WHITE PAPER

X-pure
ADVANCING MEDICAL SCIENCE

GELATIN FOR SAFE MICRO- AND SUBMICRON PARTICLE APPLICATIONS

X-PURE® GELATINS, THE SAFE CHOICE FOR THE PRODUCTION AND APPLICATION OF GELATIN MICRO- AND SUBMICRON PARTICLES

Gelatin-based micro- and submicron particles have gained increased interest in the fields of drug delivery and regenerative medicine. However, these micro- and submicron particle formulations can contain endotoxins, which is a safety challenge in these specific applications.
A new and ultra-pure gelatin, X-Pure, is now available for preparing gelatin micro- and submicron particles with exceptionally low endotoxin levels. X-Pure can be used for clinical drug/biomolecules delivery and regenerative medicine applications. It meets the highest regulation and quality standards, thereby providing maximum safety. This white paper provides background information on gelatin-based micro- and submicron particles properties and application examples.

By Jos Olijve, Principal scientist and R&D Project Manager, Rousselot Biomedical

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INTRODUCTION

Recently, gelatin-based micro- and submicron particles are gaining ground in the fields of drug delivery and regenerative medicine[1-3]. Endotoxins are extremely important for these applications, since remaining endotoxins may cause significant adverse immunogenic effects. X-Pure® is an ultrapure, low-endotoxin gelatin, which combines the unique advantages of natural gelatins, in terms of biochemical versatility and biocompatability, with an unprecedented safety profile. With X-Pure®, it is now possible to prepare gelatin-based formulations that comply with the highest safety and quality standards. This white paper provides an overview of the wide range of applications of gelatin micro- and submicron particles in drug delivery and regenerative medicine.

Gelatin - a natural, adaptable and trusted excipient and biomaterial

Gelatin has a long history as a trusted excipient in the pharmaceutical industry, meeting the highest standards of safety and regulatory compliance. Gelatin is formed by the partial hydrolysis of collagen, the most abundant protein in the body and the most prevalent macromolecule of the extracellular matrix (ECM). Gelatins used for pharmaceutical applications are produced in the same way as gelatins for food applications via the breakdown of collagen into Type A (acid hydrolysis) or Type B (alkaline hydrolysis) gelatin. This chemical versatility enables a broad range of applications.

Due to the extensive deamination of asparagine and glutamine in Type B gelatin, the isoelectric point (IEP) of type B gelatin (4.9-5.1) is lower than that of Type A gelatin (7.0-9.0). Consequently, these gelatins are positively or negatively charged, respectively, at neutral physiological pH. Moreover, the abundant presence of cell-recognition motifs such as the well-known Arginine-Glycine-Aspartic acid (RGD) sequence in gelatin macromers, favors cell attachment, spreading and proliferation[4]. Finally, the free amine and carboxyl groups of gelatin allow for facile chemical modification of gelatin at a molecular level to achieve the desired properties for specific applications.

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After graduating in Biochemistry, Jos Olijve worked as a Researcher in the Department of Molecular Genetics at the University of Groningen. Since 2012, he has been responsible for the development of new gelatin-based products and applications at Rousselot. Jos Olijve has published 15 patent applications and is the author/co-author of 10 scientific papers.

ENDOTOXINS - A CHALLENGE TO THE SAFE USE OF GELATIN IN MEDICAL APPLICATIONS

Despite the unique properties of gelatin, the use of traditionally manufactured gelatin in medical applications is challenged by the presence of endotoxins. Endotoxins are large, highly immunogenic lipopolysaccharides that are the major component of the outer membrane of Gram-negative bacteria. They are highly heat-resistant, which complicates heat-induced inactivation for biomaterials like gelatin. When exposed to the immune system, endotoxins initiate an immune response, which can lead to tissue inflammation, increased sensitivity to other allergens and risk of fatal shock[5]. Endotoxins mediate their effect via the inflammasome and TLR4 receptor present on cells, resulting in the release of pro-inflammatory cytokines[6-7].
Even exposure to very low levels of endotoxin may cause significant immune responses. Consequently, the FDA has imposed severe restrictions on the endotoxin content for a number of medical devices (FDA, 2012). Endotoxin limits for medical devices are set at 2.15 Endotoxin Units (EU) per device (or 0.06 EU/ml) for devices exposed to the central nervous system via cerebrospinal fluid, and at 20 EU/device (or 0.5 EU/ ml) for devices in peripheral applications reached by the cardiovascular and lymphatic systems.

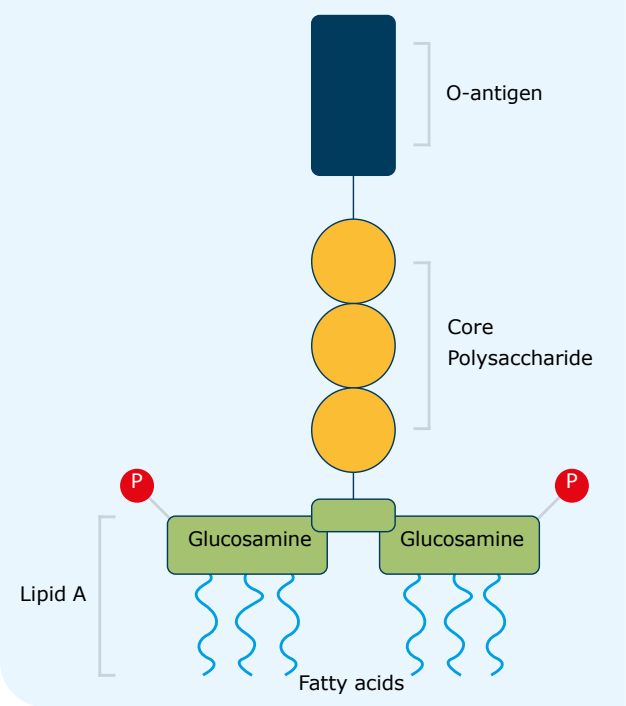

Figure 1. Structure of an endotoxin - Adapted from Lieder, Petersen and Sigurjonsson (2013)

GELATIN MICRO- AND SUBMICRON PARTICLES

The natural origin and tunable physicochemical properties of gelatin render this material ideal for processing into micro- and submicron particles. Gelatin micro- and submicron particles can be easily fabricated and the resulting lyophilized particles can be stored for sustained time periods. Compared to pure gelatin, the gelatin micro- and submicron particles can be re-formulated through a promising "bottom-up" strategy (formation of a tissue engineering scaffold through assembly of the basic units, including gelatin particles, cells and drug molecules in a modular way[8]) to design functional, shape-specific and even personalized bulk materials[9]. This new class of re-formulated material offers a virtually unlimited degree of freedom with respect to their capacity for spatial-temporal release of multiple drugs at predetermined release rates. These unique properties open up new application areas for gelatin micro- and submicron particles in drug delivery and regenerative medicine. Moreover, gelatin submicron particles can initiate strong interactions with mammalian cells[10], which might facilitate intracellular drug delivery.

Preparation of micro- and submicron particles

Gelatin micro- and submicron particles have been widely used to facilitate tissue regeneration. Gelatin microparticles are generally prepared using a water-in-oil emulsion method[11-12], while the gelatin submicron particles can be prepared through two-step desolvation[9, 12], coacervation-phase separation[13], emulsification-solvent evaporation[14] or nanoprecipitation methods[15-16]. Physical or chemical cross-linking are required to increase the thermal and mechanical stability of gelatin particles.

PROPERTIES OF GELATIN MICRO- AND SUBMICRON PARTICLES

Submicron gelatin particles are defined as particles with a size between 0.1-1.0 µm, whereas microparticles have a size of > 1.0 µm. The morphology of the gelatin micro- and submicron particles is depicted in Figure 2.

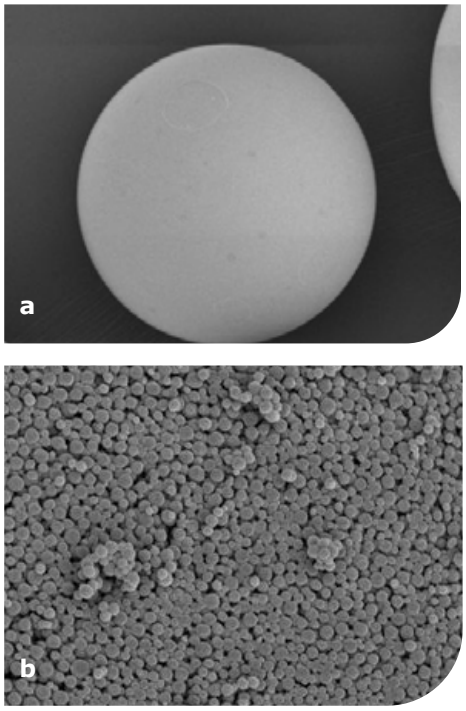

Figure 2. Scanning electron micrograph of type B gelatin micro- (a) and submicron particles (b).

Both positively and negatively charged gelatin micro- and submicron particles can be prepared by selecting the right type of gelatin (Type A or B) or post-modification strategies. The charge and degradation rate of the gelatin micro- and submicron particles can be fine-tuned by adjusting their cross-linking densities (Figure 3). Moreover, dried gelatin micro- and submicron particles can absorb approximately 10 times their own volume in water. The extent of water absorption can be controlled by varying cross-linking density.

In contrast to gelatin microparticles, gelatin submicron particles can be processed into injectable nanostructured colloidal gels. These materials exhibit a strong capacity for self-healing (Figure 4) due to the reversible non-covalent electrostatic and hydrophobic interactions between the gelatin submicron particles[9, 12]. This unique property confirms that gelatin submicron particles are highly useful for fabricating injectable formulations to be applied in defected/injured sites, especially in view of how they optimally fill irregular shaped tissue defects using minimally invasive delivery[9, 17]. Additionally, these injectable colloidal gels can maintain their structural integrity in case of severe network destruction[12].

Gelatin particle degradation at 40°C at different crosslinking densities

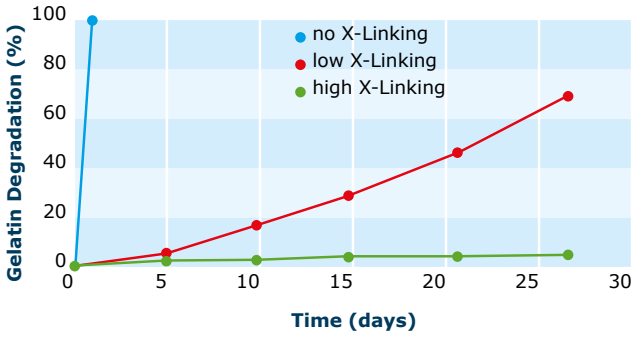

Figure 3. Enzymatic degradation profiles of gelatin submicron particles with different cross-linking densities at 37°C. Non-crosslinked gelatins degraded within a short time and rapidly lost their integrity.

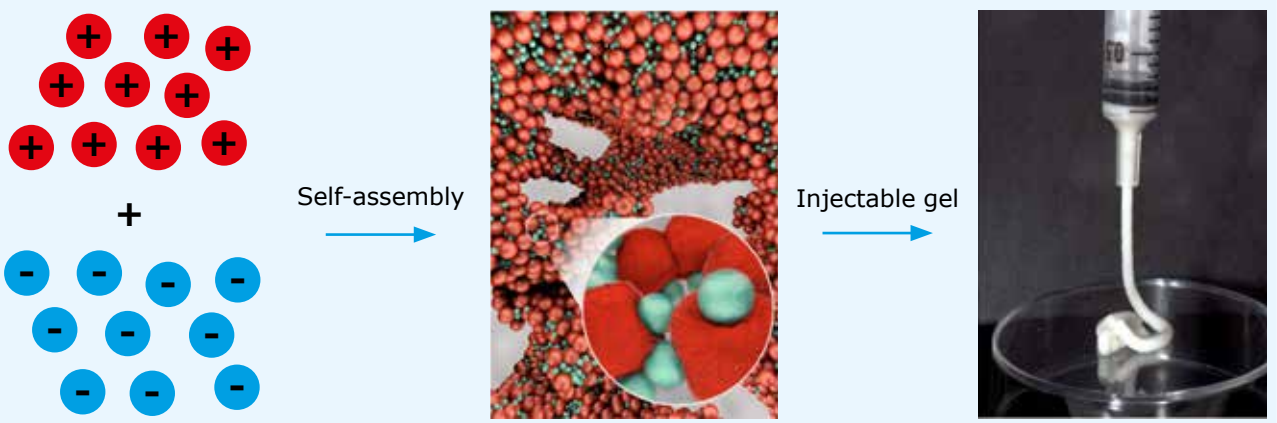

Figure 4. Injectable gelatin colloidal gels composed of oppositely charged gelatin submicron particles

GELATIN MICRO- AND SUBMICRON PARTICLES FOR DRUG DELIVERY

Owing to their unique biodegradation and charge properties, gelatin micro- and submicron particles have been widely used as embolic agents and drug delivery carriers, respectively. The charged nature of gelatin allows for controlled delivery of oppositely charged biomolecules. Therefore, gelatin micro- and submicron particles are ideal materials for the delivery of large biomolecules[1-2], such as growth factors, polysaccharides, as well as small therapeutic molecules[18-20], like antibiotics and anti-osteoporosis drugs. The advantage of using gelatin micro- and submicron particles as drug carriers can be attributed to their easy synthesis, tunable drug release kinetics and long shelf-life as lyophilized/dried powders.

Drug molecules can be loaded onto these carriers through a diffusional (enhanced by the strong water binding properties) post-loading method (Figure 5), which effectively facilitates absorption of biomolecules by the carrier matrix, thereby preserving the bioactivity of the loaded biomolecules[4]. Using this method, the biomolecules are not harmed by harsh processing conditions or organic solvents, which is highly beneficial from an application perspective.

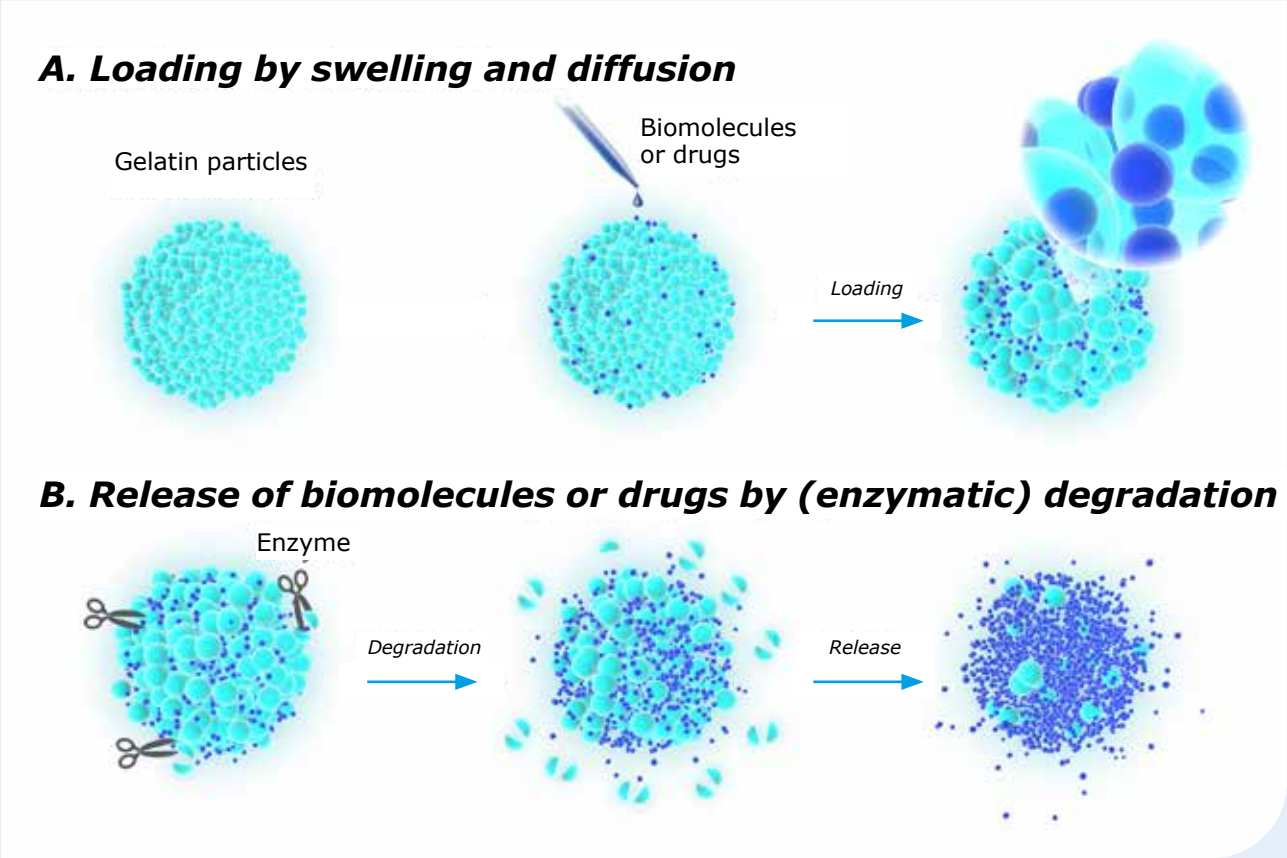

Figure 5. Loading of biomolecules onto gelatin particles through a diffusional post-loading method (A) and release of loaded biomolecules or drugs from gelatin carriers by enzymatic degradation (B).

The mechanism of controlled release of macromolecules from micro- and submicron particles involves loading bioactive molecules through polyion complex formation and release of macromolecules via the enzymatic degradation of gelatin matrices[3] (Figure 5). Many macromolecules (e.g. growth factors) are positively charged at physiological pH, since their IEPs are higher than a neutral pH value of 7[21]. For instance, positively charged growth factors can be electrostatically complexed with negatively charged Type B gelatin molecules. Once the charged macromolecules are complexed with an oppositely charged gelatin carrier, they cannot be released from the gelatin matrix unless gelatin degradation occurs[22]. Therefore, the release kinetics of macromolecules from gelatin micro- and submicron particles can be fine-tuned by adjusting their degradation rates, which can be achieved by controlling the cross-linking densities of the gelatin carriers during

synthesis. Compared to gelatin microparticles, gelatin submicron particles exhibit a much higher specific surface area. These characteristics facilitate more and stronger polyion complexation of biomolecules and drugs with oppositely charged gelatin submicron particles. The larger specific surface area of submicron particles enhances the (control over) the amount of drugs loaded as compared to microparticles.

Examples of drug delivery applications for gelatin based micro- and submicron particles

• Chemoembolization

Owing to their adjustable size and biodegradability, gelatin microparticles have been widely used as a temporary occlusive material. Specifically, gelatin microparticles can be used as a temporary embolic agent. By occluding the blood vessels at the tumor site using gelatin microparticles with specific dimensions, the supply of nutrients or oxygen is stopped, thereby impeding the further proliferation of tumor cells[23-24] (Figure 6). Additionally, the gelatin microparticles can be used as drug carriers to achieve local delivery of chemotherapeutics. Anticancer drugs can be loaded onto the gelatin microparticles to realize sustained release of chemotherapeutics with improved therapeutic efficacy and minimized systemic toxicity[25].

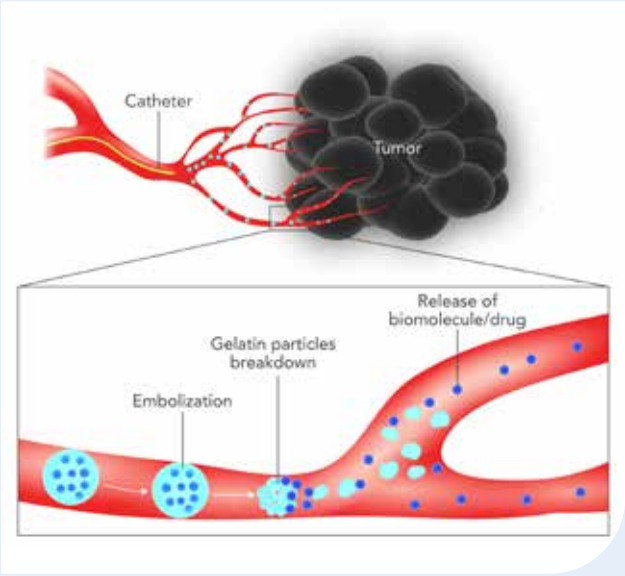

Figure 6: Illustration of the use of gelatin microparticles as smart embolic agents.

• Delivery of growth factors

Gelatin microparticles, especially the gelatin submicron particles, have been successfully used for the controlled release of growth factors like BMP-2[11]. These BMP-2 loaded gelatin particles can also be incorporated into hydrogels[26] or calcium phosphate cements[27] to prepare graft materials for bone regeneration purposes. Besides BMP-2, gelatin particles can also be used for sustained delivery of other growth factors like bFGF[28-29], TGF-β1[30-31] and VEGF[11].

• Delivery of nucleotides

Polyanionic macromolecules such as DNA or small interfering RNA (siRNA) can electrostatically bind to cationized gelatin microparticles to form electrostatic complexes, which allow for controlled release of these macromers upon enzymatic degradation of gelatin carriers[32-34].

• Delivery of antibacterial agents

Molecules such as antibiotics can also be loaded onto gelatin micro- and submicron particles provided that sufficiently strong electrostatic and/or hydrophobic interactions can be formed between these antibiotics and gelatin macromers[18, 35]. In that case, the release of antibiotics can be controlled by the degradation of gelatin particles. This system allows for local and sustained delivery of high amounts of antibiotics at infected sites. This strategy can minimize the systemic toxicity of antibiotics and reduce antibacterial resistance.

• Delivery of anti-osteoporosis drugs

The surface of gelatin submicron particles composes abundant free amine and carboxyl groups. These groups provide ample opportunities for grafting biomolecules onto the surface. When there is gelatin degradation, these grafted drug molecules can be released in a controlled manner to treat diseases such as osteoporosis. For example, the anti-osteoporosis drug alendronate has been conjugated onto the surface of gelatin submicron particles and controlled delivery of alendronate was shown to stimulate the regeneration of defects in osteoporotic bone *in vivo*[19].

• Examples of other applications for gelatin micro- and submicron particles

- Both gelatin micro- and submicron particles can absorb extensive extensive amounts of water (approximately 10 times their own volume). This property makes micro- and submicron particles highly useful for hemostatic applications.
- The porous structure of gelatin based submicron colloidal gel is an attractive property for cell culturing applications.

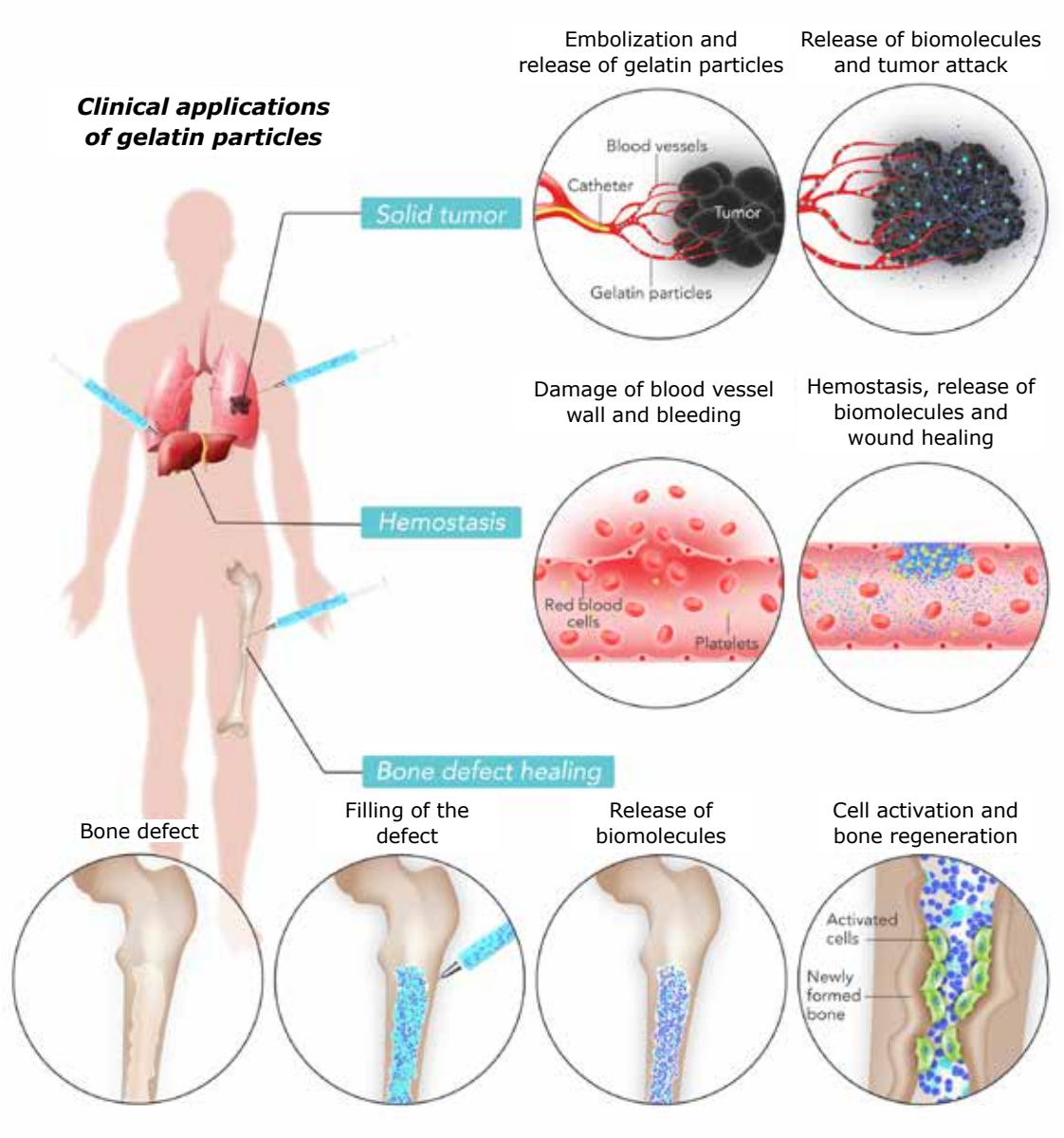

Figure 7. Examples of clinical applications of gelatin particles.

CONCLUSION AND FUTURE PERSPECTIVES

Gelatin micro- and submicron particles are highly promising biomaterials for drug delivery in regenerative medicine. These particles exhibit a strong capacity for delivering various large and small biomolecules at controlled release kinetics. Their easy processing, storage, transport and functionalization are strong assets for designing customized applications in biomedicine such as tissue regeneration, anticancer chemotherapy and pharmacy. However, these clinical applications can only be realized when endotoxin levels meet strict regulatory requirements. These levels can now be met with Rousselot's X-Pure® gelatins, which comply with the highest safety, quality and regulatory standards for drug delivery and regenerative medicine.

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