RHEOLASER MASTER

# Gelation behavior of platelet lysates

## Control of gelation properties

## INTRODUCTION

Platelet lysates have attracted attention in cell therapy, as they provide a 100% human origin alternative with human growth factors. These improve significantly the proliferation of cells concerning conventional culture media. However, in contact with Ca2+ ions in cell culture media, platelets tend to clot due to fibrin hydrogel formation. Although very weak, the gel formation reduces proliferation capacity. Heparin (usually from animal sources) is therefore added to avoid gelation. Recently gamma irradiation, which is already used for sterilization is used to inhibit the gelation capacity of the platelet lysates. This application note shows how Rheolaser Master can measure weak gels, due to a non-contact method and so evaluate fast and accurately the effectiveness of radiation on the platelet lysate samples.

**Platelet lysates** **Weak gel** **Gelation**

## HOW IT WORKS

Rheolaser Master is based on Diffusing Wave Spectroscopy (DWS), a multiple light scattering technique. Light is backscattered by scatterers in the sample. The microstructure motion inside the sample (droplets, crystallites, etc.), creates an interference pattern (Speckle Image). Variation of this image in time is directly related to the mobility of the scatterers. (Figure 1). The faster the Speckle Image changes in time, the higher the mobility of the microstructure.

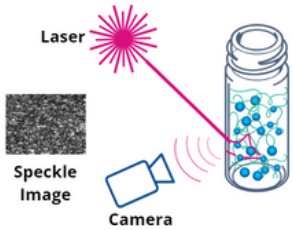


**Figure 1. Schematical representation of the measurement set-up.**

By mathematical treatment, Mean Square Displacement (MSD) curves are obtained (Figure 2), which contain the viscoelastic information. Short straight lines (blue curves) indicate the liquid behavior of the sample, whereas curves with a plateau, the so-called elastic plateau (red curves), indicate gel-like or solid-like behavior.

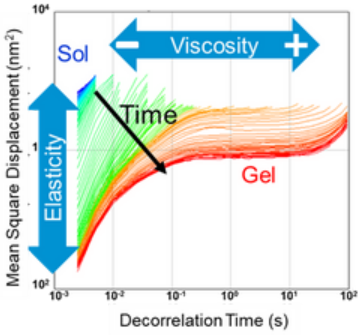


**Figure 2. Typical evolution of MSD curves showing a gelation with liquid curves (bleu) adn viscoelastic curves (red).**

## SAMPLE AND PROTOCOLE

Five different platelet lysates were studied (table 1). Two different plasma concentrations (high, and low) were studied with and without gamma irradiation. The fifth sample is a reference sample with a high plasma content, but with heparin addition, preventing gelation. All samples were analyzed by mixing them with a standard cell culture medium (Hemeda et al., 2014; Walenda et al., 2012) at 37°C with the Rheolaser Master. 0.1% (wt%) of Latex particles were added to increase backscattering intensity.

**Table 1. List of human platelet lysate samples**

| Sample | Plasma concentration | Radiation |
|---|---|---|
| **PL 1 - native** | High | No |
| **PL 1 - irradiated** | High | Yes |
| **PL 2 - native** | High | No |
| **PL 2- irradiated** | Low | Yes |
| **PL 3 (reference)** | High | No, but heparin added |

## RESULTS AND DISCUSSION

Platelet lysates are used in cell culture for their excellent properties to improve cell growth and efficiency. This is mainly due to the presence of human growth factors. The main drawback of human platelet lysates is the formation of a weak gel, when in contact with the Ca2+ ions of the cell culture media.

# Gelation behavior of platelet lysates
## Control of gelation properties

Figure 3 shows the typical evolution of the Elasticity Index (EI) during the gelation of a platelet lysate without any gel inhibitors. The higher this index is, the higher the sample's elasticity, thus the gel strength.

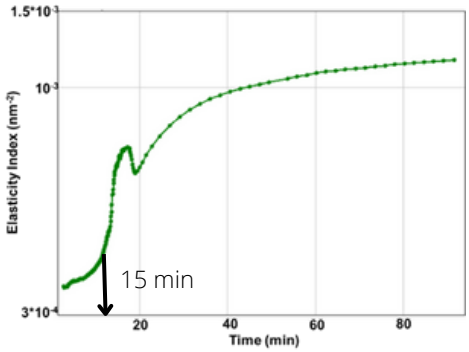


**Figure 3. Evolution of Elasticity Index during gelation of a platelet lysate "PL1 - native" at 37°C**

Figure 4 shows the comparison of the gelation kinetics of all samples. The different platelet lysates were in contact with the cell culture media. The samples "PL1-native", "PL1 - irradiated" and "PL2-native" started to gel, indicated by an increase in the Elasticity Index. The samples "PL2 - irradiated" and "PL3 (reference)" did not gel. PL3 was the reference sample, which is not supposed to gel, because of heparin addition. The slight increase in EI is due to flocculation, however, no continuous gel phase was formed.
PL1 contains the highest percentage of plasma, which is the source of fibrinogen. As it was not irradiated, it gelled in 15 minutes after contact with the Ca2+ ions.

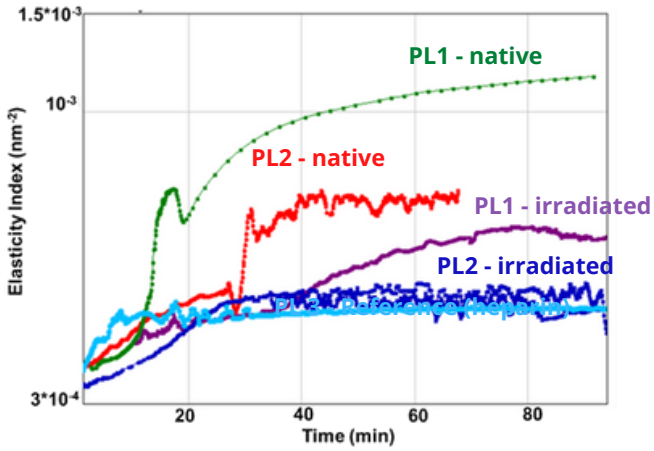


**Figure 4. Comparison of gelation kinetics of different platelet lysate samples at 37°C in contact with cell culture media.**

"PL2 - native" is composed of less plasma than "PL1 - native", thus, gel formation is needed twice the time, i.e. 30 minutes. "PL1 - irradiated" has the same plasma concentration as "PL1 - native", but was irradiated with gamma rays. The efficiency of the irradiation is translated by a delayed gelling, however, a slight gelation is observed by the increase of the EI at about 40min. The gamma irradiation dose was not high enough to completely prevent gelation.

"PL2 - irradiated" is of the same composition as "PL2 - native" but irradiated. Irradiation was sufficient to inhibit completely the gelling. Only flocculation was observed, in the same way as for sample PL3 (Reference), the reference solution. Table 2 shows the gelation times and the elasticity index after 60 minutes.

**Table 2. Gelation time and gel strength of different platela**

| Sample | Gel Time (min) | EI (* 10-3 nm) |
|---|---|---|
| **PL 1 - native** | 15 | 1.1 |
| **PL 1 - irradiated** | 40 | 0.6 |
| **PL 2 - native** | 30 | 0.8 |
| **PL 2 - irradiated** | N/A | 0.4 |
| **PL 3 (reference)** | N/A | 0.4 |

## CONCLUSION

Rheolaser Master is a powerful tool for the analysis of platelet lysate gelation. The gels formed in contact with the culture media are very weak and break down if disturbed. Rheolaser Master uses an optical non-destructive and non-intrusive method, making it a perfectly adapted instrument to study the gel behavior of such weak gels. In addition, the 6 measurement positions allow fast screening of different preparation. It was shown, that Rheolaser Master can differentiate between samples of different plasma compositions, as well as between samples that are γ-irradiated. The instrument can therefore help to adjust the right plasma concentration or the right irradiation dose to obtain a non-gelling sample or the right gelation time.