A QCM-D Assessment of Binding Properties of a Collagen Mimetic Peptide for Use in Ocular Indications

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PURPOSE

Collagen is one of the main proteins of the extracellular matrix throughout the body. In the eye, its intact structure is fundamental for homeostatic maintenance of both neural and non-neural tissues[1]. Collagen mimetic peptides (CMPs) bind and, in so doing, repair collagen cleaved by metalloproteinases (MMPs)[2]. We have shown that CMPs protect ARPE-19 cells damaged by MMPs *in vitro* and neurons subjected to ocular stress using *in vivo* models of glaucoma, optic nerve injury, and dry eye[3,4,5,6]. Here, our purpose was to evaluate changes over small time scales in thickness, mass, and viscosity of collagen-I during adsorption, cleavage by MMP-1, and treatment with a type I CMP using the Quartz m Crystal Microbalance with Dissipation Monitoring. (QCM-D) platform. This platform measures real-time changes in frequency and dissipation of the quartz sensor related to the deposited mass and associated viscoelastic properties[6].

METHODS

A QSense Analyzer equipped with standard flow modules was used in this study (Biolin Scientific, Gothenburg, Sweden). Collagen-I (cat #5007, Advanced BIOMATRIX, Carlsbard, CA) was adsorbed onto gold-coated sensors (cat #QSX301, Biolin Scientific). Intact collagen was cleaved by MMP-1 (Enzo Life Sciences, Inc., Farmingdale, NY, USA) in DIW, and the interaction between CMP (cis-Flp-Hyp-Gly)$_7$, Bachem, AG (Torrance, CA, USA) and cleaved collagen-I was tested with CMP in DIW. The temperature was maintained at 37°C. CMP binding to either intact collagen-I or bare gold-coated sensors was used as the negative control. Changes in frequency and dissipation were recorded using QSoft401 software (Biolin Scientific), and the Kelvin-Voigt viscoelastic model was used to analyze changes in thickness, mass, and viscosity of collagen-I using the QSense data analysis software (Biolin Scientific).

RESULTS

1. Collagen-I adsorption monitored using QCM-D onto gold-coated sensor

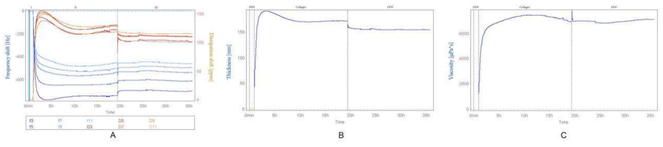

Figure 1. A. Collagen-I adsorption monitored using QCM-D onto gold-coated sensor. The evolution of the frequency and dissipation versus time profiles at the 3rd, 5th, 7th, 9th, and 11th overtones are shown. The different stages of the flow procedure are as follows: (i) initial DIW baseline, (ii) collagen solution flow at 10ug/ml, (iii) DIW wash to remove unbound collagen to a stable baseline. **B.** Collagen-I adsorbed on gold coated sensor leads to a positive change in thickness. The evolution of the changes in thickness with time is shown. **C.** Collagen-I adsorbed on the gold-coated sensor leads to a positive change in viscosity. The evolution of the changes in viscosity with time is shown. The Kelvin-Voigt viscoelastic model was used to analyze changes in the thickness and viscosity of human collagen-I using QSense data analysis software.

2. Collagen-I cleaved by MMP-1 monitored using QCM-D onto gold-coated sensor

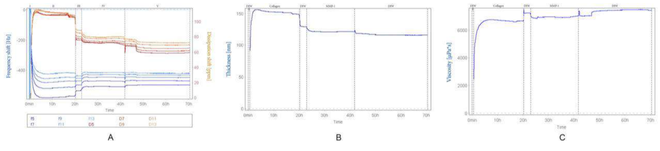

Fig. 2. A. Collagen-I cleavage by MMP-1 was monitored using QCM-D on a gold-coated sensor. The evolution of the frequency and dissipation versus time profiles at the 5th, 7th, 9th, 11th, and 13th overtones are shown. Different stages of the flow procedure are indicated as follows: (i) initial DIW baseline, (ii) collagen solution flow (10ug/ml) (iii) DIW wash to remove unbound collagen to a stable baseline,(iv) MMP-1 solution in DIW (10ug/ml) to cleave collagen-I, (v) final DIW wash to remove unbound MMP-1 to a stable baseline. **B.** MMP-1 cleavage of collagen-I effect on collagen thickness. The enzymatic activity of MMP-1 leads to a reduction in thickness. The evolution of changes in thickness versus time are shown. **C.** MMP-1 cleavage of collagen-I affects collagen viscosity. The enzymatic activity of MMP-1 leads to a decrease in the viscosity of broken collagen compared with the previous DIW stable line period. The evolution of changes in viscosity versus time is shown.

3. CMP binding to cleaved collagen-I monitored using QCM-D onto gold-coated sensor

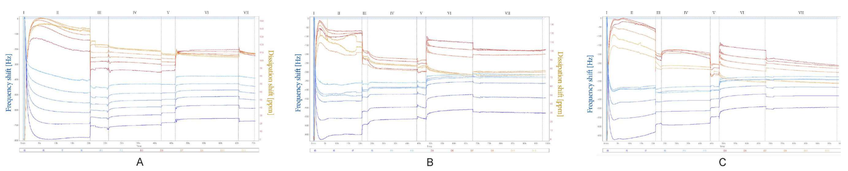

Fig. 3. A-C. Binding of CMP to cleaved collagen-I was monitored using QCM-Dated sensor. The evolution of the frequency and dissipation versus time profiles at the 3rd, 5th, 7th, 9th, 11th, and 13th overtones are shown. Different stages of the flow procedure are indicated as follows: (i) initial DIW baseline, (ii) collagen-I solution flow (10ug/ml) (iii) DIW wash to remove unbound collagen to a stable baseline,(iv) MMP-1 (10ug/ml) solution flow in DIW to cleave collagen, (v) DIW wash to remove unbound MMP-1 to a stable baseline. (v) CMP solution flow (1mg/ml) in DIW, (vii) final DIW wash to a stable baseline. Three plots are shown for the three different experiments.

3. CMP binding to cleaved collagen-I monitored using QCM-D onto gold-coated sensor

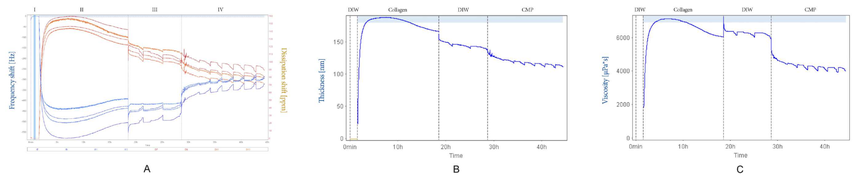

Fig. 5 A. CMP showed no binding to intact human collagen-I, as monitored using QCM-D, onto a gold-coated sensor. The evolution of the frequency and dissipation versus time profiles at the 7th, 9th, 11th, and 13th overtones are shown. Different stages of the flow procedure are indicated as follows: (i) initial DIW baseline, (ii) collagen-I solution flow (10ug/ml), (iii) DIW wash to remove unbound collagen-I to a stable baseline, (iv) CMP solution flow (1mg/ml) in DIW. **B**. Addition of CMP to intact collagen-I leads to a decrease in thickness. The evolution of changes in thickness versus time are shown. **C.** The addition of CMP to intact collagen-I leads to a decrease in viscosity. The evolution of changes in viscosity versus time is shown. .

4. Analysis of changes in thickness, mass and viscosity of collagen-I, cleaved collagen and repaired collagen by CMP

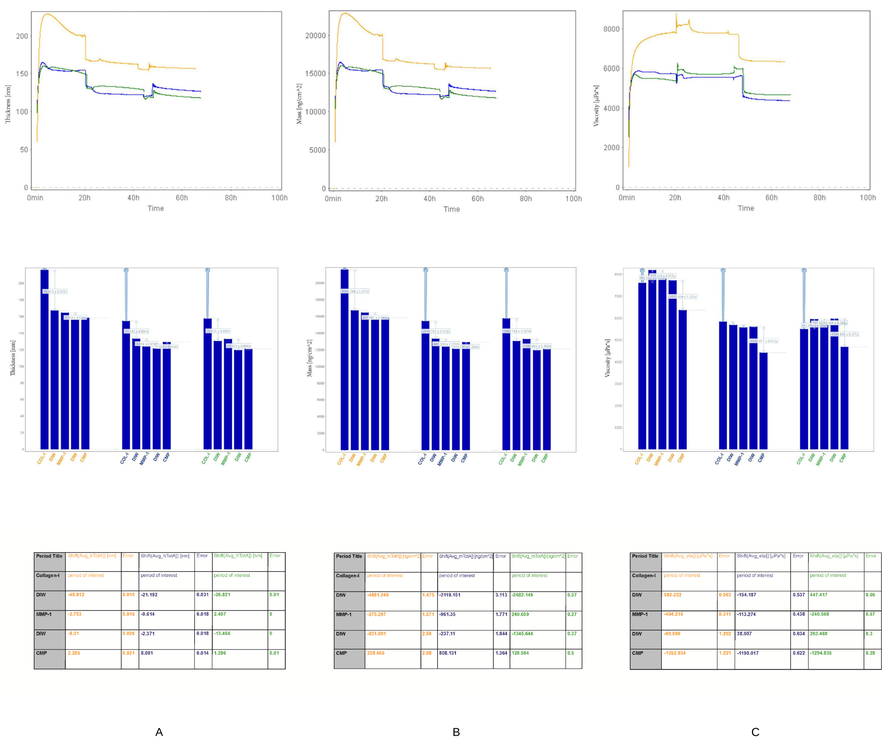

Fig. 4 A-C. Qualitative and quantitative analyses of changes in thickness, mass, and viscosity of intact, cleaved, and repaired collagen-I in three different experiments. **Upper panel.** The evolution of the changes in thickness, mass, and viscosity versus time is shown. Repairing broken collagen-I by CMP induces a slight increase in thickness and mass and a decrease in viscosity compared to the previous DIW stable line period. **Middle panel.** Quantitative analysis of changes in the thickness, mass, and viscosity of collagen-I at different periods**. Lower panel.** Tables showing the average shift values between the selected periods. The Kelvin-Voigt viscoelastic model was used to analyze changes in thickness, mass, and viscosity of cleaved collagen I repaired by CMP using the QSense data analysis software.

3. CMP binding to cleaved collagen-I monitored using QCM-D onto gold-coated sensor

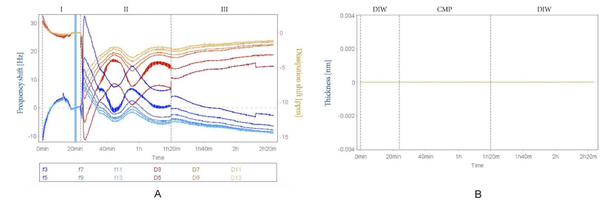

Fig. 6. A. CMP shows no binding to the bare gold-coated sensor monitored using QCM-D onto a gold-coated sensor. The evolution of frequency and dissipation versus time profiles at the 3rd, 5th, 7th, 9th, 11th, 13th overtones are shown. Different stages of the flow procedure are indicated as follows: (i) initial DIW baseline, (ii) CMP solution flow (1mg/ml) in DIW, (iii) final DIW wash to a stable baseline. **B.** The addition of CMP to the bare gold-coated sensor does not lead to an increase in thickness. The evolution of changes in thickness versus time is shown.

CONCLUSIONS

- Our results using QCM-D strongly suggest that CMP specifically binds to cleaved collagen-I and restore its native structure.
- Our findings provide a starting point for assessing the distinct affinities of different CMPs for cleaved collagens and their various effects on morphological, biochemical and structural changes of the ECM.
- The present data provide further support that CMPs represent novel collagen-targeting approaches for diseases that challenge the integrity of collagen in the extracellular matrix.

References

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Acknowledgments

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Commercial Relationships Disclosure

Stefania Marsili: Commercial Relationship(s);Code N (No Commercial Relationship)
Robert O Baratta: Commercial Relationship(s);Code E (Employment):Stuart Therapeutics Inc.
Brian J Del Buono: Commercial Relationship(s);Code E (Employment):Stuart Therapeutics Inc.
Eric Schlumpf: Commercial Relationship(s);Code E (Employment):Stuart Therapeutics Inc.
David J Calkins: Commercial Relationship(s);Code C (Consultant/Contractor):Stuart Therapeutics Inc.