaging-based studies while enabling translation toward clinical applications, requiring that key parameters such as conjugate stability, reproducibility, biocompatibility, and batch-to-batch consistency meet defined regulatory and performance standards.

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Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: N/A Ethical approval: N/A

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