

Impact of Autoclave Sterilization of Gelatin on Endotoxin Level and Physical Properties Compared to Surfactant Purified Gelatins

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Abstract : Introduction and Purpose: Endotoxins are found in the outer membrane of gram-negative bacteria and have profound in vitro and in vivo responses. They can trigger strong immune responses and negatively affect various cellular activities particular cells expressing toll-like receptors. They are therefore unwanted contaminants of biomaterials sourced from natural raw materials, and their activity must be as low as possible. Collagen and gelatin are natural extracellular matrix components and have, due to their low allergenic potential, suitable biological properties, and tunable physical characteristics, high potential in biomedical applications. The purpose of this study was to determine the influence of autoclave sterilization of gelatin on physical properties and endotoxin level compared to surfactant purified gelatin. Methods: Type A gelatin from Sigma-Aldrich (G1890) with endotoxin level of 35000 endotoxin units (EU) per gram gelatin and type A gelatins from Rousselot Gent with endotoxin activity of 30000 EU per gram were used. A 10 w/w% G1890 gelatin solution was autoclave sterilized during 30 minutes at 121°C and 1 bar over pressure. The physical properties and the endotoxin level of the sterilized G1890 gelatin were compared to a type A gelatin from Rousselot purified with Triton X100 surfactant. The Triton X100 was added to a concentration of 0.5 w/w% which is above the critical micellar concentration. The gelatin surfactant mixtures were kept for 30-45 minutes under constant stirring at 55-60°C. The Triton X100 was removed by active carbon filtration. The endotoxin levels of the gelatins were measured using the Endozyme recombinant factor C method from Hyglos GmbH (Germany). Results and Discussion: Autoclave sterilization significantly affect the physical properties of gelatin. Molecular weight of G1890 decreased from 140 to 50kDa, and gel strength decreased from 300 to 40g. The endotoxin level of the gelatin reduced after sterilization from 35000 EU/g to levels of 400-500 EU/g. These endotoxin levels are however still far above the upper endotoxin level of 0.05 EU/ml, which resembles 5 EU/g gelatin based on a 1% gelatin solution, to avoid cell proliferation alteration. Molecular weight and gel strength of Rousselot gelatin was not altered after Triton X100 purification and remained 150kDa and 300g respectively. The endotoxin levels of Triton X100 purified Rousselot gelatin was < 5EU/g gelatin. Conclusion: Autoclave sterilization of gelatin is, in comparison to Triton X100 purification, not efficient to inactivate endotoxin levels in gelatin to levels below the upper limit to avoid cell proliferation alteration. Autoclave sterilization gave a significant decrease in molecular weight and gel strength which makes autoclave sterilized gelatin, in comparison to Triton X100 purified gelatin, not suitable for 3D printing.

Keywords : endotoxin, gelatin, molecular weight, sterilization, Triton X100

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