Influence of autoclave sterilization of gelatin on physical properties and endotoxin level and the influence of endotoxin level on endothelial cellular activity

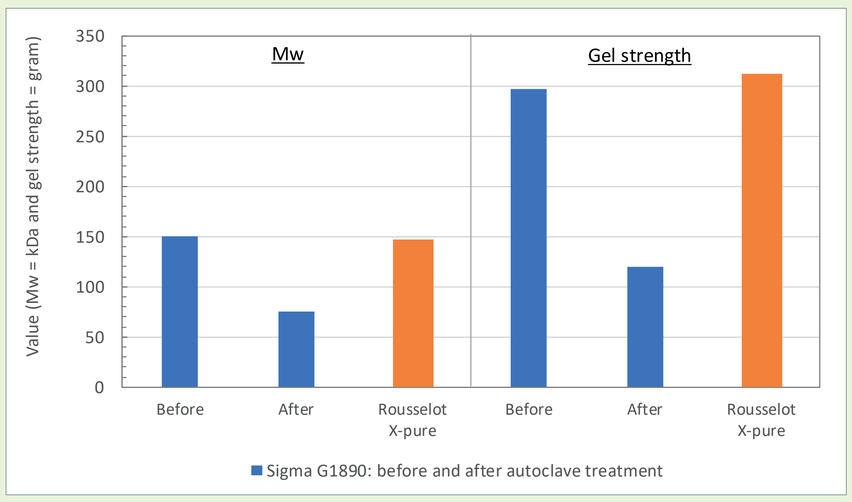
Barbara Vanhoecke and Jos Olijve - Rousselot, Ghent, Belgium (jos.olijve@rousselot.com)

Introduction and purpose: Gelatin is a traditional and safe excipient extensively used in pharmaceutical applications. It is derived from collagen which is part of the natural extracellular matrix. Due to its low allergenic potential, suitable biological properties, and tunable physical characteristics, gelatin is an interesting material for (bio)medical applications. However, since gelatin is sourced from natural raw material it might contain unwanted contaminants like endotoxins. Endotoxins are found in the outer membrane of gram-negative bacteria and under certain circumstances they can provoke profound negative reactions in the body such as strong immune responses. Underlying mechanisms are the binding of endotoxins to specific receptors, namely toll-like receptors, which might trigger pro-inflammatory pathways. The aim of this study was to determine the influence of autoclave sterilization of gelatin on physical properties and on endotoxin level. Additionally, the influence of endotoxin level on cellular viability of an endotoxin-sensitive endothelial cell line was measured.

Methods: A 10% solution of a type A gelatin from Sigma-Aldrich (G1890) with endotoxin level of 20000 endotoxin units per gram gelatin (EU/g) was autoclave sterilized during 30 minutes at 121°C and 1 bar overpressure. The molecular weight, gel strength and the endotoxin level of the sterilized G1890 gelatin was compared to a purified (WO2016085345) X-pure Rousselot gelatin with endotoxin level < 10 EU/g. The endotoxin levels of the gelatins were measured using the Endozyme recombinant factor C method from Hyglos GmbH (Germany). A 1% solution was used to measure the effect of both gelatins (Sigma and Rousselot) on mitochondrial activity and cellular viability of an endotoxin-sensitive endothelial cell line.

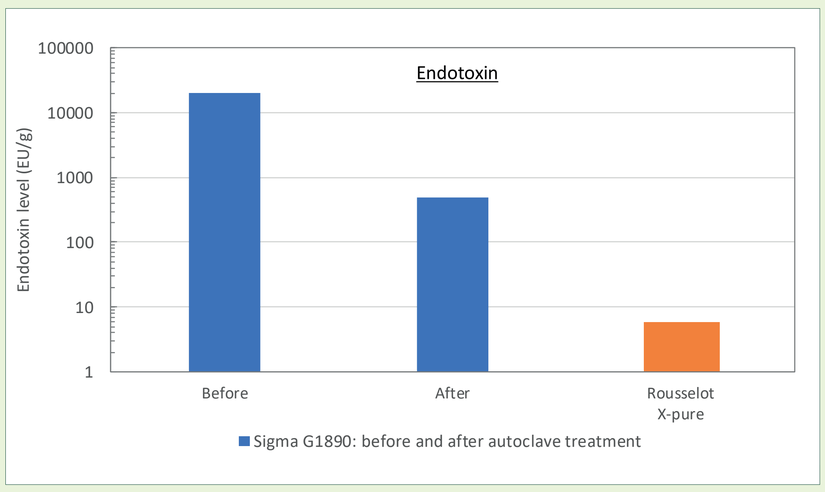
Results:

Figure 1. Average molecular weight (Mw) and gel strength of Sigma G1890 gelatin before and after autoclave treatment compared to Rousselot X-pure gelatin.



A significant decrease in average molecular weight and gel strength was observed for G1890 gelatin after autoclave treatment compared to non-autoclaved Rousselot X-pure gelatin.

Figure 2. Endotoxin level of Sigma G1890 gelatin before and after autoclave treatment compared to Rousselot X-pure gelatin.



Autoclave treatment reduced the endotoxin level of G1890 gelatin, however the endotoxin level is still significantly higher compared to non-autoclaved Rousselot X-pure gelatin.

Figure 3. Effect of Sigma G1890 and Rousselot X-Pure gelatin solution (1%) on cell viability (measured by mitochondrial cell activity) of an endotoxin-sensitive endothelial cell line.

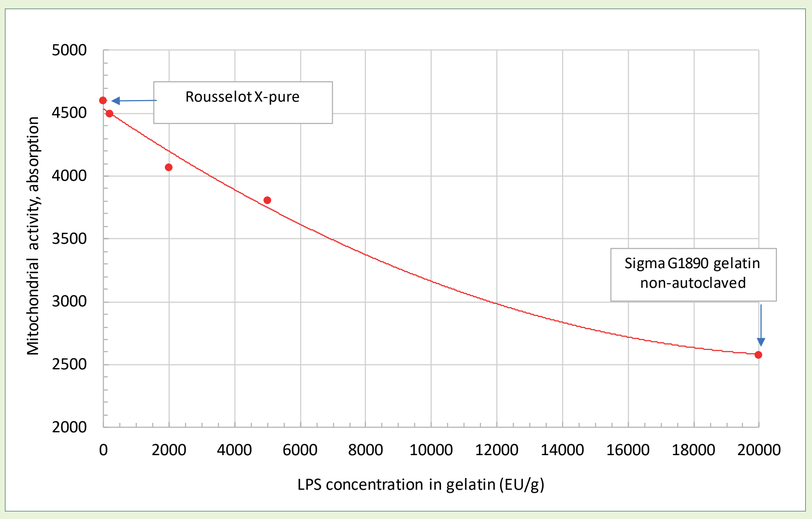
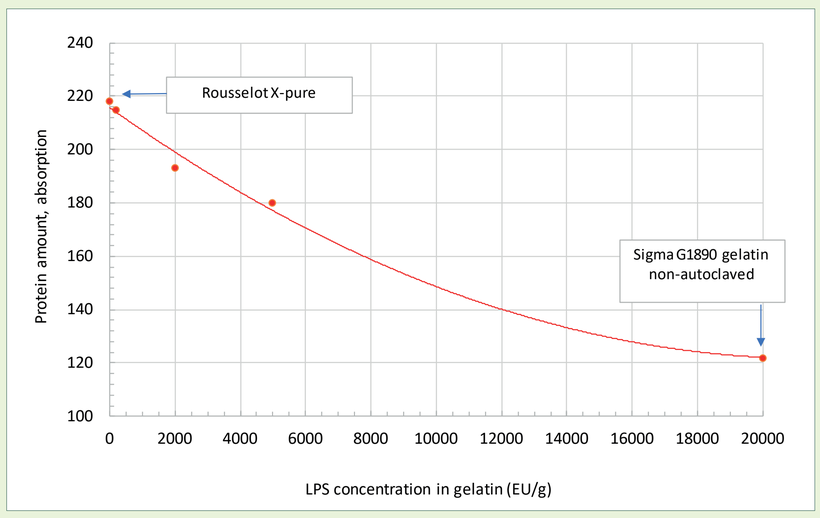


Figure 4. Effect of Sigma G1890 and Rousselot X-Pure gelatin solution (1%) on cell growth (measured by protein amount) of an endotoxin-sensitive endothelial cell line.



The cellular activity and growth of the endothelial cell line was largely influenced by endotoxin. The G1890 Sigma gelatin with endotoxin level of 20000EU/g gave significantly lower cellular activity compared to purified Rousselot X-pure gelatin with endotoxin level <10EU/g.

Conclusion:

- Autoclave sterilization of Sigma G1890 gelatin gave a significant decrease in gelatin physical properties which makes autoclave sterilization less suitable for applications where physical properties are important, like bioprinting.
- Autoclave sterilization is not sufficient to inactivate endotoxin in gelatin.
- A clear reduction in endothelial cellular activity and viability was observed with increasing endotoxin levels which indicates that low endotoxin levels are important in applications such as tissue engineering using living cells.

August 2018

References:
1. 1. Olijve J.H. et.al. WO2016085345
2. Sivisubramaniyan K, et.al. Regen Med. 2008 Jan;3(1):23-31.
3. Hirayama et al, J. of Chromatography 781, (2002) 419-432.

Rousselot

DARLING INGREDIENTS