Endotoxins – The Unwanted Contaminants in Biomaterials for Regenerative Medicines

Elien Gevaert, Principle Scientist at Rousselot Biomedical, talks about these disrupters of *in vitro* and *in vivo* cell growth and how they can stifle research and delay lab to clinic translation

In biomedical applications, the presence of lipopolysaccharides (LPS) is a significant cause for concern. What are LPS and the associated risks?

Elien Gevaert: LPS, also known as endotoxins, are toxins found in the outer membrane of gram-negative bacteria. They can initiate strong immune responses, mainly via interaction with Toll-like receptor 4 (TLR-4) expressed on the cell membranes of a variety of cell types. The human body can react to the presence of LPS with fever, chills, shaking, and sometimes respiratory symptoms. The presence of LPS in the circulation can lead to endotoxemia and possible anaphylactic shock, while chronic exposure to LPS can result in systemic inflammation and can aggravate existing diseases or create novel health problems.

Unsurprisingly, the FDA imposes strict regulations on the levels of endotoxins allowed for medical devices. For devices targeting the cardiovascular system, the endotoxin limit is 20 endotoxin units (EU)/device, while for those in contact with cerebrospinal fluid, the limit is even lower, at 2.15 EU/device.

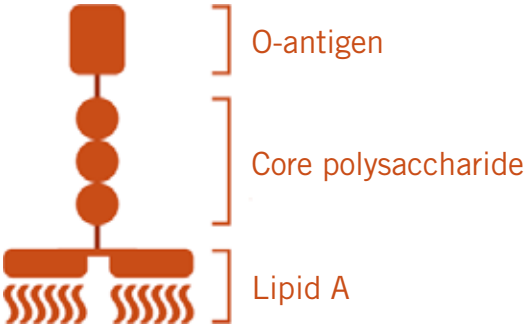
The selection of endotoxin-purified biomaterials for in-body applications is, therefore, a crucial step in ensuring regulatory compliance.

Purity is key, yet many biomaterials still have unregulated levels of LPS. Why is this?

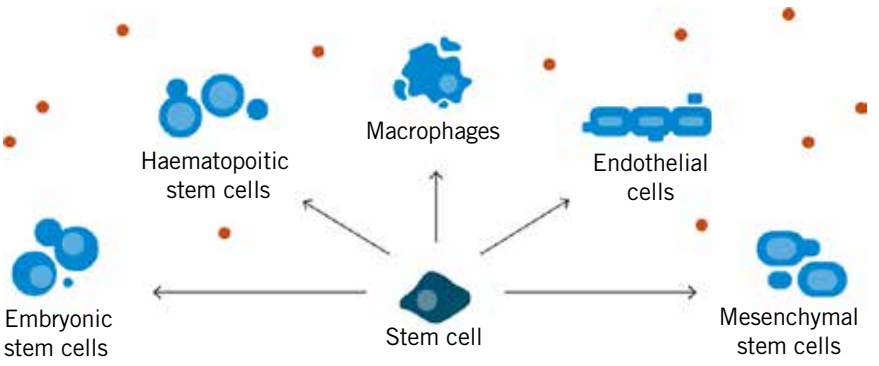
LPS form supramolecular structures (micelles and vesicles) with a size up to >1000kDa, making them extremely difficult to remove. Added to this, they are UV and heat stable, requiring very high temperatures (>180˚C) for inactivation, which risks altering the biomaterial. Consequently, most commercially available biomaterials are non-endotoxin purified and are, therefore, often not suitable for clinical applications.

LPS can significantly impact clinical applications. Does the need for purity translate to non-clinical biomedical applications?

Absolutely. At the cellular level, research has shown that endotoxins can negatively impact cell viability, proliferation, functionality, and differentiation. Moreover, the use of gelatins with unknown endotoxin levels in *in vitro* research can contribute to irreproducible results and/or delaying laboratory to clinic translation.



Lipopolysaccharides (LPS) can initiate strong immune responses.



LPS can cause the misinterpretation of results by affecting:

Thinking about specific cell types, how do LPS impact cells involved in the immune response?

As LPS induce an innate immune response, LPS contamination of lab equipment, buffers, and compounds is a major concern when working with immune cells. Many other cell types, such as epithelial cells, endothelial cells, and structural component cells, play an important role, both in initiating and driving inflammation as part of the immune response. Consequently, a large fraction of primary isolated cells and cell lines express TLR-4 and are sensitive to LPS.

The impact of LPS on non-immunologic cell systems is often unrecognised, but evidence shows that LPS contaminants can actively interfere with cell viability, proliferation, and functional performance in those systems.

A significant proportion of regenerative medicine research focuses on stem cells. How do LPS impact these cells?

Gelatin is ideally suited for stem cell bioengineering, but the presence of even trace amounts of endotoxins can negatively impact viability and differentiation of stem cells.

Literature shows that the viability of human umbilical cord mesenchymal stem cells (MSCs) is inversely correlated to LPS concentration, and that time-dependent LPS exposure or fluctuations of LPS can lead to opposite therapeutic outcomes. It has also been shown that TLR-4 stimulation can affect the characterisation of MSCs, leading to disruption in intended therapeutic functions, and undesired production of growth factors, such as VEGF, FGF2, HGF, and IGF-1. Moreover, LPS has been shown to affect cell differentiation, for example, downregulating osteo/odontogenic differentiation, and inhibiting osteogenic differentiation in stem cells.

We investigated the effect of gelatin methacryloyls (GelMAs) containing LPS on the chondrogenic differentiation of equine mesenchymal stromal cells. Glycosaminoglycans production at day 28 of differentiation was higher in GelMA with lower LPS levels.

In addition, to predict the inflammatory response upon cell transplantation, equine peripheral blood mononuclear cells were cultured on gelatin hydrogels for 24 hours, followed by assessment of tumour necrosis factor (TNF)-α as an inflammatory marker. Unsurprisingly, the levels of TNF-α production increase with increased LPS levels in the gelatin.

Why is gelatin so beneficial for biomedical applications?

Gelatin has a long history in medicine and is used today in a range of pharmaceutical and biomedical applications, including haemostatics, drug delivery, and cell cultures.

Based on this well-established track record of versatility and reliability, it is no surprise that gelatin is now playing a key role in groundbreaking regenerative medicine. Gelatin-based hydrogels offer tuneable physicochemical properties, biocompatibility, and biodegradability, and promote cell adhesion and proliferation, proving an ideal biomaterial for regenerative medicine.

How can the use of low-endotoxin gelatin improve research and accelerate lab to clinic translation?

In the lab, the use of gelatins with uncontrolled LPS content has been shown to interfere with cell viability, proliferation, functionality, and differentiation, leading to unexpected or irreproducible results. Caution with the translation from *in vitro* to *in vivo* studies is, therefore, warranted when using non-purified gelatins. In the clinic, safety, quality, and purity are paramount when biomaterials are used in the body. Due to strict regulations on the levels of endotoxins allowed, the use of a low-endotoxin gelatin is essential.

Dr Elien Gevaert holds a Master of Science in Biochemistry and Biotechnology. After obtaining a PhD in Biomedical Sciences in the field of liver tissue engineering, she joined the upper airways research lab at the University of Ghent/University Hospital, Belgium. There, Elien investigated the immunological mechanisms underlying airway inflammatory diseases as a postdoc for six years. Her academic career has resulted in more than 30 scientific publications in leading scientific journals. In 2020, she joined **Rousselot** as Principal Scientist. In her current position, she leads and coordinates several R&D projects with the aim of developing new and innovative gelatin-based products for nutritional or biomedical applications.