A comprehensive investigation of copper binding properties of metformin using on-disc magnetic microbead agglomeration with real-time analysis

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A comprehensive investigation of copper binding properties of metformin using on-disc magnetic microbead agglomeration with real-time analysis

Authors & affiliations:

Rokon Uddin, Xueling Quan, Marco Donolato, Robert Burger and Anja Boisen
Department of Micro-and Nanotechnology, Technical University of Denmark
rokud@nanotech.dtu.dk

Introduction: Metformin is a widely used type-2 diabetes drug. Its copper-binding properties are known to be crucial but still not fully understood. We present a comprehensive investigation of its interaction with L-cysteine-Cu complex using a magnetic microbead (MB)-based assay on a microfluidic disc. The assay scheme is similar to the one presented in our previous study, where optomagnetic readout and magnetic nanobeads were used [1]. In this study, we have significantly simplified the detection by using an optical scanning method [2] and micrometer-sized beads. Additionally, we can measure the effect of *100-fold lower concentration of metformin* than in our previous study. Our results clearly illustrate the strong metformin-Cu interaction and provide the opportunity of real-time analysis.

Methods:

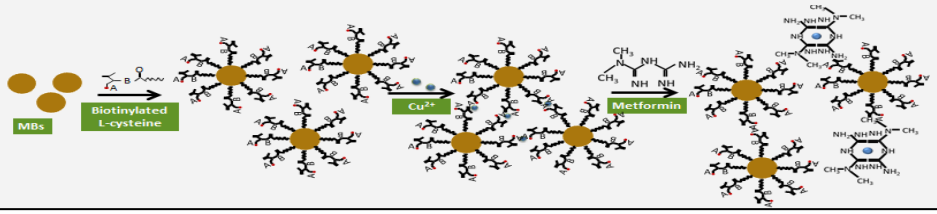

Fig1: Streptavidin-coated MBs are functionalized with biotinylated L-cysteine. Addition of Cu^{2+} molecules causes the formation of multiple L-cysteine-Cu complexes resulting in the formation of MB clusters. Addition of metformin breaks the L-cysteine-Cu complex causing the breakage of the clusters followed by formation of several metformin-Cu complexes. [1]

The assay platform is a microfluidic disc made of Poly(methylmetacrylate) (PMMA) bonded together using pressure-sensitive adhesive (Fig.2a). For studying L-cysteine-Cu interaction, Cu solution and 1µm streptavidin-coated MBs (1mg/ml) functionalized with biotinylated L-cysteine (volume ratio 1:11) were incubated for 10 minutes with gentle shaking and then loaded into the disc. For studying interaction between metformin and L-cysteine-Cu complex, metformin was added to a separately pre-incubated L-cysteine-Cu (volume ratio 2:1) solution, further incubated for 5 minutes and loaded into the disc. Finally, all samples were incubated on-disc under permanent magnetic field with continuous shaking for 10 minutes before scanning by oCelloScope (Fig.2b).

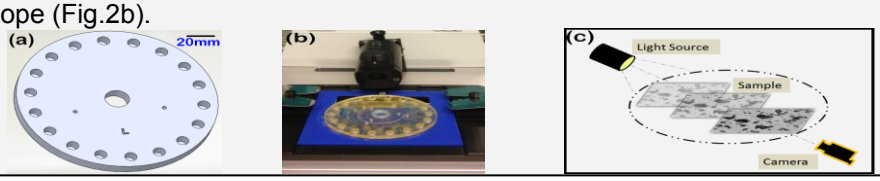

Fig2: (a) Schematics of microfluidic disc with 18 pools. The disc format has been used for its rotational advantages during magnetic incubation and easy paralysation. (b) Optical scanning setup (oCelloScope, Philips Biocell) (c) Functional unit of oCelloScope [2]. It uses an optical sectioning principal by tilting the focal plane few degrees for recording series of images along the scan direction and forms an image stack resulting in capturing and characterizing all the objects of interest along all axes.

Results: Scanning results of four different samples are illustrated in Fig.3.

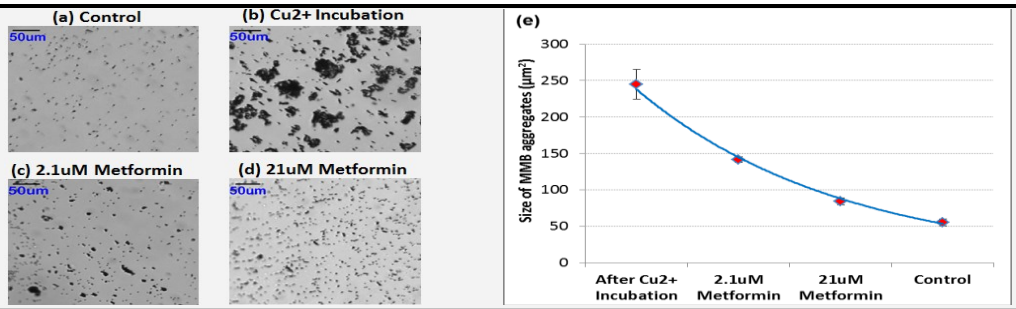

Fig3: (a) Magnetic beads functionalized with biotinylated L-cysteine used as the control sample (b) L-cysteine functionalized MBs incubated in Cu solution followed by 10min magnetic incubation. MB clusters form because of the formation of L-cysteine-Cu complex. (c) & (d) After the addition of 2.1μM and 21μM metformin into two separate mixtures of Cu and L-cysteine functionalized MB followed by magnetic incubation, the MB-clusters are broken in respective order for the breakage of L-cysteine-Cu complex and formation of metformin-Cu complex. (e) Measured MB cluster size vs amount of metformin in the sample: we conclude that samples with 21μM and 2.1 μM metformin have 57.9% and 34.6% reduced mean cluster size respectively than the sample with only Cu. Scale bar: 50μm.

We performed real-time analysis of cluster formation as illustrated in Fig4.

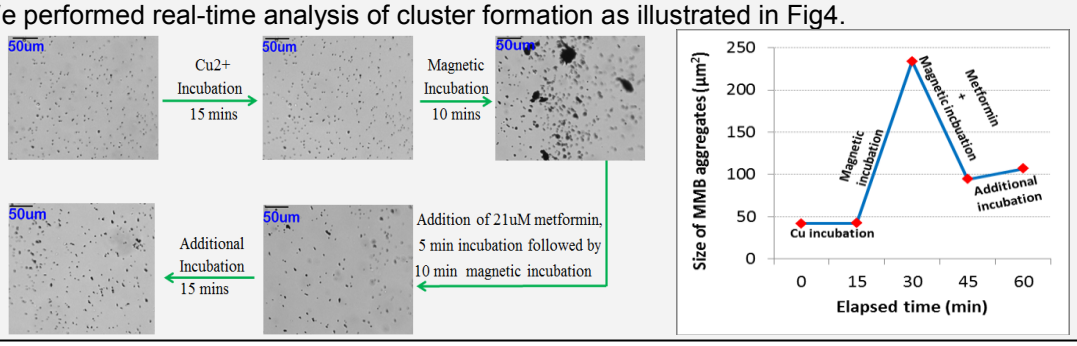

Fig4: L-cysteine coupled MBs were incubated in Cu^{2+} solution for 15 minutes, which resulted in an insignificant visible change in size of particles. Magnetic incubation for 10 mins resulted in the formation of large clusters (mean area: 233.68μm^2) which shows the significance of an optimized magnetic incubation. Next, 5 mins incubation with metformin followed by 10 min magnetic incubation showed MB clusters of about 94.24μm^2 mean area i.e. addition of metformin caused 40% averaged reduction of the L-cysteine-Cu-MB cluster size. Additional incubation of 15 mins was performed showing no significant changes.

Discussion: The presented results show that metformin, even at low concentration, is able to break the L-cysteine-Cu complex, which can affect mitochondrial function [3]. The investigation provides in-depth insight on the metformin-Cu interactions by quantifying the size of the clusters formed by molecular reaction. Thus, the novel detection strategy and kinetics analysis could be used for studying metal-binding properties of metformin-analogues.

References:

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3. L Logie et al., 'Cellular responses to the metal binding properties of metformin', Diabetes, 2012, 61, 1423–1433.