TROMBOGUARD® - FIRST AID WOUND DRESSING

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Abstract
Recently, the market of medical devices shows serious need for wound dressings for first aid and temporary traumatic wounds. Such a wound dressing should show primarily the hemostatic effect and antibacterial activity allowing protection against infection. Tromboguard® topical wound dressing is a three-layer dressing with active layer. It contains chitosan, sodium alginate/calcium alginate with addition of silver salt. The wound dressing provides protective performance as well as is characterized by the ability to immediately stop bleeding and antibacterial activity allowing protection against infection and occurrence of secondary infection without the need for or with limited participation of antibiotics. Tromboguard® topical wound dressing is a firs aid dressing designed for the uniform services (military and homeland) and rescue.

Key words: *wound dressings, microcrystalline chitosan sponges, preclinical studies, medical devices.*

1. Introduction

Market of medical devices recently shows serious damage for innovative wound dressings for first aid and temporary traumatic wounds treatment. Such a wound dressing should show primarily the hemostatic effect and antibacterial activity allowing protection against primary and secondary infection. Especially it is more important for the first-help treatment of dirty wounds or traumas.

Tromboguard® topical wound dressing is a three-layer dressing with active layer directly contacted with the surface of the wound. The active layer contains chitosan – a natural polysaccharide, sodium alginate/calcium alginate with addition of silver salt to enhance the antimicrobial effect. The dressing provides protective performance as well as is characterized by the ability to immediately stop bleeding and antibacterial activity allowing protection against infection and occurrence of secondary infection without the need for or with limited participation of antibiotics.

Tromboguard® topical wound dressing is a firs aid dressing designed for the uniform services (military and homeland) and rescue.

The aim of this study was to confirm the safety and performance (in scope of multifunctionality) of Tromboguard® wound dressing in the wide range of preclinical and clinical studies.

2. Materials and methods

2.1. Materials

Tromboguard® wound dressing consist in three layers (***Figure 1***):

- external – made of semipermeable, microporous film selectively passing gasses and protecting against external factors;
- middle – made of hydrophilic polyurethane foam with innovative structure "pore-in-pore";
- directly contact with wound layer – made of the mixture of chitosan, sodium/calcium alginate and silver salt.

2.2. Methods

2.2.1. *Physical and mechanical properties*

Physical and mechanical properties of Tromboguard® wound dressing were assessed according to PN-EN 13726 Standards in range described in ***Table 1***.

The range of studies was selected based aspects of the safety and performance aspects of the risk analysis according to PN-EN ISO 14971:2009 Standard [5].

2.2.2. Antibacterial activity

Antibacterial activity of Tromboguard® wound dressing in vitro was performed according to JIS L 1902:2002 Standard [6].

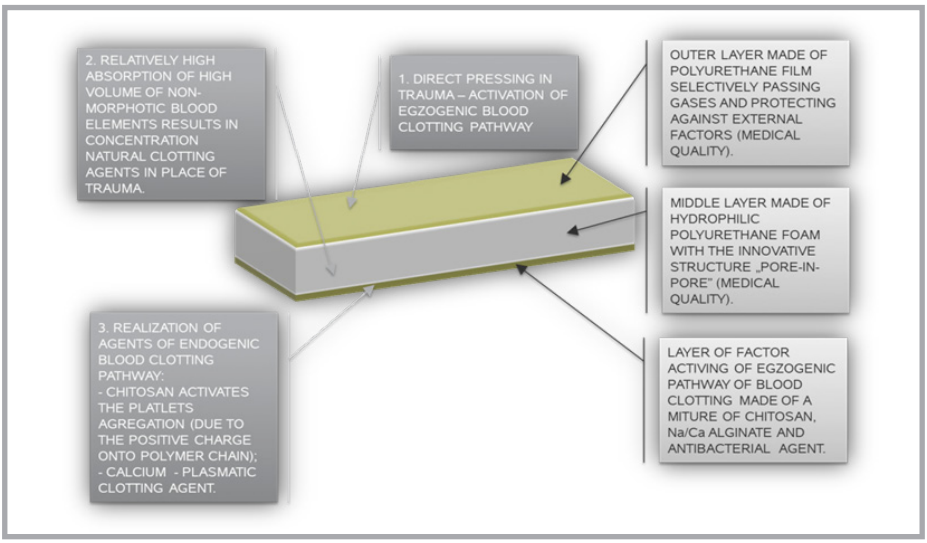


Figure 1. *Schema of Tromboguard® wound dressing design and its mode of action.*

Table 1. *Range of physical and mechanical study [1 - 4].*

TITLE OF STANDARD	STANDARD	DETERMINED PARAMETERS
„Test methods for primary wound dressings - Part 1: Aspects of absorbency"	PN-EN 13726-1:2005 [1]	■ Free swell absorptive capacity; ■ Fluid handling capacity;
„Test methods for primary wound dressings - Part 2: Moisture vapour transmission rate of permeable film dressings"	PN-EN 13726-2:2005 [2]	Moisture vapour transmission rate (MVTR) of a wound dressing when in contact with: ■ Water vapour; ■ Liquid.
„Non-active medical devices - Test methods for primary wound dressings - Part 3: Waterproofness"	PN-EN 13726-3:2005 [3]	Waterproofness.
„Non-active medical devices - Test methods for primary wound dressings - Part 4: Conformability"	PN-EN 13726-4:2005 [4]	■ Extensibility; ■ Permanent set.

2.2.3. Accelerated ageing

Accelerated ageing of Tromboguard® wound dressing was performed according requirements of ASTM 1998F:2002 Standard [7].

2.3.4. Biocompatibility studies

The biocompatibility studies were performed on base of requirements of PN-EN ISO 10993-1:2009 Standard [8] for medical devices contacted with the damaged skin for not longer than 24 h. The studies were performed:

- at Department of Experimental Surgery and Biomaterials Research, Wroclaw Medical University;

- at Nofer Institute of Occupational Medicine, Lodz;
- at TZMO, Torun.

The range of the biocompatibility studies with the accompanying harmonized standards are shown in ***Table 2***.

2.3.5. Clinical Studies

Clinical study was performed in Military Institute of Medicine (Warszawa) under supervising Wojciech Witkowski according to PN-EN ISO 14155-1/2:2003 Standard.

3. Results and discussion

3.1. Physical and mechanical properties

3.1.1. Aspects of Absorbency

Tromboguard® wound dressing was tested according to [1] Standard in range of:

- free swell absorptive capacity;
- fluid handling capacity.

Figure 2 shows the results of free swell absorptive capacity and fluid handling capacity for Tromboguard® wound dressing.

Tromboguard® wound dressing, due to the presence of innovative foam structure ("pore-in-pore") indicated significantly high and invariable in time fluid handling capacity determined after 24 h or 48 h. The fluid handling capacity after 48 h showed the increase by approx. 8%.

3.1.2. Aspects of Moisture Vapour Transmission (MVTR)

Figure 3 shows the moisture vapour transmission of Tromboguard® wound dressing when contact with water liquid or water vapour.

Table 2. *Range of biocompatibility studies [9 – 12].*

Biocompatibility test	Harmonized standard
Cytotxicity (in direct test)	PN-EN ISO 10993-5:2001
Irritation	PN-EN ISO 10993-10:2003
Subacutous reactivity	PN-EN ISO 10993-10:2002
Delayed-type hypersensitivity (allergenicity)	PN-EN ISO 10993-10:2003
Haemoactivity Absorption of blood plasma	PN-EN ISO 10993-4:2002 in vivo and in vitro tests
Wound healing in vivo	Some aspects of PN-EN ISO 10993-6:2007
Bacterial endotoxin	Polish Pharmacopoeia ed. VIII
Pirogenicity	European Pharmacopoeia

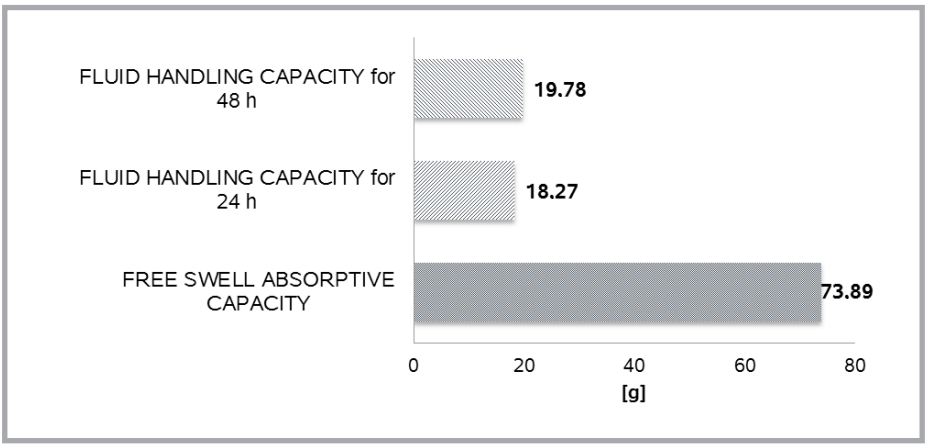


Figure 2. *Free swell absorptive capacity and fluid handling capacity for Tromboguard® wound dressing.*

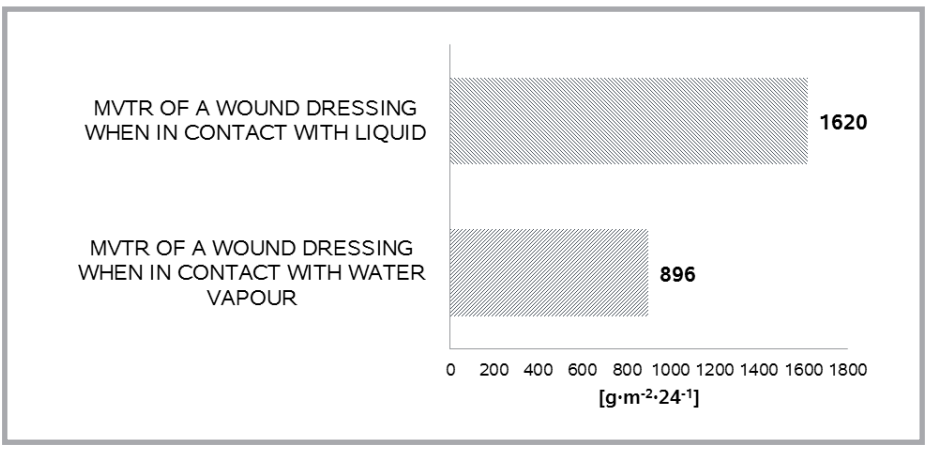


Figure 3. *MVTR (contacting with water liquid or water vapour) of Tromboguard® wound dressing.*

Tromboguard® wound dressing showed higher MVTR (by only 2 times) when was contacted with water liquid. The application of semipermeable film as external surface of wound dressing yielded in limited evaporation of liquid. MVTR parameters determined both when contacted directly with water or water vapour have comparable value for competitive wound dressing for wet wound treatment applied.

3.1.3. Aspect of waterproofness

Tromboguard® wound dressing are durable for the application of hydrostatic head of 500 mm of water for 300 s to the circular area. The wound dressing met the waterproofness requirements of [3].

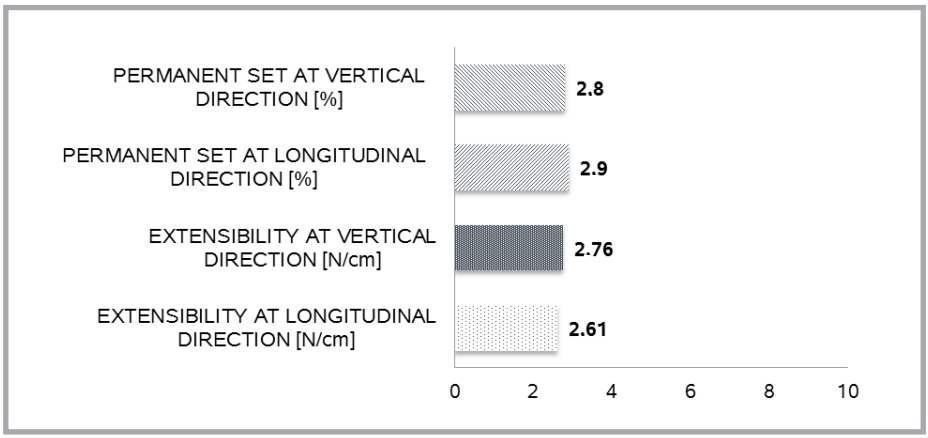


Figure 4. *Permanent set and extensibility both at vertical and longitudinal direction of Tromboguard® wound dressing.*

3.1.4. Aspects of conformability

The conformability test of wound dressing showed full compliance of Tromboguard® with requirements of [4] Standard in range of permanent set and extensibility both at vertical and longitudinal direction. ***Figure 4*** presents the results of studies.

3.2. Antibacterial activity assay

The results of antibacterial activity *in vitro* of Tromboguard® wound dressing against *Staphylococcus aureus* or *Escherichia coli* in range of antiseptic and bacteriostatic activity is shown on ***Figure 5***.

Tromboguard® wound dressing indicated the both high antibacterial behavior and high bacteriostatic activity *in vitro* against *Staphylococcus aureus* or *Escherichia coli* which promotes the medical devices to be effective to protect the wound and trauma against primary and secondary infection. Dual and synergistic action of chitosan (as a natural antibacterial agent) and silver salt yielded in stable and high antibacterial effect *in vitro*.

3.3. Accelerated ageing

Accelerated ageing of Tromboguard® wound dressing was performed according requirements of [7]. The presumed time of use of Tromboguard® wound dressing was 1 year. It is very important to know the changes in the crucial parameters of wound dressing during the time, due to the storing of typical wound dressing before use.

Figure 6 shows the changes in mechanical as well as physical parameters of Tromboguard® wound dressing.

There are not critical changes in parameters of Tromboguard® wound dressing after accelerated ageing both corresponding to ageing at real conditions for 1 year. The highest

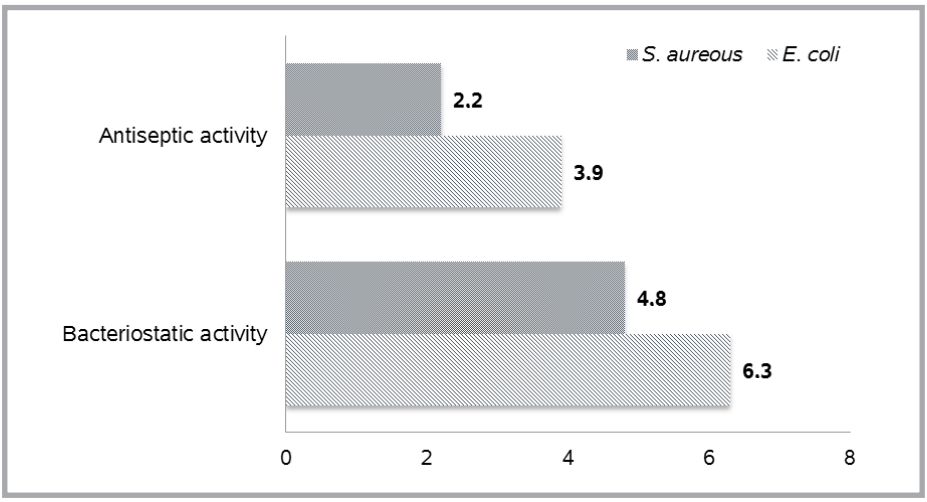
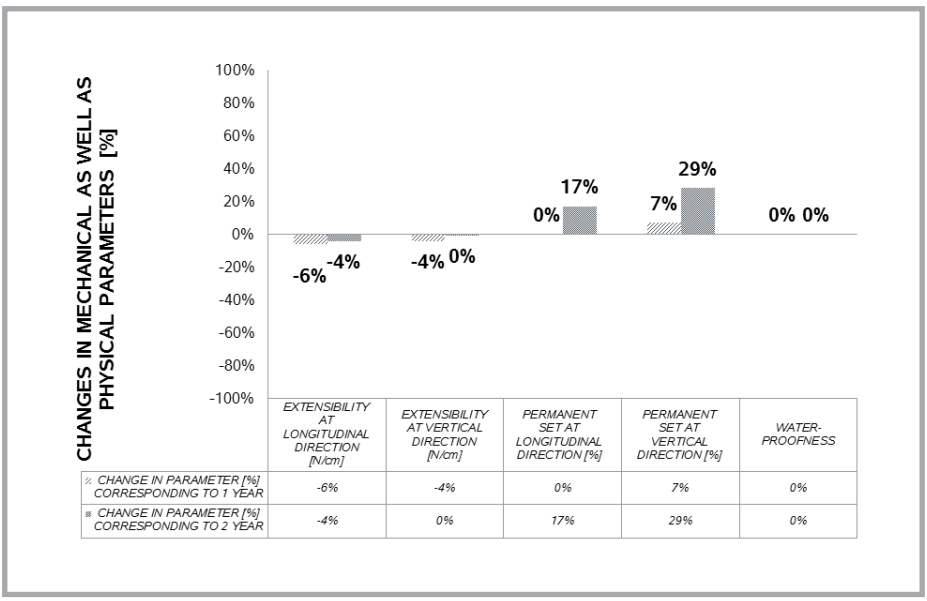


Figure 5. *Antibacterial behavior of Tromboguard® wound dressing against Staphylococcus aureus or Escherichia coli.*



	EXTENSIBILITY AT LONGITUDINAL DIRECTION [N/cm]	EXTENSIBILITY AT VERTICAL DIRECTION [N/cm]	PERMANENT SET AT LONGITUDINAL DIRECTION [%]	PERMANENT SET AT VERTICAL DIRECTION [%]	WATER-PROOFNESS
½ CHANGE IN PARAMETER [%] CORRESPONDING TO 1 YEAR	-6%	-4%	0%	7%	0%
■ CHANGE IN PARAMETER [%] CORRESPONDING TO 2 YEAR	-4%	0%	17%	29%	0%

Figure 6. *The changes in mechanical and physical parameters of Tromboguard® wound dressing after accelerated ageing corresponding to 1 year or 2 years of storing at real conditions.*

changes in permanent set was observed, however the resulted values are still below permissible limits. Also prolongation of accelerated study resulted in changes that may affects the performance of the wound dressings in range of permanent set parameter.

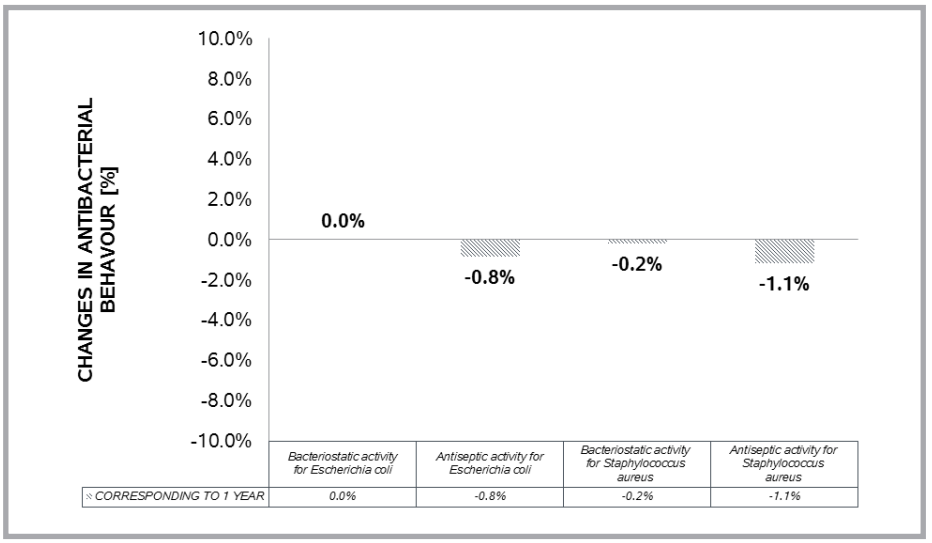


Figure 7. *The changes in antibacterial behavior of Tromboguard® wound dressing after accelerated ageing corresponding to 1 year or 2 years of storing at real conditions.*

Figure 7 presents the changes in antibacterial behavior *in vitro* of Tromboguard® wound dressing after accelerated ageing corresponding to 1 year of storing at real conditions.

The accelerated aging study show insignificant reduction in antiseptic activity of Tromboguard® wound dressing (for *Staphylococcus aureus* – 11,4% and *Escherichia coli* – 8,3%), whereas no changes in antiseptic activity was found after accelerated aging corresponding to 1 year of storing at real conditions.

3.4. Biocompatibility Studies

The results of biocompatibility studies of Tromboguard® wound dressing are presented in ***Table 3*****.**

3.5. Clinical Studies

The clinical study of Tromboguard® wound dressing has been two aims:

- Primary: estimation of the safety and performance, effectiveness of hemostatic and antibacterial behavior of Tromboguard® multifunctional wound dressing for short time after clinical use (5 days) and in long-term period (estimation of place of application after 1 and 3 months);
- Secondary: comparison of action effectiveness (Tromboguard® vs. control wound dressings consists in Surgicel® supported by Medisorb® Silver Pad).

The mode of clinical study was consisted in:

- Comparison of direct hemostatic effect;
- Comparison of hemostatic effect stability after 15 min., 24 h, 48 h and 5 days;

Table 3. *Results of biocompatibility studies of Tromboguard® wound dressing.*

BIOCOMPATIBILITY TEST	HARMONIZED STANDARD	RESULTS
Cytotxicity (in direct test)	PN-EN ISO 10993-5:2001	Absence
Irritation	PN-EN ISO 10993-10:2003	Absence
Subacutous reactivity	PN-EN ISO 10993-10:2002	Light
Delayed-type hypersensitivity (allergenicity)	PN-EN ISO 10993-10:2003	Absence
Haemoactivity Absorption of blood plasma	PN-EN ISO 10993-4:2002 *in vivo* and *in vitro* tests	■ Confirmed clotting activation, ■ Confirmed high absorption of blood plasma.
Wound healing *in vivo*	Some aspects of PN-EN ISO 10993-6:2007	Reduction in time of wound healing
Bacterial endotoxin	Polish Pharmacopoeia ed. VIII	≤ 12.5 EU
Pirogenicity	European Pharmacopoeia	Absence

- Comparison of comfort of use, easiness of removal, clinical advantages;
- Comparison of antibacterial effectiveness base on the inoculation form wounds;

The clinical study endpoints for Tromboguard® were defined as fallow:

- Significant reduction in bleeding time/control of bleeding from wound;
- Maintenance of haemostatic effect during 24 h;
- Prevention of wound infection for 24 h.

The clinical studies of Tromboguard® wound dressing indicates its quick and repeatable hemostatic effectiveness, high antibacterial effectiveness, clinical universality and safety.

4. Conclusions

Preclinical studies of Tromboguard® wound dressing confirmed its:

- high chemical and microbiological purity;
- absence of cytotoxicity;
- absence of the irritation and allergenicity;
- reduction in time of wound healing;
- haemostatic and antibacterial mode of action.

Clinical studies of Tromboguard® wound dressing confirmed its:

- high hemostatic effectiveness in scope of the clinical repeatability (100% patients) and quick hemostasis (till 3 min.);
- high antibacterial effectiveness. Tromboguard® wound dressing protects wounds against infection till 5 days;
- clinical universality: mode of action of Tromboguard® wound dressing is independent on hematopexis and synergistic with blood clotting pathway;
- safety: Tromboguard® wound dressing indicates absence of allergenicity, adverse effects connecting with the hematology, biochemistry, urine analysis, microbiology.

Acknowledgments

The composition of multifunctional wound dressing as well as the method of the manufacture have been patented in 2010 (patent application No. P390253). The patent application has been partially (ITB "MORATEX") supported by European Regional Development Found in range of Innovative Economy – National Cohesion Strategy 2007 – 2013, agreement No. UDA-POIG.01.03.02-10-015/08-00.

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4. *PN-EN 13726-4:2005 „Non-active medical devices - Test methods for primary wound dressings - Part 4: Conformability".*
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11. *PN-EN ISO 10993-4:2002 "Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood".*
12. *PN-EN ISO 10993-6:2007 "Biological evaluation of medical devices - Part 6: Tests for local effects after implantation".*