APPLICATION OF CHITOSAN IN MEDICAL DEVICES DESIGN

Marcin H. Struszczyk
1. Chitin and chitosan – main definitions;
2. EU general requirements for medical devices;
3. EU requirements for medical devices utilizing animal tissue derived substances;
4. Sterilization aspects;
5. Quality aspect;
6. Biocompatibility aspects;
7. Examples of commercial medical devices utilizing chitin or chitosan;
8. Conclusions.
Chitin is a copolymer: poly[β-(1-4)-2-acetamido-2-deoxy-D-glucopyranose] containing a low percentage of 2-amino-2-deoxy-β-glucopyranose residual units.

Chitin possesses a highly ordered structure with an excess of crystalline regions and appears in three polymorphic forms.
Chitosan is chemically defined as a copolymer of two residues:

- 2-acetamido-2-deoxy-β-D-glucopyranose and
- 2-amino-2-deoxy-β-glucopyranose.

The proportion of glucosamine is higher than N-acetylglucosamine, producing much better solubility in an aqueous solution of organic and numerous inorganic acids.

The difference between chitin and chitosan lies in the degree of deacetylation (DD), usually defined as the ratio of the number of glucosamine to the total amount of N-acetylglucosamine and glucosamine, being the most important parameter determined for chitosan and chitin.
**Occurrence of Chitin in Nature**

| **Articulated Brachiopoda** | • Occurrence: calcified cuticles, intersegments membranes, hardened cuticle;  
|                           | • Chitin fractions [%]: 20.0 - 85.0;  
|                           | • Crystal polymorphic form: α. |
| **Pogonophora** | • Occurrence: tubes;  
|                | • Chitin fractions [%]: 33.0;  
|                | • Crystal polymorphic form: β. |
| **Mollusca** | • Occurrence: shell plates, mantle bristles redula, shell, redula, jaws, stomacal plates, calcified shell, pen, jaws, redula, shells;  
| Polyplacophora, Gastropoda, Cephalopoda, Lamellibranchia | • Chitin fractions [%]: 0.1 - 36.8;  
|                | • Crystal polymorphic form: α, β, γ. |
| **Annelida** | • Occurrence: chaetae, jaws;  
| Polychaeta | • Chitin fractions [%]: 0.28 - 38.0;  
|                | • Crystal polymorphic form: β. |
| **Fungi** | • Occurrence: cell walls and structural membranes of mycelia stalks and spores;  
| Ascomyceta, Basidiomyceta, Phycomyceta, Imperfecti | • Chitin fractions [%]: traces - 45.0;  
|                | • Crystal polymorphic form: α. |
| **Algae** | • Occurrence: cell wall components;  
| Chlorophyceae | • Chitin fractions [%]: traces;  
|                | • Crystal polymorphic form: α. |
| **Protozoa** | • Occurrence: cyst wall, shell, perisarc, coenosteum, skeleton, podocyst;  
| Rhizopoda, Ciliata, Cindaria, Hydrozoa, Anthozoa, Scyphozoa | • Chitin fractions [%]: 3.2 - 30.3;  
|                | • Crystal polymorphic form: α. |
| **Brachiopoda** | • Occurrence: stalk cuticle, stalk cuticle, shell;  
| Articulata, Inarticulata | • Chitin fractions [%]: 3.8 - 29.0;  
|                | • Crystal polymorphic form: β, γ. |

Four European Directives regulate the requirements for marketing and putting into service medical devices:

Materials used for design of medical device and coatings, including biological materials, shall be acceptably compatible in their useable 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 10993-1:2009 Standard, or
- by selection from the materials found suitable by proven clinical use in similar applications.
The most crucial aspects affecting the safety and performance of medical device made of chitin and its derivatives are:

- **purity (medical grade chitosan processes the chitin under clean-room conditions in a series of verifiable purification steps);**
- **re-productivity of chitin sources;**
- **absence of European Standards regulating directly requirements for chitin and its derivatives in design of medical devices;**
- **compliance with the European Standards, e.g. EN ISO 22442-1/2/3 Standards;**
- **sterilization of medical devices containing chitin or its derivatives – critical process may affect their biocompatibility.**
Chitin and its derivatives are derived from the animals.

According of the 17th rule of MDD all medical devices containing chitin or its derivatives are incorporated in the III class.
For medical devices that utilize animal tissues or derivatives of animal tissues, controls shall be applied in accordance with the requirements of:

- **EN ISO 12442-1:2008 Standard** (analysis and management of risk),
- **EN ISO 22442-2:2008 Standard** (controls on sourcing, collection and handling) and
- **EN ISO 22442-3:2008 Standard** (validation of the elimination and/or inactivation of viruses and transmissible agents).
EU standardized methods of sterilization include:

- exposition to dry heat or saturated steam at various temperature (126 °C or 131 °C);
- ethylene oxide (EO) or
- irradiation ($\gamma$ – irradiation or accelerated electrons).
EO sterilization generates cross-linking, by-products and residues, such as:

- Ethylene oxide residue;
- Ethylene chlorhydrin residue;
- Ethylene glycol residue.

Above-mentioned residues are able to be absorbed into sterilized medical devices.
Ethylene oxide (EO):

- *is irritating to body surfaces and highly reactive*;
- *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.*
Porous medical devices could contain ethylene oxide residue affecting:

- cytotoxicity;
- irritation;
- mutagenicity and
- genotoxicity.
Ethylene chlorhydrin:

• *is irritating to body surfaces*;
• *is acutely toxic*;
• *is readily absorbed through the skin in toxic amounts*;
• *has weak mutagenic potential*;
• *has some potential to produce fetotoxic and teratogenic changes*;
• *can produce injury to several organ systems in the body including lungs, kidneys, central nervous system and cardiovascular system.*
Exposure to **dry heat** of chitosan (in solid state) resulted in:

- lower aqueous solubility,
- insolubility in acidic aqueous media

**Saturated steam** was also found to:

- increase in insolubility of chitosan,
- reduction in the tensile strength
IRRADIATION STERILIZATION

A $\gamma$ - irradiation caused in chitosan:

- *main chain scission events*;
- *increase in tensile strength*;
- *cross-linking*;
- *decrease in the swelling index*.

Applying anoxic conditions during irradiation significantly reduced the changes in chitosan properties.

On the basis of these findings, it may be concluded that $\gamma$ - irradiation under anoxic conditions provides the best means of sterilization for medical devices containing chitosan.
STERILIZATION – QUESTION

Chitosan

Sterilization
- EO sterilization or
- Irradiation or
- Steam sterilization

Co-products, sterilization residues, cross-linking, degradation ...
- Chitosan or ... ?
QUALITY CHARACTERIZATION AND IDENTIFICATION OF CHITOSAN PURITY FOR APPLICATION IN THE MEDICAL DEVICES

- Struszczyk M.H., Global requirements for medical applications of chitin and its derivatives, Polish Chitin Society, Monograph XI, 2006, 95-102
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 the 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 some implantation or cytotoxicity without acceptable control group or statistical sampling.
WOUND DRESSINGS

PRIMARY WOUND DRESSING
- NON-WOVENS
- HYDROGELS
- SEMIPERMEABLE FILMS
- TOPICAL GELS
- SPONGES
- DRESSING CONTAINING BIOACTIVE AGENTS
- NON-WOVENS
- GAUZE
- SEMIPERMEABLE FILMS
- SPONGES
- POWDERS
- BANDAGES
- MULTILAYERED STRUCTURES
- GAUZE

SECONDARY WOUND DRESSING
- NON-WOVENS
- GAUZE
- SEMIPERMEABLE FILMS
- SPONGES
- POWDERS
- BANDAGES
- MULTILAYERED STRUCTURES
- GAUZE

HAEMOSTATIC TOPICAL AGENTS
- NON-WOVENS
- GAUZE
- SEMIPERMEABLE FILMS
- SPONGES
- POWDERS
- BANDAGES
- MULTILAYERED STRUCTURES
- GAUZE

WOUND DRESSING UTILIZING CHITIN OR ITS DERIVATIVES
### COMMERCIAL WOUND DRESSING FOR DERMIS REGENERATION UTILIZING CHITIN OR ITS DERIVATIVES

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choriochit®</td>
<td>Biological dressing manufactured by the lyophilization of human placenta blended with microcrystalline chitosan. ChorioChit® was characterized by good handling, good wound isolation and the ability to the reduction of the pathogens growth. The wound dressing was not CE-certificated and did not agree by FDA. Available in Poland till 2004.</td>
</tr>
<tr>
<td>Beschitin®</td>
<td>The wound dressing in form of non-woven manufactured using chitin. It accelerates granulation phase and affects no scar formation. The wound dressing was not CE-certificated and did not agree by FDA. Available only in Japan.</td>
</tr>
<tr>
<td>ChitiPack® S</td>
<td>The wound dressing made by the freeze-drying of squid pen chitin dispersion. The usable form of wound dressing is sponge-like product. The wound dressing favors the acceleration of wound healing and no scar formation. The wound dressing was not CE-certificated and did not agree by FDA. Available only in Japan.</td>
</tr>
<tr>
<td>ChitiPack® P</td>
<td>The wound dressing is designed by the drifting the chitin suspension onto poly-[ethylene terephthalate] non-woven fabric. The recommended range of application is: the large skin defects, especially with difficulty to suture. The wound dressing was not CE-certificate and did not agree by FDA. Available only in Japan.</td>
</tr>
<tr>
<td>Chitopack® C</td>
<td>The fibrous wound dressing made by spinning of chitosan acetate solution coagulated in bath containing mixture of ethylene glycol (humectant), cold water and sodium or potassium hydroxide. The application range considers the regeneration and reconstruction of body tissue, subcutaneous tissue as well as skin. The wound dressing was not CE-certificate and did not agree by FDA. Available only in Japan.</td>
</tr>
<tr>
<td>Chitodine®</td>
<td>Powdered chitosan containing elementary iodine. Applicable for disinfection and cleaning of wounded skin and as a primary wound dressing. Wound dressing has got CE-mark.</td>
</tr>
<tr>
<td>Vulnsorb®</td>
<td>The composition of collagen and chitosan in form of freeze-dried sponges. Wound dressing has got CE-mark since 1996.</td>
</tr>
<tr>
<td>Trade name/Company</td>
<td>Description</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HemCon® Bandage ChitoFleX® Hemostatic Dressing HemCon Medical Technologies</td>
<td>Hemcon products are made of positively charged chitosan (chitosan acetate) by lyophilization process. Positively charged chitosan salt has strong affinity to bond with red blood cells, activates the platelets and forms a clot that stops massive blood bleeding. HemCon® Bandage was approved by FDA in 2002, whereas ChitoFleX® Hemostatic Dressing in 2007.</td>
</tr>
<tr>
<td>Syvatek® Patch Marine Polymer Technologies</td>
<td>Syvatek® Patch has been implemented for the control of bleeding at vascular access sites in interventional cardiology and radiology procedures. It consists of poly-N-acetylglucosamine (pGlcNAc) isolated in a unique fiber crystalline structural form. It is able to significantly reduce the fibrin clot formation time and has the ability to cause aggregation of red blood cells form. Approved by FDA and CE-certificated.</td>
</tr>
<tr>
<td>Clo-Sur® PAD Scion Cardio-Vascular</td>
<td>Clo-Sur® Pad is made as a non-woven sealed by soluble form of chitosan. Approved by FDA and CE-certificated.</td>
</tr>
<tr>
<td>ChitoSeal® Abbott Vascular Devices</td>
<td>ChitoSeal® is made on soluble chitosan salt. It is intended for external temporary use to control moderate to severe bleeding. Approved by FDA and CE-certificated.</td>
</tr>
<tr>
<td>Traumastat® Ore-Medix</td>
<td>Traumastat® is made on the non-woven substrate comprised of porous polyethylene fibers highly filled with precipitated silica. This substrate is coated with chitosan (ChitoClear™). It is intended for external temporary use to control moderate to severe bleeding. Approved by FDA and CE-certificated.</td>
</tr>
<tr>
<td>ExcelArrest® Hemostasis LLC Co.</td>
<td>ExcelArrest® is comprised of modified chitin particles and polysaccharide binders. Hemostat is manufactured in form of foam by lypholization process from the chitin and polysaccharides suspension. Approved by FDA in 2007.</td>
</tr>
<tr>
<td>Tromboguard® TRICOMED SA</td>
<td>Multifunctional Tromboguard® shows multifunctional behavior: local hemostasis, antibacterial and acceleration of wound healing. During CE-certification.</td>
</tr>
</tbody>
</table>
CHARACTERIZATION OF MULTIFUNCTIONAL TROMBOGUARD® WOUND DRESSINGS – ASSUMPTION DATE

1. DIRECT PRESSING IN TRAUMA – ACTIVATION OF EGZOGENIC BLOOD CLOTTING PATHWAY

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2. RELATIVELY HIGH ABSORPTION OF HIGH VOLUME OF NON-MORPHOTIC BLOOD ELEMENTS RESULTS IN CONCENTRATION NATURAL CLOTTING AGENTS IN PLACE OF TRAUMA.

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3. REALIZATION OF AGENTS OF ENDOGENIC BLOOD CLOTTING PATHWAY:
- CHITOSAN ACTIVATES THE PLATLETS AGREGATION (DUE TO THE POSITIVE CHARGE ONTO POLYMER CHAIN);
- CALCIUM - PLASMATIC CLOTTING AGENT.

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- CALCIUM - PLASMATIC CLOTTING AGENT.

OUTER LAYER MADE OF POLYURETHANE FILM SELECTIVELY PASSING GASES AND PROTECTING AGAINST ETERNAL FACTORS (MEDICAL QUALITY).

OUTER LAYER MADE OF POLYURETHANE FILM SELECTIVELY PASSING GASES AND PROTECTING AGAINST ETERNAL FACTORS (MEDICAL QUALITY).

MIDDLE LAYER MADE OF HYDROPHILIC POLYURETHANE FOAM WITH THE INNOVATIVE STRUCTURE „PORE-IN-PORE” (MEDICAL QUALITY).

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LAYER OF FACTOR ACTIVING OF EGZOGENIC PATHWAY OF BLOOD CLOTTING MADE OF A MUTURE OF CHITOSAN, Na/Ca ALGINATE AND ANTIBACTERIAL AGENT.

LAYER OF FACTOR ACTIVING OF EGZOGENIC PATHWAY OF BLOOD CLOTTING MADE OF A MUTURE OF CHITOSAN, Na/Ca ALGINATE AND ANTIBACTERIAL AGENT.
## Preclinical Studies – Accelerated Ageing

**acc. ASTM 1998F:2002 Standard**

<table>
<thead>
<tr>
<th>Changes in Mechanical as well as Physical Parameters [%]</th>
<th>Extensibility at Longitudinal Direction [N/cm]</th>
<th>Extensibility at Vertical Direction [N/cm]</th>
<th>Permanent Set at Longitudinal Direction [%]</th>
<th>Permanent Set at Vertical Direction [%]</th>
<th>Water-Proofness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Parameter [%] Corresponding to 1 Year</td>
<td>-6%</td>
<td>-4%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>Change in Parameter [%] Corresponding to 2 Year</td>
<td>-4%</td>
<td>0%</td>
<td>17%</td>
<td>29%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Changes in Mechanical as well as Physical Parameters [%]**

- Extensibility at Longitudinal Direction: -6% (1 Year), -4% (2 Years)
- Extensibility at Vertical Direction: -4% (1 Year), 0% (2 Years)
- Permanent Set at Longitudinal Direction: 0% (1 Year), 7% (2 Years)
- Permanent Set at Vertical Direction: 17% (1 Year), 29% (2 Years)
- Water-Proofness: 0% (1 Year), 0% (2 Years)

**Antiseptic activity**
- **S. aureus**: 4.8%
- **E. coli**: 3.9%

**Bacteriostatic activity**
- **S. aureus**: 6.3%
- **E. coli**: 2.2%

**Changes in antibacterial behaviour [%]**
- **Bacteriostatic activity for Escherichia coli**: 0.0%
- **Antiseptic activity for Escherichia coli**: -0.8%
- **Bacteriostatic activity for Staphylococcus aureus**: -0.2%
- **Antiseptic activity for Staphylococcus aureus**: -1.1%

*CORRESPONDING TO 1 YEAR* 0.0% -0.8% -0.2% -1.1%
### Preclinical Studies
**Biocompatibility ACC. EN ISO 10993-1:2003**

<table>
<thead>
<tr>
<th>Biocompatibility Test</th>
<th>Standard</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity (in direct test)</td>
<td>PN-EN ISO 10993-5:2001</td>
<td>Absence</td>
</tr>
<tr>
<td>Irritation</td>
<td>PN-EN ISO 10993-10:2003</td>
<td>Absence</td>
</tr>
<tr>
<td>Subacuteous Reactivity</td>
<td>PN-EN ISO 10993-10:2002</td>
<td>Light</td>
</tr>
<tr>
<td>Delayed-Type Hypersensitivity (Allergenicity)</td>
<td>PN-EN ISO 10993-10:2003</td>
<td>Absence</td>
</tr>
<tr>
<td>Absorption of Blood Plasma (in vivo and in vitro tests)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound Healing in vivo</td>
<td>Aspects of PN-EN ISO 10993-6:2007</td>
<td>Reduction in time of wound healing</td>
</tr>
<tr>
<td>Amount of Bacterial Endotoxine</td>
<td>Polish Pharmacopoeia ed. VIII</td>
<td>≤12.5 EU</td>
</tr>
<tr>
<td>Pirogenicity</td>
<td>European Pharmacopoeia</td>
<td>Absence</td>
</tr>
</tbody>
</table>

- Zakład Chirurgii Eksperymentalnej i Badania Biomateriałów, AM, Wrocław;
- Instytut Medycyny Pracy, Łódź;
- TZMO, Toruń.
Aim of clinical study

Primary: estimation of the safety and performance: effectiveness of hemostatic and antibacterial behavior of multifunctional wound dressing for short time after use (5 days) and for long-term (estimation of place of application after 1 and 3 months).

Secondary: comparison of action effectiveness (TROMBOGUARD® vs. control wound dressings consists in Surgicel® supplemented with Medisorb® Silver Pad).

Clinical study was performed in Military Institute of Medicine (Warszawa) under supervising płk dr n. med. Wojciech WITKOWSKI

3 min. after application
T - TROMBOGUARD®
C – control wound dressing

15 min. after application
T - TROMBOGUARD®
C – control wound dressing

24 h after application
T - TROMBOGUARD®
C – control wound dressing

5 days after application
T - TROMBOGUARD®
C – control wound dressing
PRECLINICAL STUDIES OF TROMBOGUARD® CONFIRMED ITS:

• HIGH CHEMICAL AND MICROBIOLOGICAL PURITY;
• ABSENCE OF CYTOTOXICITY;
• ABSENCE OF THE IRRITATION AND ALLERGENICITY;
• REDUCTION IN TIME OF WOUND HEALING;
• HEMOSTATIC AND ANTIBACTERIAL MODE OF ACTION.

CLINICAL STUDIES OF TROMBOGUARD® CONFIRMED ITS:

• HIGH HEMOSTATIC EFFECTIVENESS: repeatable (100% patients), quick hemostasis (till 3 min.);
• HIGH ANTIBACTERIAL EFFECTIVENESS: protection of wounds against infection till 5 days;
• CLINICAL UNIVERSALITY: mode of action is independent on hematopexis and synergistic with blood clotting pathway;
• SAFETY: absence of allergenicity, adverse affects connecting to hematology, biochemistry, urine analysis, microbiology).

M. KUCHARSKA, M. H. STRUSZCZYK, A. NIEKRASZEWICZ, D. CIECHAŃSKA, E. WITCZAK, S. TARKOWSKA, K. FORTUNIAK, A. GULBAS-DIAZ, A. ROGACZEWSKA, I. PŁOSZAJ,
HAEMOSTATIC DRESSING AND METHOD OF MANUFACTURE OF HAEMOSTATIC DRESSING,
PATENT APPL. NO. P 390253, 2010
Hernia mesh made of polypropylene and chitosan yarns

Polypropylene hernia mesh coated by chitosan

Mechanical coating by chitosan film to cover both side of non-resorbable polypropylene knit

Non-resorbable knit made of polypropylene monofilament:
- high pore surface 3.3 – 7.6 mm²
- low surface weight << 80 g/m²

The crucial limitations in the use of chitin and its derivatives for design of medical devices are:

- collection of the appropriate quality raw materials;
- difficulty to receive reproducible product batches with various sources of raw materials or various collection times;
- cost of the manufacture is still too high however the efficiency should be improved in future;
- absence of the knowledge on the exact physiological mechanism of chitosan sources requiring for advanced applications in medicine;
- absence of the standardization for chitinous raw materials in aspects of their use in medical applications;
The crucial limitations in the use of chitin and its derivatives for design of medical devices are:

- absence of validated process of biopolymers manufacture;
- unavailability of a good quality assessment system for chitinous derivatives manufactures (such as: ISO 9001) or manufactures implemented the designed medical devices (such as: ISO 13485 Standards);
- absence of standardization of product quality and product assay methods for chitin and its derivatives in scope of medical application;
- high cost of biocompatibility studies as well as clinical assessment (including clinical studies) with high risk of defeat.
THANK YOU FOR ATTENTION