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# *In vitro* biological assay design for the inhibition of the matrix metalloproteinase, Collagenase type-1, using ethylenediaminetetraacetic acid, a metal ion chelator.

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**Coronavirus disease-2019 (COVID-19) has been a pandemic in recent years. The viral infection, mainly targeting the lungs, triggers inflammation in most cases leading to alveolar damage. The alveolar epithelial cells are especially prone to the viral infection-based inflammation related damage. Replenishment of these cells within a short period of time is very important for patients to recover which otherwise results in pulmonary disorders. Matrix metalloproteinases (MMPs) play a critical role in extracellular matrix remodeling during cellular regeneration. In this study, we used Collagenase type-1 enzyme which is also an MMP as a model enzyme to establish an enzyme inhibition assay that can be used to evaluate our newly designed MMP modulators. As a positive control, we used EDTA in this assay and evaluated the activity of Collagenase type-1 MMP in the presence and absence of EDTA using chicken liver tissue pieces. Our results suggest that EDTA indeed inhibits the enzyme activity of Collagenase type-1 MMP by chelating the metal ions in the buffer. Further the inhibitors will be evaluated in future using the EDTA as a positive control.**

**Keywords:** Metalloproteinase, Collagenase type-1, EDTA, biological assay, enzyme inhibitors.

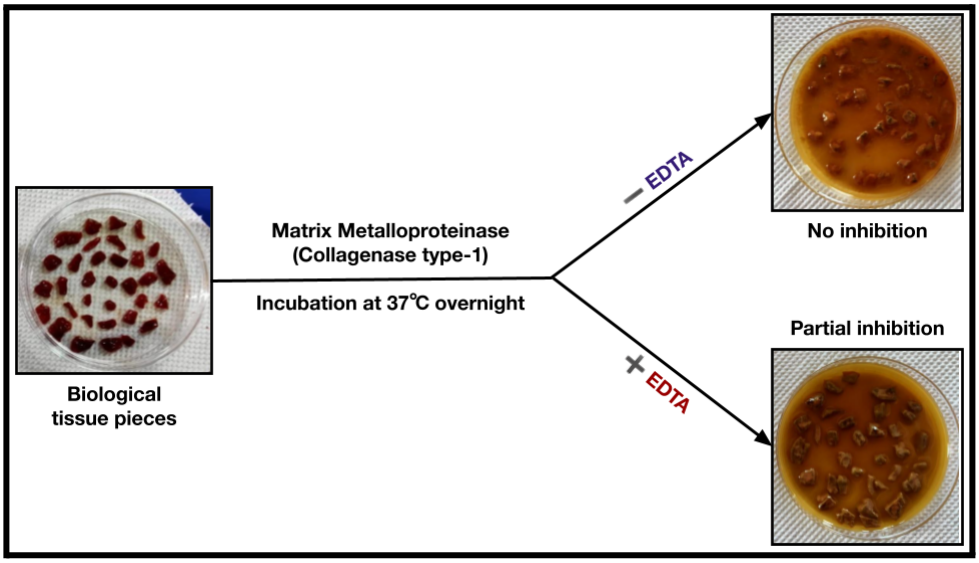


**Figure 1.** Overview of *in vitro* biological assay for Collagenase type-1, in the presence and absence of inhibitor, EDTA.

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Coronavirus disease-2019 (COVID-19) [1-9] causes severe damage to the alveolar epithelial cells in the lungs [10]. Infected patients, in order to recover faster, must not only be able to clear the viral load but also regenerate and replenish the damaged alveolar epithelium. However, during cellular regeneration replenishing the damaged tissue the remodeling of the extra -cellular matrix (ECM) is critical. In this context the matrix metalloproteinases (MMPs) are crucial in remodeling the extracellular matrix [11]. Recently we designed a novel strategy taking advantage of the MMPs and their tissue inhibitors of metalloproteinases (TIMPs) [12]. The equilibrium between MMPs and TIMPs is very important for healthy tissue remodeling which would otherwise result in scarring, fibrosis, cirrhosis, etc. [13-17].

There are several types of MMPs in humans that are secreted into the ECM. Collagenases (MMP-1, MMP-8, MMP-13, etc.), Gelatinases (gelatinase-A/MMP-2 and gelatinase-B/MMP -9) enzymes, Stromelysins (stromelysin-1/ MMP-3 and stromelysin-2/ MMP-10), Matrilysins (matrilysin-1/MMP-7 and matrilysin -2/MMP-26), Membrane type MMPs (MT-MMPs: MMP-14, MMP-15, MMP-16, MMP-24, MMP-17 and MMP-25), Other MMPs ( MMP12, MMP19, MMP20, MMP22, MMP23, and MMP28). Within the MT-MMPs, some of them also have glycoprotein anchors to the membranes.

In this study, we focused on designing an *in vitro* biological assay to evaluate the MMP-1, Collagenase type-1 using chicken liver tissue pieces by using EDTA as a control. We hypothesized that MMP function is highly dependent on the metal ion binding which will be directly affected in the presence of EDTA. We performed the Collagenase type-1 assay in the presence and absence of EDTA to observe any difference in the activity of the MMP. If a difference is observed in this assay then, it can be fine tuned further to evaluate the potency of our MMP/TIMP modulators in future.

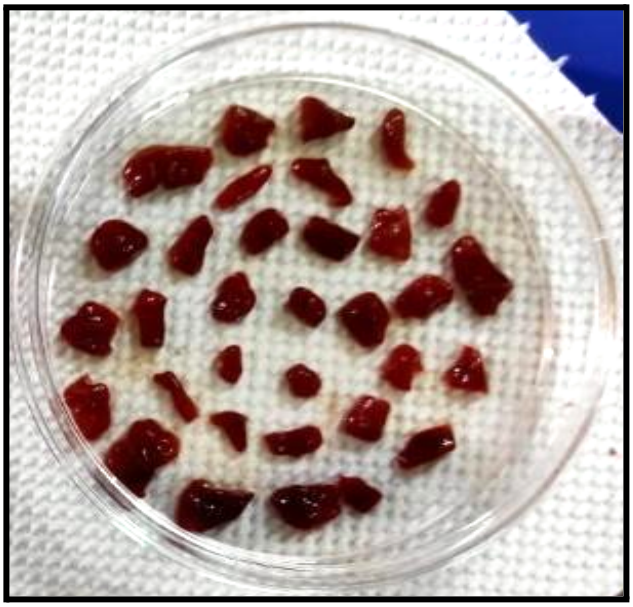

**Figure 2.** Sliced chicken liver pieces taken in a petri plate.

## Materials & Methods:

*NCBI search:* An extensive search was performed using NCBI to identify different transcript variants of MMP-1. We identified two transcript variants of the human MMP-1 with the NCBI accession numbers: NM_002421.4 and NM_001145938.2 which were further used for any sequence information. The nucleotide sequences were further aligned to evaluate the presence of any significant differences between the two variants.

*In vitro biological assay:* Chicken liver pieces were used as tissue source to evaluate the enzyme activity in the presence and absence of EDTA. Two petri plates were sterilized with 70% ethanol and were labeled as plate 1 and plate 2. The chicken liver was sliced into small pieces and were taken equally into both the petri plates. To the petri plate 1, 8ml of 1x TAE buffer and 2ml of collagenase type-1 enzyme were added. To the petri plate 2, 8ml of 1x TAE buffer, 2ml of collagenase type-1 enzyme and 1ml of 0.5 M EDTA were added. Both the plates were put in the incubator at $37^0$C overnight. After overnight incubation both the petri plates were observed for any changes.

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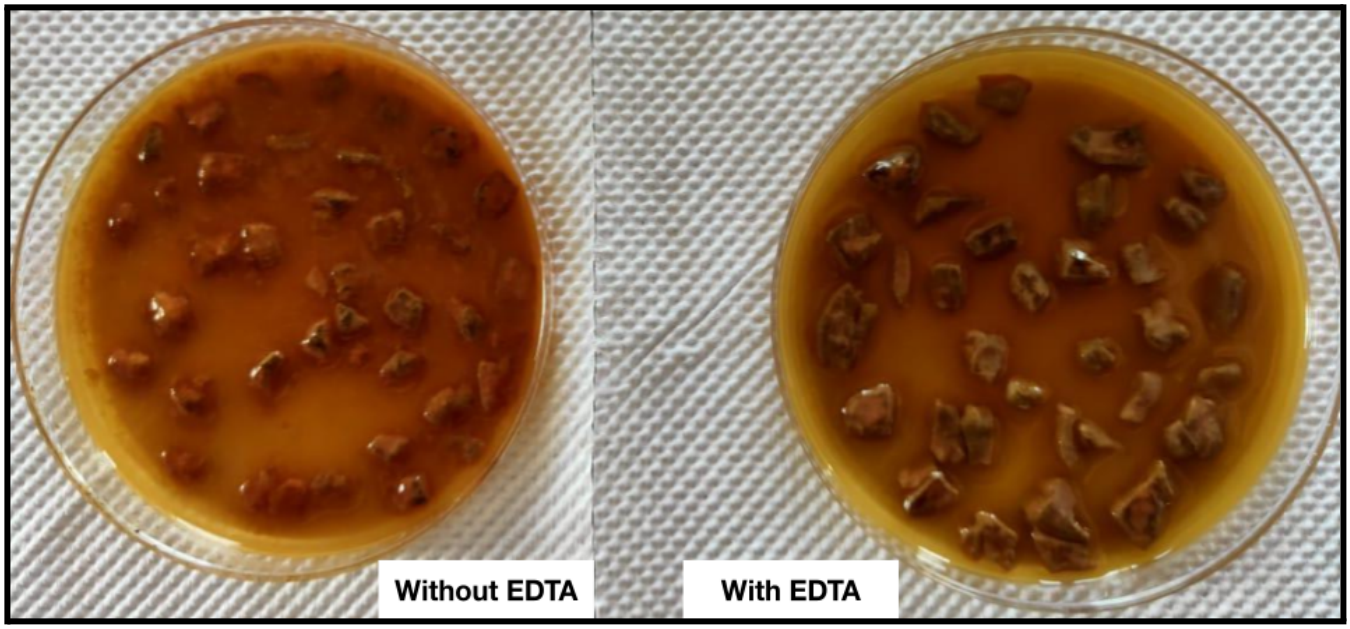


**Figure 3.** Post incubation observation of petri plates.

## Results and Discussion:

In this assay, we demonstrated that Collagenase type-1 activity can be measured *in vitro* using chicken liver pieces both in the presence and absence of inhibitors such as EDTA. As shown in Figure 3, we can observe that in petri plate 1 (left plate) the Collagenase type-1 enzyme acts on the liver tissue based on notable deformation of the liver pieces along with the cloudiness of the buffer in the plate which is due to the activity of Collagenase type-1 on the extracellular matrix. Whereas in the case of petri plate 2 (right plate) we can observe due to the addition of EDTA the collagenase enzyme action is partially inhibited from the lack of significant deformation of the liver pieces. EDTA being a metal ion chelator acts as a sponge to chelate the metal ions in the buffer leading to the slowing down of the MMP activity. The resultant degraded tissue that is mixed with the buffer is further used to measure the optical absorbance at 600 nm. Further, by using the image quantification software such as the ImageJ, the pixels can be quantified along with the O.D. 600 nm. Such O.D. measurements can also be performed systematically for different time periods.

By using different doses of inhibitors one can establish dose-response curves that can further be used to calculate $IC_{50}$ values of the inhibitors. In this way one can compare the potencies of various inhibitors. Thus, one can get a clear idea on the activity/inhibition of the MMPs in the presence and absence of the inhibitors with an appropriate control such as EDTA. This assay can thus be used in future to evaluate our in house MMP1 inhibitors.

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**Conflict of interest:** This research article is an ongoing project currently at TCABS-E, Rajahmundry, India.