STUDY OF A G-QUADRUPLEX APTAMER INHIBITING MMP-9 ON HUMAN KERATINOCYTES AND ON A 3D RECONSTRUCTED HUMAN SKIN MODEL

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Introduction

MMP-9 or 92 kDa gelatinase/type IV collagenase is a zinc-dependent endopeptidase and a major mediator of pericellular proteolysis. MMP-9 is secreted in a zymogenic form (pro-MMP) that is activated in the extracellular space[1]. This activation, mediated by plasminogen and MMPs, is an important mechanism of regulation of gelatinase activity in skin. MMP-9 promotes degradation of extracellular matrix (ECM) components in the epidermis, the dermis, the basement membrane, and cell surface-associated proteins. Type I, IV and V collagens, laminins and elastin are ECM elements degraded by MMP-9 which leads to strong damage visible on skin such as wrinkles. For protecting skin and preventing it from aging, MMP inhibitors are key compounds. However, natural, safe, specific and effective MMP9 inhibitors were not yet available for topical cosmetic use.

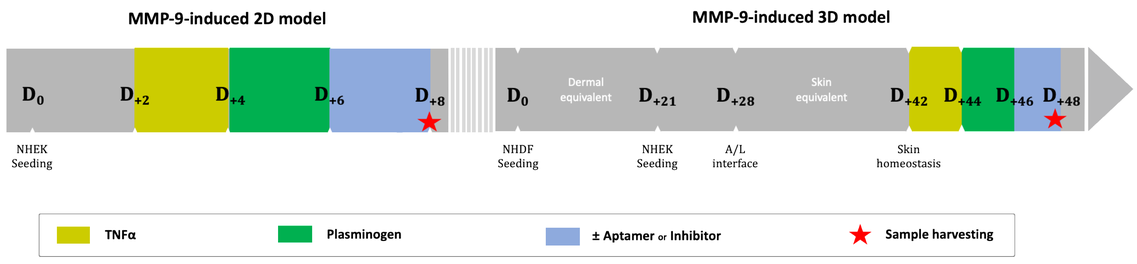
To overcome this limitation, we developed DNA aptamers targeting the MMP-9 catalytic site using SELEX (Systemic Evolution of Ligands by Exponential Enrichment) procedure[2]. This approach led to the selection of sequences exhibiting G-quadruplex structures able to interact tightly and specifically with MMP-9 catalytic site and to inhibit the enzymatic activity[3]. To further investigate the activities of one of these aptamers in a complex system, we tested it on human keratinocyte cultures and then on 3D reconstructed skin models that were both induced to produce the active form of MMP-9.

Optimal conditions for induction of MMP-9 expression and activation by TNF alpha and plasminogen were determined and then, inhibitory activity of MMP-9 aptamer was studied on keratinocytes and on a 3D reconstructed skin model.

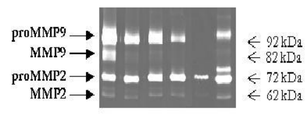
Histological observations and analysis were performed on reconstructed skin for studying the impacts of MMP-9 activity and its inhibition, on the epidermis and dermis and to visualize and quantify the effects on ECM elements.

Materials & Methods

To examine the activity of our aptamer on MMP-9 activity in cellular setting, we first developed human 2D and 3D skin cell culture models that were induced to produce active MMP-9. Based on these MMP-9-activated cellular models, we then tested the activity of our G-quadruplex aptamer candidate developed by SELEX approach at different concentrations on MMP-9.

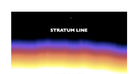


Detection of MMP9 protease activity by fluorescent Zymography



Reconstructed tissue exploration by histological and morphometry analysis

Epidermal thickness



Euclidean distance map

ECM density

Image deconvoluton and quantification

Results & Discussion

1. Modulation of MMP-9 activity by the aptamer candidate on MMP-9-activated NHEK model

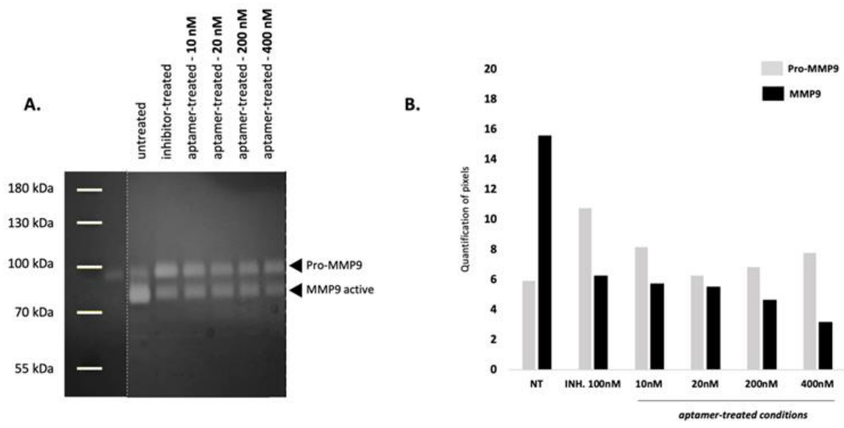
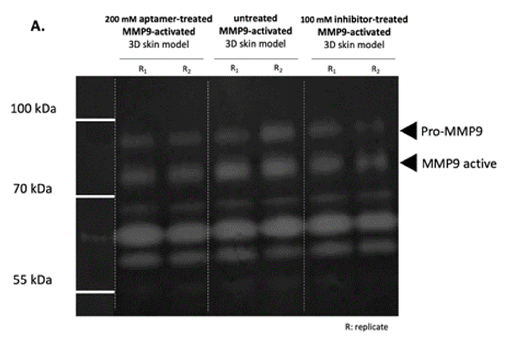


Fig.1: Zymographic analysis of gelatinase secretion (pro-MMP9 and active MMP9) after stimulation of NHEK by TNF-alpha followed by an activation with plasminogen at 30 microg/ml and treatment with aptamer candidate at different concentrations.
A. 5 microg of protein of each sample obtained from supernatants of NHEK-conditioned media were subjected to gelatin zymography. Controls: untreated and inhibitor-treated conditions.
B. Semi-quantification of gelatinase secretion by image processing and analysis.

Incubation of MMP9-activated NHEK with the aptamer led to a significant decrease of the activity of MMP-9 active form compared with the untreated control (Fig. 1A). Analysis showed a clear dose effect of the aptamer modulation. Treatment with the lowest concentration (10 nM) of aptamer showed an inhibition activity equivalent to the one observed with the MMP9-inhibitor at 100 nM.

2. Modulation of MMP-9 activity by the aptamer candidate on MMP-9-activated 3D reconstructed skin model



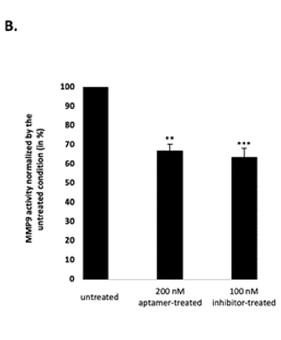


Fig.2: Zymographic analysis of gelatinase secretion (pro-MMP9 and active MMP9) after treatment of the MMP-9-activated 3D reconstructed skin model by aptamer at 200 nM or MMP-9 inhibitor at 100 nM.
A. 5 microg of protein of each sample obtained from undernatants of 3D skin model-conditioned media were subjected to gelatin zymography. Controls: untreated and inhibitor-treated conditions.
B. Semi-quantification of gelatinase secretion by image processing and analysis.

MMP-9-activated 3D skin model treated with the aptamer showed a significant inhibition of the activity of the active form of MMP-9, compared with the untreated control. MMP-9-activated 3D skin model subjected to the MMP-9 inhibitor I of reference showed also a significant 34% inhibition by the aptamer similarly to the chemical inhibitor.

3. Impact of the modulation of MMP-9 activity by the aptamer candidate on the epidermal thickness and ECM density

Skin model subjected to the aptamer at 200 nM exhibited a thicker, more cohesive and better organized epidermal compartment compared with the untreated control. The dermis seemed denser and richer in fibers compared with the untreated condition. Same effects were observed in the chemical inhibitor-treated condition. Image quantification revealed a significant increase of the epidermal thickness up to 29% and 40% in the inhibitor- and aptamer-treated conditions, respectively (Fig. 3B). At the dermal level, we observed a significant increase of the fibrillar collagen content up to 53% and 27% in the inhibitor- and aptamer-treated conditions, respectively (Fig. 3C).

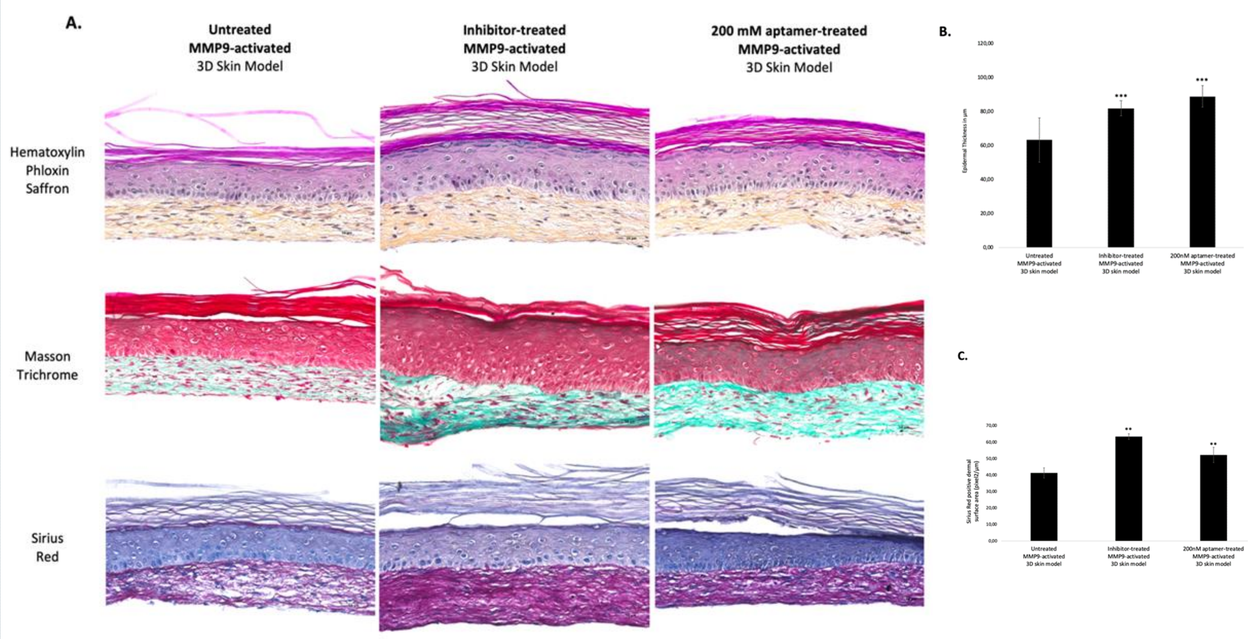


Fig.3: Histological and morphological impact of the MMP-9 inhibition by aptamer on MMP-9-activated 3D reconstructed model. **A**. Histological staining of 3D reconstructed skin stained with Hematoxylin-Phloxine-Saffron, Masson Trichrome and Sirius Red. Normal, untreated reconstructed skin control versus MMP9-activated reconstructed skin, treated with aptamer (200 nM) or MMP-9-inhibitor of reference (100 nM). Scale bar: 50µm. **B/C**. Quantification of epidermal thickness (in µm) and fibrillar collagen positive area by image analysis (pixel²/µm), respectively.

SELEX procedure has been followed to develop DNA aptamers targeting the MMP-9 catalytic site for inhibition of the enzyme activity. Selected sequences formed G-quadruplex structures and the inhibitory activity of one of these sequences has been studied in this work. On keratinocytes, we showed that our aptamer at 10 nM reduced MMP-9 activity with the same efficacy that the chemical commercial compound used as positive control at 100 nM. On a 3D reconstructed skin model, we have clearly demonstrated the negative impact exerted by MMP-9 activity on epidermis, dermis, and ECM. The MMP-9 chemical inhibitor I (100nM) or our aptamer (200nM) were able to protect epidermis, dermis and ECM from alterations caused by MMP-9 activity.

Conclusions

The field of application for aptamer technology is very wide and several examples as various as for catalytic activities or for recognition of small compounds or for macromolecular entities have been reported.
Our preliminary results are very promising and open a new way for modulating efficiently MMP-9 activity and potentially others MMPs in human skin very specifically and without side effects. The aptamer used in this study was a pure DNA sequence without any base modifications to optimize its biological activity. This innovative approach makes this aptamer a real mimic of a natural compound.
More investigations are currently on going to further explore aptamer activity on our unique MMP-9-activated 3D model and to refine the optimal aptamer dosage. Recently, Zhao N. et al., reported the use of a bifunctional aptamer-fibrinogen macromer for VEGF delivery and skin wound healing[4] and Lenn J.D. et al., demonstrated that aptamers can be delivered through intact human skin[5] making aptamers new candidates for skin care regeneration and rejuvenation.

Aknowledgments & References

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