

MEDICAL APPLICATIONS OF CHITIN AND ITS DERIVATIVES

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1. Introduction

Chitin and its derivatives show a wide range of applications such as: in agriculture, pulp and paper-making, industrial sewages detoxification and purification, for animal health care, diet supplements and pharmacy as well as the first class of non-active medical devices. However, the potential of chitin and its derivatives use in medical sector is not bring into play.

Several factors negatively affect the range of chitin and its derivatives applications in medical devices design. However, the several research works are introduced to find new innovative medical devices based on chitin and its derivatives.

The presented work presents the new tendency of chitin and its derivatives applications for design of innovative medical devices.

2. Description of EU requirements for the application of chitin and its derivatives for medical devices design

There are three European Directives regulate the requirements for design, manufacture and putting into service as well as of medical devices:

- a) Active Implantable Medical Devices (AIMDD) - Directive 90/385/EEC - OJ L189/ 20.7.90,
- b) Medical Devices Directive (MDD) - Directive 93/42/EEC - OJ 169/ 12.7.93 (know as MEDDEV),
- c) In Vitro Diagnostic Directive (IVDD) - Directive 98/79/EC - OJ331/ 7.12.98.

The intended performance of a medical device shall be described and documented by addressing as follow:

- intended purpose(s),
- functional characteristics,
- intended conditions of use, with particular regard to safety.

Account should also be taken of:

- published Standards,
- published clinical and scientifically literature,
- validated test results.

The design attributes of medical devices to meet the intended performance shall take into account at least the following [1]:

- materials and their biocompatibility,
- physical, mechanical and chemical properties of materials, including endurance properties and aging,
- wear characteristic of materials and the effects of wear and wear products on the biomaterial and the body
- degradation characteristics of materials, and the effects of degradation, degradation products and leachable on the biomaterials and the body,
- extent and effect of leakage of substances,
- effect of manufacturing processes (including sterilization) on material characteristics and performance,
- possible effects on the implant and its function due to interactions between its constituent materials and between its constituent materials and other materials and substances,
- interconnections and their effects on the intended performance,
- interface(s) between the biomaterial and body tissue(s), particularly relative to fixation and connection, and surface conditions,
- shape and dimensions including their possible effects on tissues and body fluids,
- biocompatibility of the medical device in its usable state,
- physical and chemical effects of the body and external environment on the biomaterial,
- effects of radiation and electromagnetic fields on the medical device and consequential effects on the body,
- ability to biomaterial, to its remove and to replace,
- ability to visualize the position and orientation of the medical device, if implanted, by radiological procedures;
- microbiological and particulate contamination levels,
- suitability and effectiveness of packaging.

Medical device design attributes shall be documented. Where any of the above are not considered to be relevant, the reason shall be documented and justified.

Medical device's materials shall be selected with regard to the properties required for the intended purpose, taking also into account the effects of manufacture, handling, sterilization and storage. Possible reactions of implant materials with human tissues and body fluids,

other materials, other implants, substances and gases shall be considered. Possible effects of radiation and electro-magnetic fields on the material shall also be considered.

When a medicinal product is an integral part of a medical device, the medicinal product shall be assessed according to pharmaceutical principles. The performance of the medicinal product used in combination with the medical device shall not be affected by the implant and/or vice versa. When assessing the safety, quality and usefulness of the medicinal product incorporated as an integral part of an implant, appropriate methods, such as specified in European Directive 2001/83/EC should be employed.

Materials used for medical device and coatings, including biological materials, shall be acceptably compatible in their implantable state. The compatibility of possible wear and degradation products shall also be acceptable. The acceptability in the particular application shall be demonstrated either:

- by documented assessment in accordance with the principles of EN ISO 10993-1:2003 Standard, or
- by selection from the materials found suitable by proven clinical use in similar applications.

3. Critical aspects of chitin and its derivatives application for design of MEDDEV

The most crucial aspects affecting the safety and performance of medical device made of chitin and its derivatives are:

- purity (medical chitosan processes the chitin under clean-room conditions in a series of verifiable purification steps) and re-productivity of chitin sources,
- absence of European Standards describing minimal application requirements of chitin and its derivatives for design of medical devices,
- connection with European Standard EN 12442-1/2/3 Standard „Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices”,
- sterilization of medical devices containing chitin or its derivatives – critical process may affect the biocompatibility.

Purity aspects and the requirements for manufacture of chitin and its derivatives have been described in [16].

3.1. Medical devices utilizing animal tissues or derivatives of animal tissues

For medical devices that utilize animal tissues or derivatives of animal tissues, controls shall be applied in accordance with the requirements of EN 12442-1 (analysis and management of risk), EN 12442-2 (controls on sourcing, collection and handling) and EN 12442-3 (validation of the elimination and/or inactivation of viruses and transmissible agents) Standards. Most important factor is the safety of source used taking into account transfer of viruses and transmissible agents. Chitin and its derivatives are derived from the animals. According to rule 17 of MDD all medical devices containing chitin or its derivatives are incorporated in class III [8]. There is necessary during design of medical device containing chitin or/and its derivatives provide detailed information as follow:

- the nature of animal tissue or product derived from animal tissue used for manufacturing of the medical device (included in the device or used as an aid in the manufacturing stages),
- if applicable, documentation attesting that the animal-derived material has been previously assessed for viral safety by a Competent Authority, for the production of medicinal product or medical devices already CE marked,
- the description of the different stages of the production process (of the animal-derived material and of the medical devices) from the collection phase, and the different subcontractors involved in the process,
- the outline (flow chart) of the manufacturing process of the product derived from animal tissue, identifying the starting materials and all intermediates. The detailed description of each stage of manufacturing of the product derived from animal tissue – and of the purification process for products obtained by fermentation - including manufacturing conditions and information on reagents, solvents, catalysts, conditions of reaction where these are critical, and the following factors: duration, pH, temperature, molarity of reagents, and an approximate value of quantities mixed. The significant steps for elimination and/or viral inactivation shall be identified. The maximum size of the batch shall be stipulated,
- the output (yield) of the process shall be stipulated. Considering the maximum quantity of purified product used in the device, the equivalent starting quantity of animal tissue shall be indicated,
- description of the measures in place to ensure traceability for every production batch up to the starting material (animal tissue) - fishing area,
- the description of the measures adopted by the manufacturer in order to control source establishments and/or third party suppliers for the animal material used in the medical device,
- the characteristics of the animals as source of material:
 - a) species,
 - b) country of origin (birthplace and rearing) - fishing area,
- information concerning the starting tissue :
 - a) description of the method used for removal of tissues/organs and of precautions adopted to avoid cross-contamination during collection,
 - b) hygiene requirements adopted for collection, handling, storage, and transport: description of method, equipment, premises and providing of health certificates,
 - c) description of measures and records for traceability of the material,
- the method applied for viral removal/inactivation:
 - a) description of scientific data that support inactivation/removal factors applied during the manufacturing process with reference to literature search, reports and scientific opinions. It must be demonstrated that the specific materials of animal origin and the specific process referred to in the literature are comparable to those used for the medical device concerned,
 - b) report of the removal/inactivation validation study. The scientific basis of the study must be described. Parameters and limits that are critical to the effectiveness of the inactivation or elimination process must be identified in the final report.

3.2. Sterilization

Common methods of sterilization include exposition to dry heat, saturated steam at various temperature (at temperature of 126 °C or 121 °C), ethylene oxide (EO) or irradiation (γ – irradiation or accelerated electrons).

The effects of the sterilization method employed for medical devices shall not impair the intended function. Before any of these methods are endorsed for the sterilization of chitosan products, their effects on the properties and end performance of the polymer will have to be documented.

Sterilization processes shall be validated and routinely controlled. For terminally-sterilized medical devices the theoretical probability of there being a viable micro-organism present on or in the device shall be equal to or less than 1×10^{-6} .

If medical devices:

- are to be sterilized by ethylene oxide (EO), ISO 11135 Standards shall apply,
- are to be sterilized by irradiation, ISO 11137 Standards shall apply,
- are to be sterilized by steam, ISO 17665 Standards shall apply,
- are to be sterilized by any other terminal sterilization method, ISO 14937 Standards shall apply.

If medical devices containing materials of animal origin have to be sterilized by chemical liquid agents, ISO 14160 Standard shall apply, whereas if medical devices are to be produced by aseptic processing, ISO 13408-1 Standard shall apply.

EO sterilization generates by-products and crosslinking [15]. Ethylene oxide is a flammable gas that is irritating to body surfaces and highly reactive. It is mutagenic under many conditions, has fetotoxic and teratogenic properties, can adversely affect testicular function and can produce injury to many organ systems in the body. In cancer studies in animals, inhalation exposure produced several types of neoplastic changes including leukaemia, brain tumours and mammary tumours while ingestion or subcutaneous administration produced tumours only at the site of contact. One investigator has reported higher cancer and mortality rates in some subpopulations of exposed workers [3]. However, the results of several recent studies in workers have shown even weaker associations [2, 3]. Porous medical devices could contain ethylene oxide residue affecting cytotoxicity, irritation, mutagenicity and genotoxicity. The wet medical devices could contain ethylene glycol [10]. Ethylene chlorohydrin is a flammable liquid that is irritating to body surfaces, acutely toxic and readily absorbed through the skin in toxic amounts. It has weak mutagenic potential, has some potential to produce fetotoxic and teratogenic changes and can produce injury to several organ systems in the body including lungs, kidneys, central nervous system and cardiovascular system. It was negative in cancer bioassays in animals [10].

Exposure to dry heat resulted in lower aqueous solubility for chitosan and in extreme cases, insolubility in acidic aqueous media [11]. This was found to be related to interchain cross-link formation involving the $-NH_2$ groups in chitosan. A reduction of 60% in tensile strength and 53% reduction in the strain at break point were also experienced [12]. Saturated steam

was also found to significantly accelerate the rate and extent of thermal events in chitosan. Chitosan became water insoluble and lost 80% of its original tensile strength.

A γ -irradiation caused main chain scission events in chitosan [13]. Chitosan membranes irradiated with 2.5 Mrad in air experienced a 58% increase in tensile strength and a 22–33% decrease in the swelling index of the membrane. Applying anoxic conditions during irradiation significantly reduced the changes to membrane properties. On the basis of these findings, it may be concluded that γ irradiation at 2.5 Mrad under anoxic conditions provides the best means of sterilization for medical devices containing chitosan.

The long-term storage effects have also to be thoroughly investigated as it will have implications on the integrity of medical devices consisting in chitin or chitosan [14].

Therefore, there remains a lot of scope to explore in the area of sterilization and storage before the method of sterilization and storage conditions can be optimized.

4. Biocompatibility aspects

The range of biocompatibility studies taking into account type, time and the contact position of medical device are detailed described in EN ISO 10993-1:2003. There are relatively low numbers of validated studies published in peer review journals describing biocompatibility of chitin or its derivatives. The absence of above-mentioned studies yielded in absence of manufacturers' interest for applications of chitin or its derivatives in the design of medical devices. Several publications and research works using chitin or chitosan for design of medical devices are not validated and present some individual aspects of biocompatibility, usually implantation or cytotoxicity without acceptable control group or statistical sampling. Since chitin derivatives as well as product of their degradation can be made from the shells of shrimp, crab, and other shellfish, people with shellfish allergy or iodine hypersensitivity may have an allergic reaction to glucosamine products. A serious hypersensitivity reaction including throat swelling has been reported with glucosamine sulfate. A small pilot study of six patients with known systemic reaction to shellfish, showed no reaction when challenged with glucosamine.

The review of validated studies showing the biocompatibility (mostly toxicological aspect) of chitosan is presented in Table 1.

5. Commercial applications of chitin and its derivatives for manufacture of medical devices

There are a few application of chitin derivatives or degraded chitosan for the manufacture of medical devices, such as HemCon Bandage (HemCon Medical Technologies) bandage that significantly outperformed the standard of care (gauze) with significant improvements in survival, blood loss, and the number of applications of either pressure or dressings required to achieve haemostasis [19]. Hemcon Bandage is made of positively charged chitosan material bonding with red blood cells and forms a clot that stops hemorrhaging. It is biocompatible and forms an antibacterial barrier, reducing the potential for infection. It adheres aggressively to tissue surfaces when in contact with blood or moisture forming a strong, flexible barrier that seals and stabilizes the wound. Extremely robust, the bandage tolerates

Table 1. Toxicological, validated studies of chitosan application based on the FDA literature review [16].

Source	Description
Human Data	No epidemiological studies or case reports investigating the association of exposure to chitosan and cancer risk in humans were identified in the available literature
Animal Data	No 2-year carcinogenicity studies of chitosan were identified in the available literature. Two studies of the acute toxicity of chitosan were identified in the available literature. Hirano [4] reported that the oral LD ₅₀ was 16 g/kg in mice. The intraperitoneal LD ₅₀ of sodium chitosan sulfate in rats was reported to be 208 mg/kg [5]
Short-Term Tests:	No <i>in vitro</i> or <i>in vivo</i> studies evaluating chitosan for mutagenic effects were identified in the available literature. It is possible that such information exists in the Drug Master Files.
Metabolism:	Chitosan is a polymer not hydrolyzed by human digestive enzymes [6]. Its function as a dietary supplement is dependent on its lack of absorption in the human body.
Other Biological Effects:	
Antigenotoxicity/ Anticarcinogenicity	Chitosan reduced the genotoxicity of aqueous solutions of the hydrophobic mutagens, 4-nitroquinoline-N-oxide and dinitropyrene, as measured in a sister chromatid exchange assay. Under similar conditions, chitosan did not show antigenotoxic activity for mitomycin C, but it reduced the genotoxicity of adriamycin by 78 %. These effects were pH dependent for mitomycin C but not for adriamycin [7].

highly compressive forces while retaining adequate flexibility to conform to irregular wound surfaces.

Based on the strength of the innovative technology, the HemCon Bandage was ushered through the FDA in October 2002 and is the second fastest approval of a medical device granted by the FDA. The HemCon Bandage has now evolved into the standard treatment for severe hemorrhaging and is included in the recommended guidelines for all three casualty management phases of "Tactical Combat Casualty Care". FDA-cleared, HemCon Bandages are non heat-producing and easily removed with saline or water. Simply packaged and stable at room temperature.

Other haemostatic bandage, the Syvek Patch (Marine Polymer Technologies), has been introduced in the recent past for the control of bleeding at vascular access sites in interventional cardiology and radiology procedures [18]. This product consists of poly-N-acetyl glucosamine (pGlcNAc) isolated in a unique fiber crystalline structural form. Clo-Sur PAD (Scion Cardio-Vascular) and ChitoSeal (Abbott Vascular Devices), are also available as patch hemostats. These two products both use chitosan, another N-acetyl glucosamine containing glycosaminoglycan, as their active ingredient. Structural, chemical, and biological comparisons of Syvek pGlcNAc and chitosan reveal a number of important differences. Syvek pGlcNAc fibers have DD of 50%, high molecular weight pGlcNAc molecules in a crystalline, three-dimensional β -structure array, and are insoluble. Chitosan is a low molecular weight mixed amorphous cationic polymer with no regular structure as a solid, and is water-soluble taking on a random coil configuration when in solution. These structural dissimilarities result in differences in the haemostatic properties of the two materials. Syvek pGlcNAc is able to significantly reduce the *in vitro* fibrin clot formation time of platelet-rich

plasma samples and has the ability to cause aggregation of red blood cells in vitro. Syvek Patch is able to control the bleeding and cause haemostasis in a coagulopathic swine spleen-bleeding animal model 100% of the time, whereas Clo-Sur PAD was completely unsuccessful (0%) and ChitoSeal (25%) was worse than a gauze pad control (50%) in the same model. Syvek pGlcNAc fibers have structural and chemical properties that provide a unique basis for their ability to interact with blood components to cause haemostasis.

6. Conclusions

Table 2. shows the assumption of favorable or adverse aspects of chitin and chitosan that are able to effect the potential applications of discussed biopolymers in the design of medical device.

Table 2. Favorable and adverse effect of chitin or chitin derivatives use for designing medical device.

Discussed aspect	Adverse attribute	Favorable attribute
Dermal regeneration and accelerated wound healing behaviour		☺ design of wound dressings
Formation of hydrogels, pastes, hydrocolloids, sponges itself or with the combination of various polysaccharides or proteins (collagen, gelatin, keratin, etc.)		☺ design of wound dressings
Antimicrobial activity or good medium for bacteria growth		☺ for medical devices apply in case of high risk of infection
Biodegradation/resorption	☹ disintegration after use (long-term or permanent implants)	☺ disintegration with growing natural tissue (connective tissue, bone, etc.)
Anti-adhesion barrier		☺ implantation in regions giving high risk of adhesion, fistula formation, such as implantation of surgical meshes in intraperitoneal position
Blood anticoagulant or blood haemostatic agent	☹ sealing for vascular prostheses, vascular patches, endovascular stents	☺ haemostatic pads, haemostatic topical agents
Absorption of (Ca ²⁺) – induction of calcification		☺ bone cements accelerating osteoblast integration and bone formation
Sterilization	☹ lost of mechanical strength, crosslinking, lost dissolution, change in bioactivity and biocompatibility	☺ sterilizeable using most sterilization methods (irradiation, steam, EO)
Biocompatible in the most aspects		☺ possible to use in most applications (skin contact, implants including permanent implants).
Allergenicity and pirogenicity	☹ high risk of allergenic reaction and increase of body temperature after use	

Taking into account above effects it can be concluded that:

- chitin and its derivatives show wide range of potential application as a source for design of innovative medical devices,
- the several aspects, such as: sterilization, purity and re-productivity as well as economical aspect of chitin and chitosan use should be take into account during design new, innovative medical devices,
- the critical limitation of chitin and its derivatives for the design of medical devices is the absence of European Standards as well as global and the local requirements characterizing the properties of mentioned biopolymers applied for the medical application.

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