EU sourcing requirements for animal-derived materials

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Abstract

Regenerative medical products derived from animal tissue have been used to successfully treat millions of patients. As they are manufactured from animal sources, there are bio-contamination and biocompatibility risks that must be addressed in accordance with ISO 22442 for receipt of the CE Mark and subsequent EU commercialisation. This article discusses important regulatory animal sourcing requirements associated with medical devices that cover these biological risks. These requirements include risk management, animal health controls, quality system elements, and demonstration of safety related to potential transmissible pathogens. The necessary information needs to be presented in the form of reports, risk analyses, and other documentation.

Regenerative medicine is a complex, interdisciplinary field that utilises bioactive substances, cells, and biomaterials with the goal of repairing and restoring bodily tissues and organs of humans. An area of extensive research and development of regenerative implants involves the use of animal-derived tissues as a standalone regenerative construct or as a delivery platform for biological agents. Clinically, regenerative applications of animal-derived tissues have widespread use in wound care, orthopaedics, dentistry, cardiology, general surgery, urogynaecology, neurology, and other medical fields. Some commonly known animal-derived products include advanced wound dressings, synthetic bone grafting materials, dural substitutes, and biological heart valves.

The fundamental scientific reason why animal-derived tissues are ideal implantable materials is that there is extensive homology between animal extracellular matrices and human analogues. This relationship is mainly due to collagen and other macromolecules such as elastin, hyaluronic acid, and sulphated glycosaminoglycans, which are the principal components of all extracellular matrices across species and are remarkably similar in their structures among mammals. Given this extensive similarity of extracellular matrix molecules, the main concern for implantation of animal tissues relates to the human antigenic response against animal cells and nucleic acids that reside within the tissue. Accordingly, an entire field has emerged that involves the engineering and manufacturing of extracellular matrices and collagen that remove the unwanted animal cellular and nucleic acid components, with the goal of leaving the extracellular matrix intact. In this regard, animal-derived, decellularised extracellular matrix tissues

Porcine heart valves

become almost "humanised" in their resemblance to human counterparts.

Given their abundance, relative low cost, and controls due to governmental and industrial regulations, tissues from swine (porcine) and cattle (bovine) are by far the most utilised in commercially approved animal-derived medical products, both in Europe and elsewhere. No matter the animal source, however, compliance with several important standards must be demonstrated for receipt of the CE Mark for a medical device. Of these standards, the animal tissue sourcing requirements of the ISO 22442 series[1-4] are the most relevant and will be discussed in this article.

ISO 22442-1 application of risk management

Risk management requirements are detailed in ISO 22442-1,[1] which primarily relate to the risks of product bio-contamination and bio-incompatibility due to the use of animal-derived tissues. The device manufacturer needs to provide a risk analysis that considers the following possible product hazards:

1. Parasites and unclassified pathogenic entities,
2. Bacteria, moulds, and yeasts,
3. Viruses,
4. Transmissible spongiform encephalopathy (TSE) agents, and
5. Pyrogenic, immunological, and toxicological reactions.

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Another important aspect related to ISO 22442-1 compliance is a justification for why animal tissues are required *in lieu* of synthetic alternative materials, materials from less risky animal species, or from human origin. Typically, a justification involves a scientific and clinical review of the relevant literature and an overall assessment of the product risk to benefit ratio. Finally, ISO 22442-1 requires surveillance of animal zoonosis to provide on-going re-assessment of the risk analyses. As an example, porcine derived materials are inherently less risky than their equivalent bovine materials, since a TSE agent, bovine spongiform encephalopathy (BSE), has been found in bovines, but to date, porcine animals have not been found to be infected with a TSE agent. Accordingly, a manufacturer of porcine derived materials used in their medical device must continuously monitor the scientific literature for evidence of a possible porcine spongiform encephalopathy agent (PSE), which of course, if ever found, could greatly impact the risk profile of their medical device.

ISO 22442-2 controls on sourcing, collection and handling

ISO 22442-2[2] provides the necessary controls for animal-derived tissues and substances, at all levels of the supply chain. Namely, requirements for farms, abattoirs, and device manufacturers are stipulated, with traceability requirements defined based on risk management. At the farms, veterinarian oversight is required for monitoring animal health, and animals must be deemed fit for human consumption with a post-mortem inspection by an animal health official. Procedures must be in place to prevent cross-contamination and to provide specific instructions for the collection and handling of tissue, storage, transportation, labelling requirements, and auditing responsibilities.

ISO 22442-3 and ISO 22442-4 validation and principles concerning the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

As sterile medical devices must meet a sterility assurance level (SAL) of 10^{-6}, there is a similar concept that must be demonstrated to establish viral safety for devices derived from animal sources. A manufacturer must establish that the manufacturing process inactivates or eliminates potential viral contaminants to a level that renders the animal tissue-based device a low viral risk. This level is quantified as a log reduction value (LRV) via a viral clearance study. Typically, the process begins with a formal literature review with a protocol, whose requirements are stipulated by ISO 22442-3.[3] Viruses that are relevant for the specific zoonosis situation, based on tissue source, are identified through the review. This enables the manufacturer to identify specific viral risks and determine the approach required in the manufacturing process to eliminate any identified zoonotic viruses. In addition, this information is utilised in the selection of a virus panel used for upcoming viral inactivation studies.

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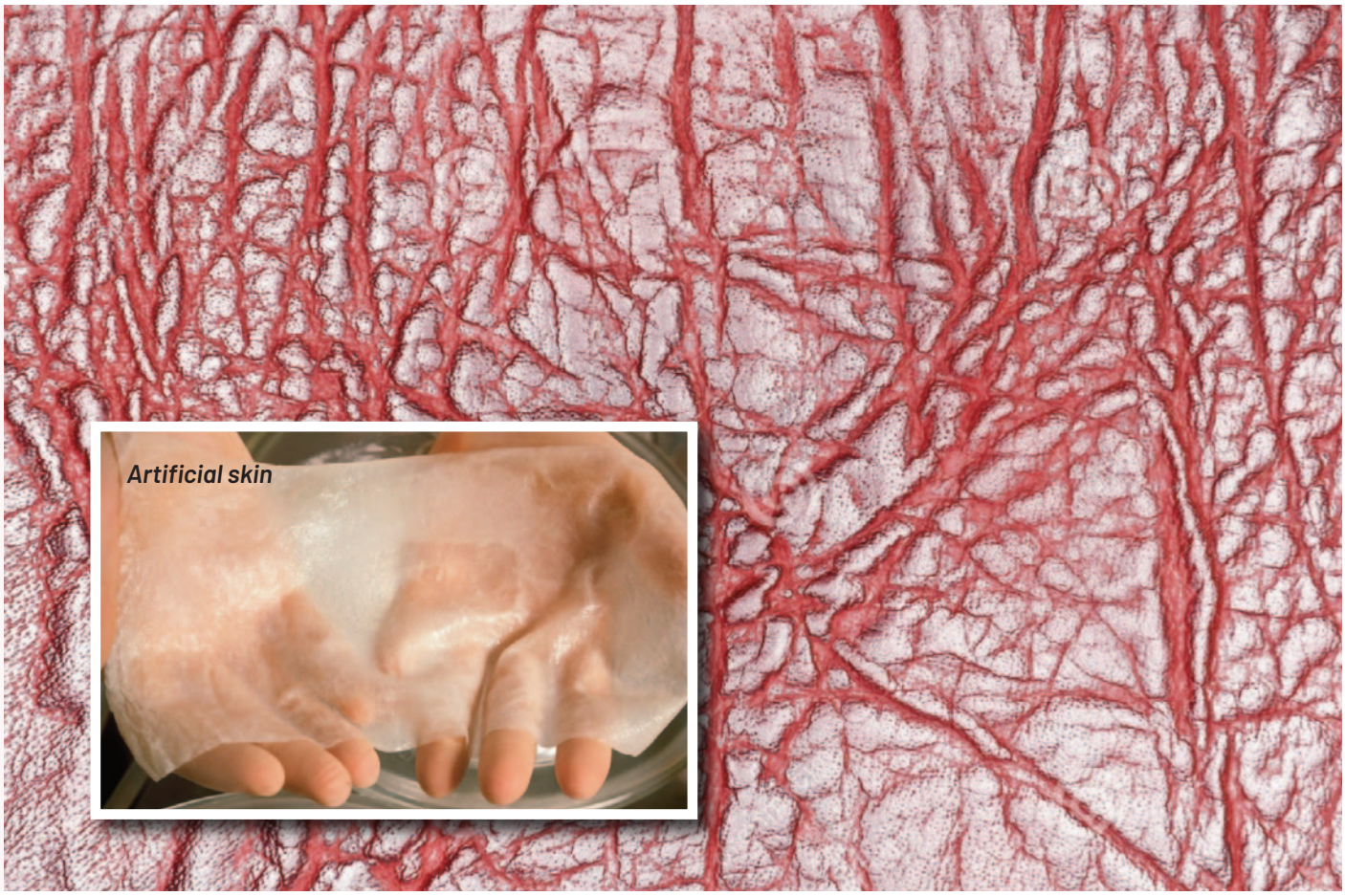

Another important aspect of the literature review is the determination of which manufacturing steps may inactivate and/or eliminate viruses, and what theoretical viral reduction levels may be obtained by these processes according to the literature. Sterilisation usually plays a key role in viral inactivation as well; for example, radiation and chemical sterilisation methods usually offer significant viral inactivation capabilities. In this planning phase, key considerations for the inactivation of viruses are also evaluated, including the effects of the tissue substrate and preceding manufacturing steps on viral inactivation efficiency. Finally, the literature review identifies the key processing parameters that affect viral inactivation, which are essential for establishing critical process controls that must be maintained for eventual viral inactivation validation.

In most cases, a medical device manufacturer will proceed with a viral inactivation study that evaluates the efficacy of the selected manufacturing steps to inactivate and/or eliminate viruses, with the goal of generating LRV data. However, in some circumstances, a manufacturer may rely upon the literature and a risk-based approach *in lieu* of performing a prospective viral inactivation study. This may occur when literature studies are so highly relevant that they closely match a viral inactivation step of the manufacturing process, and the generated log reduction values from the literature studies are directly applicable. Manufacturers may also proceed with a hybrid approach that is both literature-based and utilises viral inactivation studies.

The design and execution of viral elimination/inactivation studies must be performed in accordance with the requirements ISO 22442-3. Elimination is a process where viruses remain intact but are removed from the tissue substrate whereas inactivation is causing the alteration of the virus by the manufacturing process that renders a virus non-infectious. Prior to executing formal elimination/activation studies, a protocol should be written that documents the following:

1. Risks identified per ISO 22442-1,
2. Anticipated zoonotic viral agents,
3. A relevant virus panel that usually includes four virus types: RNA and DNA viruses, both enveloped and non-enveloped,
4. Identification of the manufacturing processes selected to eliminate/inactivate viruses,
5. Demonstration of the validity of a scaled down process used in a viral testing laboratory in comparison with the actual full-scale production process, and
6. Methods for the calculation of viral reduction factors and the method for the estimation of reduction kinetics, when applicable.

Viral elimination/inactivation studies are then conducted in accordance with the protocol, and log reduction values for each evaluated manufacturing step are obtained.

The last requirement of the ISO 22442-3 process is the writing of a final report that includes the literature review and information obtained from executed viral elimination/

inactivation studies. The efficacy of the overall manufacturing process to reduce the four virus types (RNA, DNA; enveloped and non-enveloped viruses) is provided. Finally, the report identifies critical manufacturing parameters with limits that need to be maintained during the production process for assurance of viral inactivation.

For animal sources that represent a TSE risk, such as cattle, ISO 22442-4[4] provides analogous information to the viral inactivation requirements provided in ISO 22442-3 for TSE inactivation. However, TSE inactivation studies for tissue and collagen materials are rarely performed, since the proven methods required to inactivate the TSE agent, i.e., abnormal prion protein, are impractical to use in creating a medical device. These treatments include incineration, chlorination, and strong alkali in combination with substantial heat, all of which effectively destroy tissue and collagen preparations. Therefore, when TSE inactivation studies are unable to be performed, the risk of potential TSE contamination is mitigated by an alternative risk management strategy per ISO 22442-1, which usually depends on sourcing controls. Generally, almost all tissue and collagen preparations are derived from connective tissue, which are known as a low TSE infectivity risk. Combined with strong governmental agricultural controls, usually the risk management strategy of sourcing control is accepted by regulatory bodies as a means of demonstrating an acceptable risk with respect to TSE transmission.

Special current zoonotic concerns

As discussed previously, zoonosis monitoring is an important requirement of ISO 22442-1 to understand if the risk profile associated with animal tissue sourcing has changed. Current major zoonosis concerns are related to the SARS-CoV-2 coronavirus (cause of COVID-19 pandemic) and in the case of porcine-derived materials, African swine fever virus.

Given the concern related to the SARS-CoV-2 coronavirus, manufacturers have incorporated this transmissible agent into their surveillance. Fortunately, commercial swine and cattle livestock have not been shown to be infected with this virus. Even in the event of a livestock infection, it is likely that most tissue and collagen manufacturers will determine that their existing methods are highly effective for inactivation of SARS-CoV-2 coronavirus. This is because coronaviruses are RNA enveloped and are highly susceptible to typical processes used in collagen manufacturing, which include alcohol and alkaline treatments, radiation sterilisation, and other physicochemical methods.

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African swine fever is a deadly disease of pigs that has seen widespread outbreaks across Africa, Asia, and Europe since 2007. Fortunately, the causative African swine fever virus is a threat to swine but has no impact on human health. Accordingly, the main concern for manufacturers is a supply chain issue where tissue raw materials may become limited. The animal controls instituted by governmental agencies have prevented any significant contamination of commercial swine livestock in most geographic markets, but vigilance and monitoring need to be maintained for this swine disease.

In summary, the use of tissue and collagen materials derived from animal sources are commonly used in many regenerative medical devices that have been successfully used to treat millions of patients. To protect patients from bio-contamination and bio-incompatibility risks, device manufacturers must adhere to the animal tissue sourcing requirements of ISO 22442. Compliance with these standards will ensure that animal-derived medical devices remain safe for patients.

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Disclosures and conflicts of interest

The author declares no conflicts of interest.

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