Tissue engineering, a driver for change in Medical Devices biocompatibility

Christian Pellevoisin, PhD, ERT

ADEBIOTECH
THINK TANK ONE HEALTH

TISSUE ENGINEERING FOR INDUSTRY:
3D reconstructed Tissues and their industrial applications (TEFI)
Thursday December 9, 2021

EPISKIN

3D MODELS: PHYSIOLOGICAL SITUATION

Characteristics	2D cell culture	3D cell culture	References
Morphology	Cells grow on a flat surface and have flat or stretched shape	Cells grow naturally into 3D aggregates/spheroids in a 3D environment and natural shape retained	Huang H, Ding Y, Sun XS, Nguyen TA. Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells. PLoS One. 2013;8:59482
Cell shape	Flat, Single layer	Multiple layers, volume of cells recapitulate in vivo situation	Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and Drug Development Technologies. 2014;12:207-218
Cell to cell contact	Limited cell to cell contact, only on edges	Physiologic cell to cell contact similar to *in vivo*	Li Z, Cui Z, et al. Three-dimensional perfused cell culture. Biotechnology Advances. 2014;32:243-254
Distribution of medium	Cells receive an equal amount of nutrients and growth factors from the medium during growth.	Cells do not receive an equal medium during growth. The core cell receive less growth factors and nutrients from the medium and tend to be in a hypoxic state, which is very similar to *in vivo* tissues, especially in tumors	Bell CC, Hendriks DF, Moro SM, et al. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. Scientific Reports. 2016;6:25187 Li Z, Cui Z, et al. Three-dimensional perfused cell culture. Biotechnology Advances. 2014;32:243-254
Cell proliferation	Generally, cells proliferate at a fast rate than *in vivo*	Cells proliferate faster or slower depending on the type of cell or 3D system used	Chitcholtan K, Sykes P, Evans J. The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. Journal of Translational Medicine. 2012;10:1-16 Fallica B, Mafia JS, Villa S, Makin G, Zaman M. Alteration of cellular behavior and response to PI3K pathway inhibition by culture in 3D collagen gels. PLoS One. 2012;7:48024 Luca AC, Mersch S, Deenen R, et al. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. PLoS One. 2013;8:e59689 Comparative Assay of 2D and 3D Cell Culture Models: Proliferation, Gene Expression and Anticancer Drug Response April 2018Current Pharmaceutical Design 24(15)
Protein/gene expression	Protein and gene expression profiles differ compared with *in vivo*models	Protein and gene expression profiles more similar to *in vivo* models	Price KJ, Tsykin A, Giles KM, et al. Matrigel basement membrane matrix influences expression of microRNAs in cancer cell lines. Biochemical and Biophysical Research Communications. 2012;427:343-348
Cell differentiation	Moderately differentiated	Properly differentiated	Chitcholtan K, Asselin E, Parent S, Sykes PH, Evans JJ. Differences in growth properties of endometrial cancer in three dimensional (3D) culture and 2D cell monolayer. Experimental Cell Research. 2013;319:75-78
Response to stimuli	Poor response to mechanical stimuli of cells	Good response to mechanical stimuli of cells	Li Y, Huang G, Li M, et al. An approach to quantifying 3D responses of cells to extreme strain. Scientific Reports. 2016;6:19550
Viability	Sensitive to cytotoxin	Greater viability and less susceptible to external factors	Elkayam T, Amitay-Shaprut S, Dvir-Ginzberg M, Harel T, Cohen S. Enhancing the drug metabolism activities of C3A-a human hepatocyte cell line--by tissue engineering within alginate scaffolds. Tissue Engineering. 2006;12:1357-1368
Drug sensitivity	Cells are more sensitive to drugs and drug show high efficacy	Cells are more resistant to drugs and drug show low potency	Bokhari M, Carnachan RJ, Cameron NR, Przyborsk SA. Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge. Journal of Anatomy. 2007;211:567-576
Cell Stiffness	High stiffness	Low stiffness	Dieter SM, Ball CR, Hoffmann CM, et al. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. Cell Stem Cell. 2011;9:357-365
Sub-culturing time	Allows cell to be grown in culture for up to 1 week	Allows cells to be grown in culture for almost 4 weeks	Baker BM, Chen CS. Deconstructing the third dimension—How 3D culture microenvironments alter cellular cues. Journal of Cell Science. 2012;125:3015-3024

Table from Two-Dimensional (2D) and Three-Dimensional (3D) Cell Culturing in Drug Discovery By Jitcy Saji Joseph, Sibusiso Tebogo Malindisa and Monde Ntwasa, *Open access peer-reviewed chapter November 28th 2018 - DOI: 10.5772/intechopen.81552*

Respiratory

Tracheal/bronchial

EpiAirwayFT
https://www.mattek.[illegible]/oducts/epiairway/

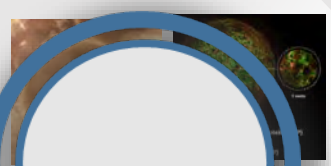
C[illegible]nparison of the basic [illegible]unction of 3D lung [illegible]es derived from several

[illegible]2020 Sep 4;1:56-69.
[illegible]2020.08.002.

Alveolar

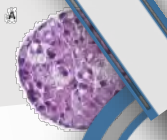
Sucre JM[illegible]et [illegible] **Primary Type II Alv[illegible]lar [illegible]typic Coculture**. Am[illegible]Resp[illegible] Aug;59(2):158-166. doi: 10.1165/rcmb.2[illegible]MA.

Brain

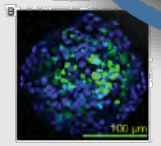


Ar[illegible]T. et al. **[illegible]ological systems: [illegible]on a chip**. In Vi[illegible]Ce[illegible]206 (2021). https[illegible]020-00532-8

Li[illegible]



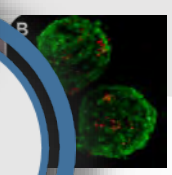
[illegible]C. Et al. **Three-[illegible]paRG Spheroid [illegible]ally Relevant [illegible]**



Intesti[illegible]

[illegible]ark[illegible]al [illegible]human small in[illegible]sti[illegible]dr[illegible]or drug per[illegible]ati[illegible]u[illegible]city assays**. In Vitro C[illegible]Rev.B[illegible]ar 57[illegible]0–173 (2021). https://doi.o[illegible]1826-020-00526-6

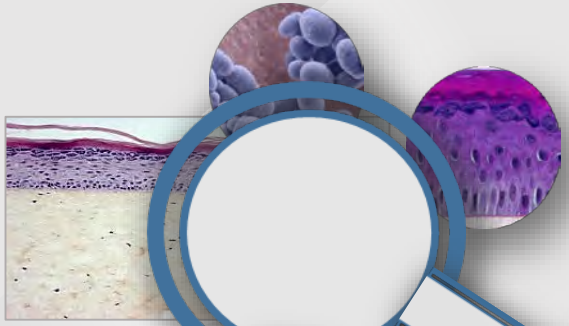
Hea[illegible]



[illegible]Optical [illegible]tion of Beating [illegible]uman Cardiac [illegible]sel). 2017;7(3):2[illegible] [illegible]od 2017 [illegible] doi:10.3390/bios7030024

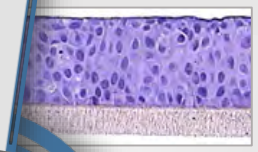
Skin/epidermis

TSKIN, RHE



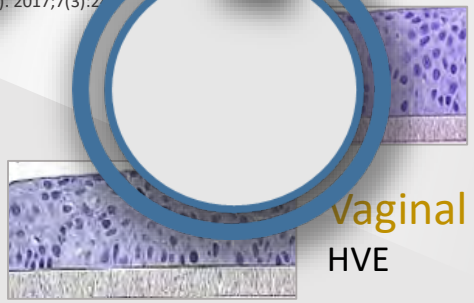
Corneal

HCE



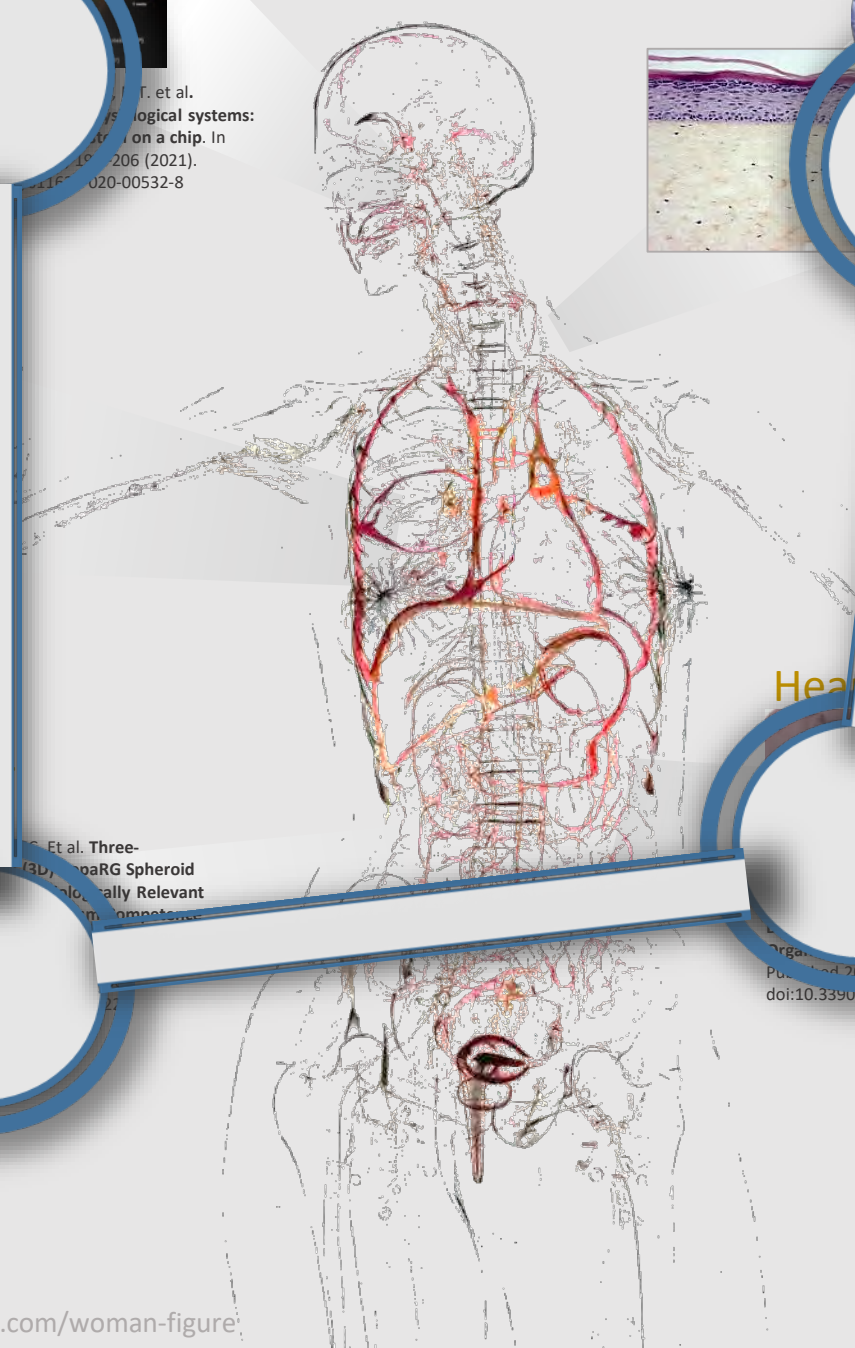
Oral

HOE



Vaginal

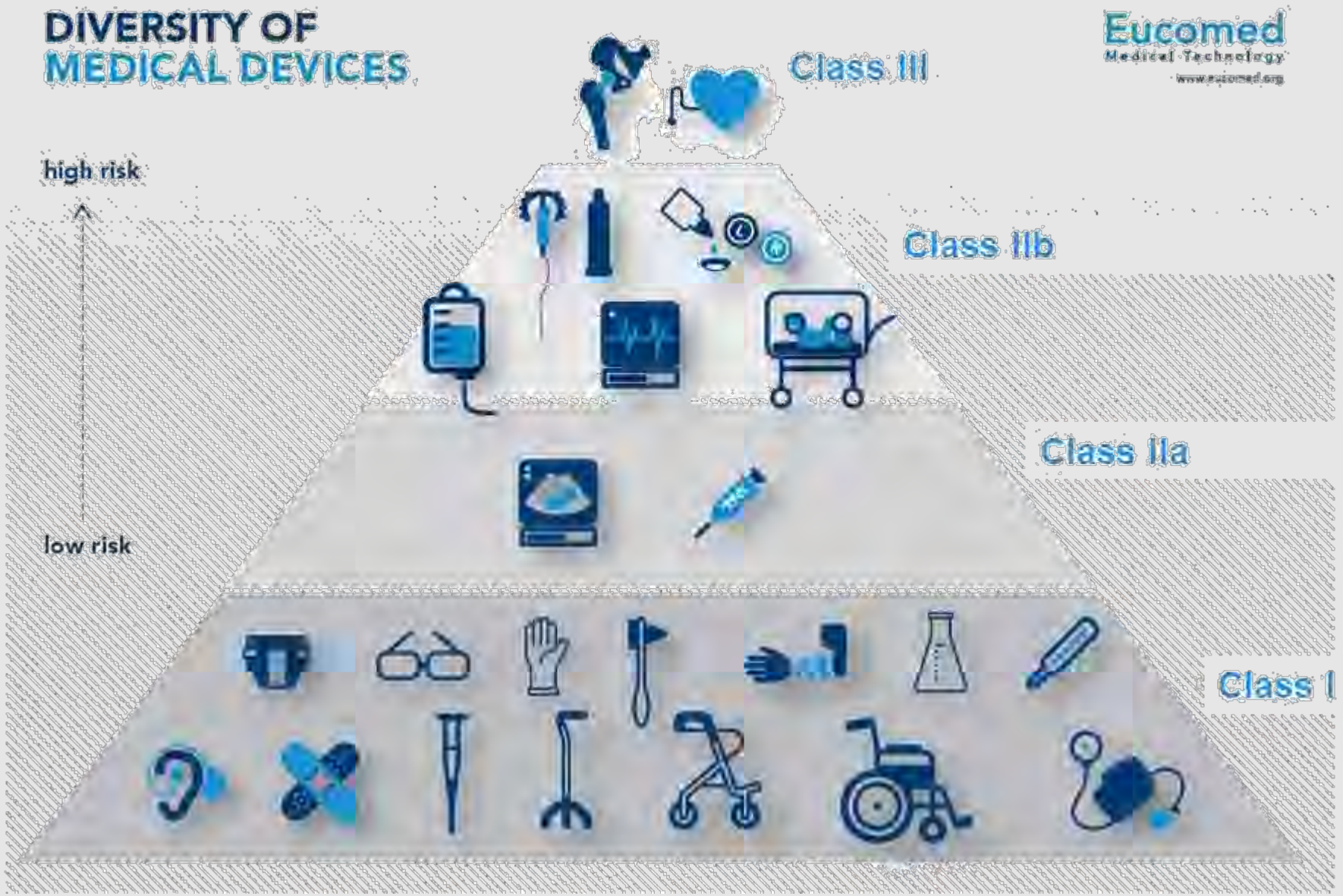
HVE



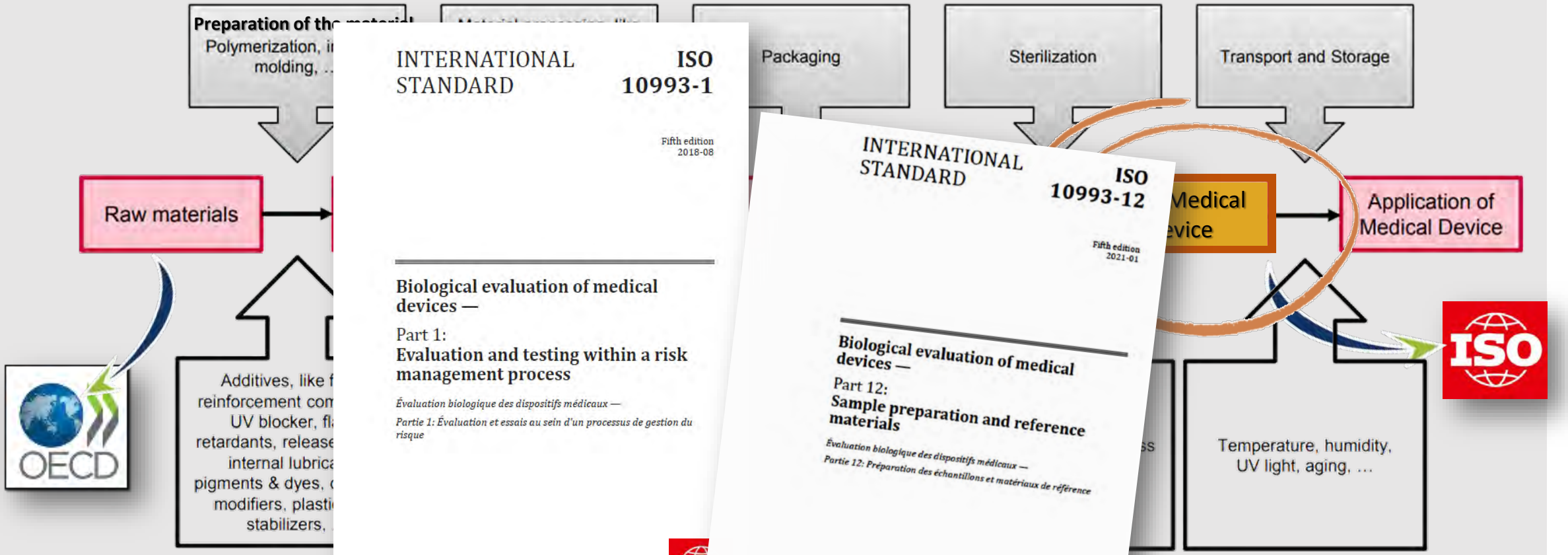
https://bryanchristiedesign.com/woman-figure

BIOCOMPATIBILITY OF MEDICAL DEVICES

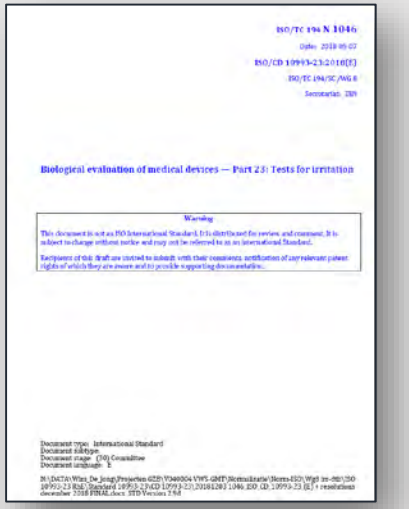
MEDICAL DEVICES



SPECIFIC CONTEXT OF MEDICAL DEVICES



IRRITATION


ISO/TC 194 N 1046

Date: 2010-05-07

ISO/CD 10993-23:2010(E)

ISO/TC 194/SC/WG 8

Secretariat: DIN

Biological evaluation of medical devices — Part 23: Tests for irritation

Warning

This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.

Recipient of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

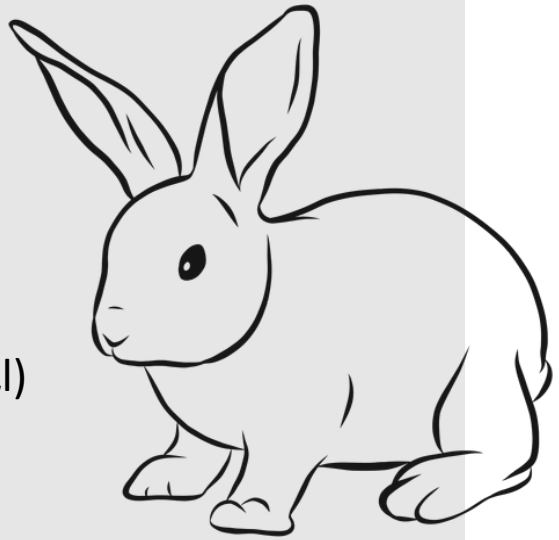
Document type: International Standard
Document subtype:
Document stage: (30) Committee
Document language: E

ISO 10993-10:2010

3 rabbits (Oryctolagus cuniculus)

Topical application 4h (500 mg or 500 µl) // **Intracutaneous** (200 µl)

Observation +1, +24, +48 and +72h (if persistence go up to 14d)



Reaction	Irritation score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Other adverse changes at the skin sites shall be recorded and reported.	

Reaction	Irritation score
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

Table 3 — Primary or cumulative irritation index categories in a rabbit

Mean score	Response category
0 to 0,4	negligible
0,5 to 1,9	slight
2 to 4,9	moderate
5 to 8	severe

Draize J.H., Woodard G. and Calvery H.O., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, Journal of Pharmacology and Experimental Therapeutics November 1944, 82 (3) 377-390;

FROM NEAT CHEMICALS TO MEDICAL DEVICES

~~OECD TG 439~~ → **Adaptation of the OECD validated method**

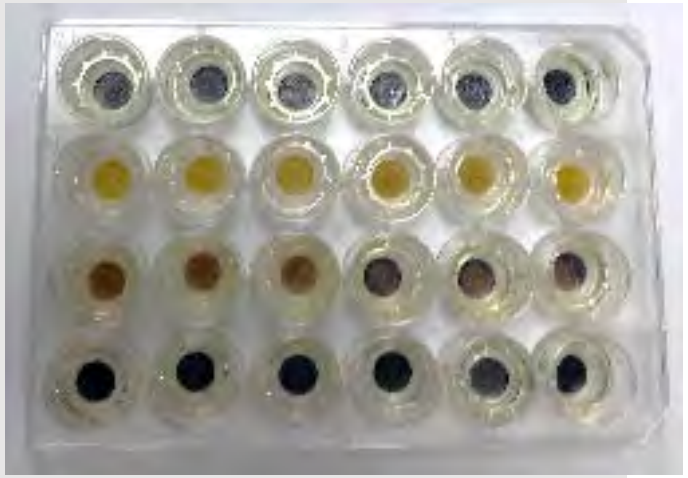
~~Pure product, small quantity~~

Extracts → mixture

~~Short time exposure~~

?

~~Long time postincubation~~



READOUT: Cell viability

→ Reduction of MTT in a blue formazan salt

Toxicology in Vitro

SUPPORTS OPEN ACCESS

Articles in press | Latest issue | Special issues | All issues | About the journal | Follow journal

Round robin study to evaluate the reconstructed human epidermis (RhE) model as an in vitro skin irritation test for detection of irritant activity in medical device extracts

Wim H. De Jong, Sebastian Hoffmann, Michelle Lee, Helena Kandárová, ... Kelly P. Coleman

SkinEthic™ RHE for in vitro evaluation of skin irritation of medical device extracts

Christian Pellevoisin, Christelle Videau, Damien Briotet, Corinne Grégoire, ... Nathalie Seyler

Pre-validation of an in vitro skin irritation test for medical devices using the reconstructed human tissue model EpiDerm™

Helena Kandarova, Jamin A. Willoughby, Wim H. De Jong, Silvia Letasiova, ... Kelly P. Coleman

Preparation of irritant polymer samples for an in vitro round robin study

Kelly P. Coleman, Thomas P. Grailer, Lori R. McNamara, Beau L. Rollins, ... Wim H. De Jong

Assessment of test method variables for in vitro skin irritation testing of medical device extracts

Daniel S. Olsen, Michelle Lee, Audrey P. Turley

Evaluation of the medical devices benchmark materials in the controlled human patch testing and in the RhE in vitro skin irritation protocol

Helena Kandárová, Hana Bendova, Silvia Letasiova, Kelly P. Coleman, ... Dagmar Jírova

EPISKIN

TOPICAL vs. INTRACUTANEOUS

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Evaluation of the medical devices benchmark materials in the controlled human patch testing and in the RhE *in vitro* skin irritation protocol

Helena Kandárová[a,*], Hana Bendova[b], Silvia Letasiova[a], Kelly P. Coleman[c], Wim H. De Jong[d], Dagmar Jírova[b]

[a] MatTek In vitro Life Science Laboratories, Bratislava, Slovak Republic
[b] National Institute of Public Health, Prague, Czech Republic
[c] Medtronic Biomaterials Department, Minneapolis, MN, USA
[d] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

		Rabbit		In vitro
	Vehicle	Patch	Intra-cutan	
1 Lactic acid, 4% solution (w/v)	Saline	-	+	+
2 Heptanoic acid, 2% solution (w/v)	SSO	-	+	+
3 Polymer Y-4 extract	Saline	-	+/-	+
4 Polymer Y-4 extract	SSO	-	+/-	+
5 Genapol X-80 0,068%	Saline	-	-	+
6 Genapol X-80 0,135%	Saline	-	+/-	+
7 Genapol X-80 0,338%	Saline	-	+/-	+
8 Genapol X-80 0,068%	SSO	-	-	-
09 Genapol X-80 0,135%,	SSO	-	+/-	-
10 Genapol X-80 0,338%,	SSO	-	+/-	+/-
11 SDS, 1% solution (v/v)	Saline	+	+	+
12 SDS, 1% solution (v/v)	SSO	+	+	+
13 Saline Vehicle	Saline	-	-	-
14 Sesame oil Vehicle	SSO	-	-	-

TOPICAL vs. INTRACUTANEOUS

Toxicology in Vitro 69 (2020) 104995

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

The suitability of reconstructed human epidermis models for medical device irritation assessment: A comparison of *In Vitro* and *In Vivo* testing results

Wim H. De Jong[a,*], Joseph W. Carraway[b], Chenghu Liu[c], Chunguang Fan[c], Jia Liu[c], Audrey P. Turley[d], Thor S. Rollins[d], Kelly P. Coleman[e,*]

[a] Formerly National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands (retired)
[b] NAMSA, Northwood, OH, USA
[c] Shandong Quality Inspection Center
[d] Nelson Labs, Salt Lake City, UT, US
[e] Medtronic, plc, Minneapolis, MN, US

Table 3
In vitro **RhE Assay and** ***in vivo*** **intracutaneous test results for extracts prepared from positive and negative irritant polymer materials[a].**

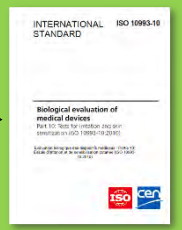
Test Sample	*In vitro* SkinEthic™ RhE model		I or NI	*In vivo* intracutaneous test rabbit		I or NI
	Saline extract	Sesame oil extract		Saline extract	Sesame oil extract	
Polyurethane E80A	98.3 ± 3.1[b]	104.2 ± 1.4[b]	NI	0.0	0.0	NI
PVC (Y-1)	99.6 ± 3.0	102.3 ± 3.5	NI	0.0	0.0	NI
Silicone	101.1 ± 2.3	104.1 ± 2.6	NI	0.0	0.0	NI
PVC Genapol X-80 (Y-4)	1.1 ± 0.2	3.3 ± 2.0	I	2.38	5.0	I
Silicone SDS	0.6 ± 0.3	109.8 ± 7.9	I	4.0	0.22	I
PVC Genapol X-100	1.2 ± 0.4	2.1 ± 07	I	1.44	5.89	I
Silicone HA[c]	103.6 ± 2.0	107.1 ± 3.2	NI[c]	0.0[c]	0.22[c]	NI[c]

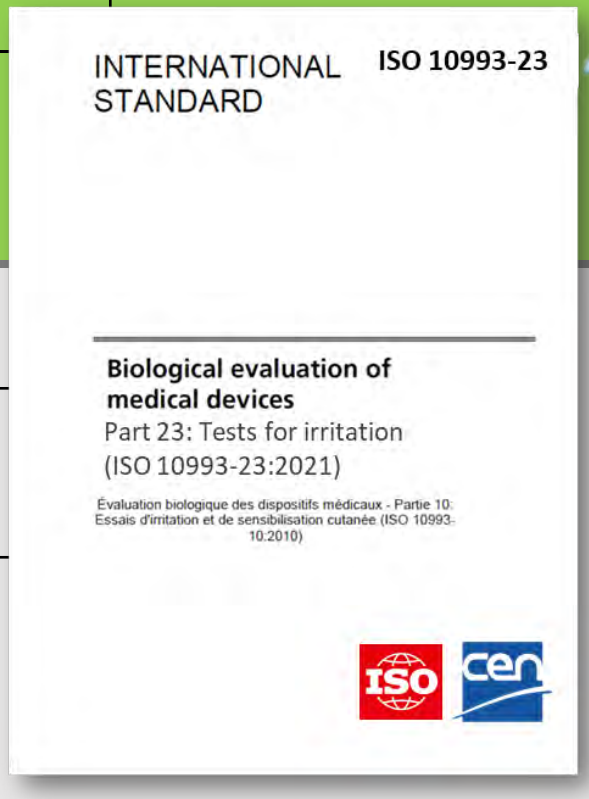
2021

EXTRACTIBLE

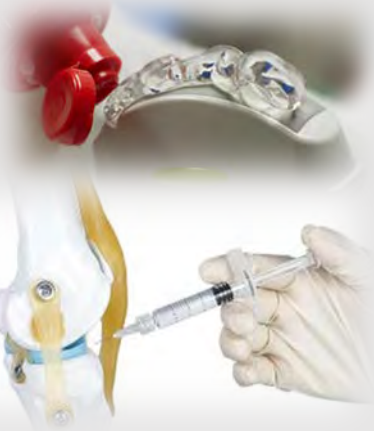
10993-1
10993-2
10993-18

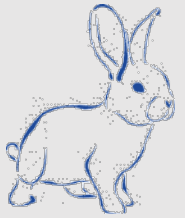
10993-12





NON-EXTRACTIBLE





SPECIAL IRRITATION TESTS (Annex D)

ANNEX B

ISO 10993-23 In vitro irritation testing for medical devices: Substantiating applicability to mild irritants and non-extractables

Christian Pellevoisin[1], Kelly P. Coleman[2], Sebastian Hoffmann[3]

1 EpiSkin, Lyon, France

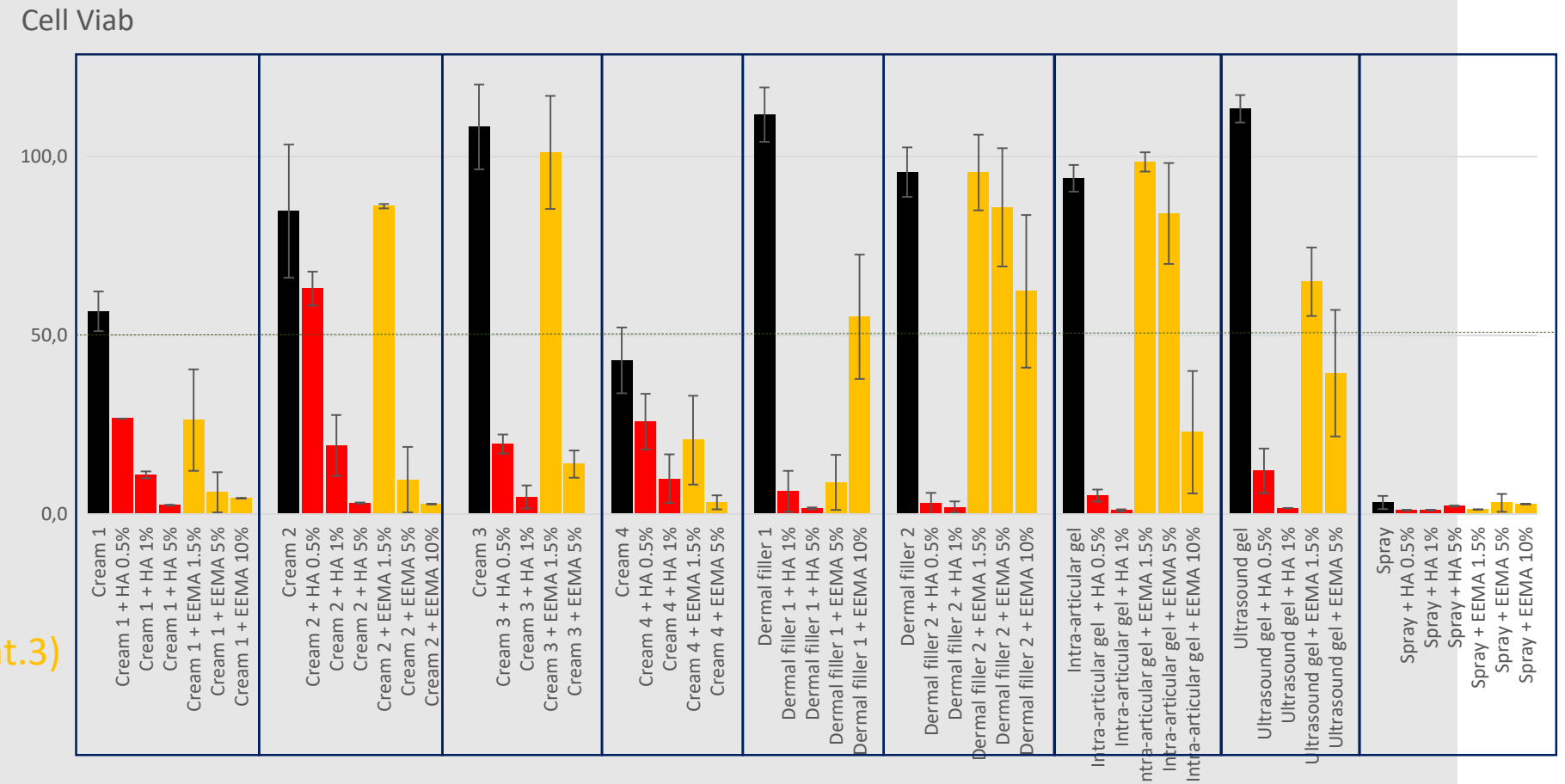
2 Medtronic plc, Minneapolis, MN, USA

3 seh consulting + services, Paderborn, Germany

Under review in

Non extractable medical devices

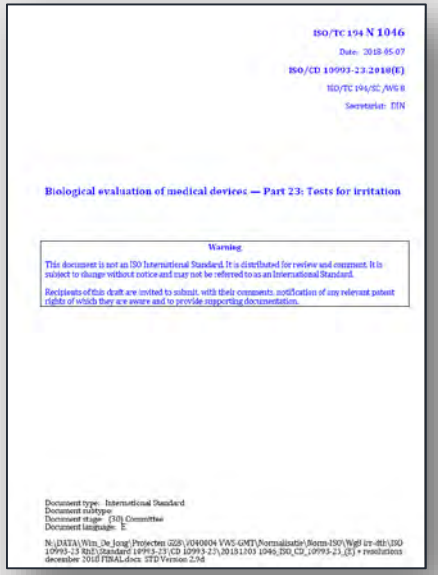
	Composition
Cream 1	Dimethicone, glycerin, water
Cream 2	Cetearyl glucoside, decyl oleate, cetearyl alcohol, sodium lauryl sulfate and sodium
Cream 3	Hyperoxygenated glycerides of essential fatty acids, vitamin E
Cream 4	Glycerol, vaseline, paraffin, excipients
Dermal filler 1	Hyaluronic acid, sodium chloride physiological solution
Dermal filler 2	Hyaluronic acid
Intra-articular gel	Hyaluronic acid, sodium chloride physiological solution
Ultrasound gel	Water, carbomer, glycol propylene
Spray	Hyaluronic acid, polysorbate, phenoxyethanol, benzoic acid



Heptanoic acid (GHS Cat.2)

2-Ethoxy-methylacrylate (GHS Cat.3)

SKIN SENSITIZATION


ISO/TC 194 N 1046
Date: 2018-05-07
ISO/CD 10993-23:2018(E)
ISO/TC 194/SC /WG 8
Secretariat: DIN

Biological evaluation of medical devices — Part 23: Tests for irritation

Warning
This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.
Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Document type: International Standard
Document subtype:
Document stage: (30) Committee
Document language: E

N:\DATA\Wim_De_Jong\Projecten (ZZS)\040604 VWS GMT\Normalisatie\Norm-ISO\Wg8 (nr-8)\ISO 10993-23 Rnf\Standard 10993-23 (CD 10993-23)\2018-1103 1046_ISO_CD_10993-23_(E) + resolutions december 2018 FINAL.docx STD Version 2.9d

ISO 10993-10:2021

Buehler test (1965)

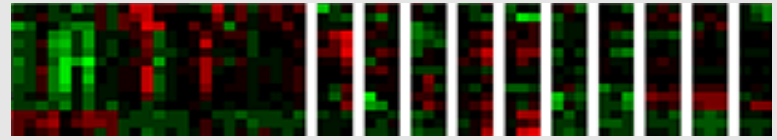
GPMT (1969)

OECD 406, EC B.6

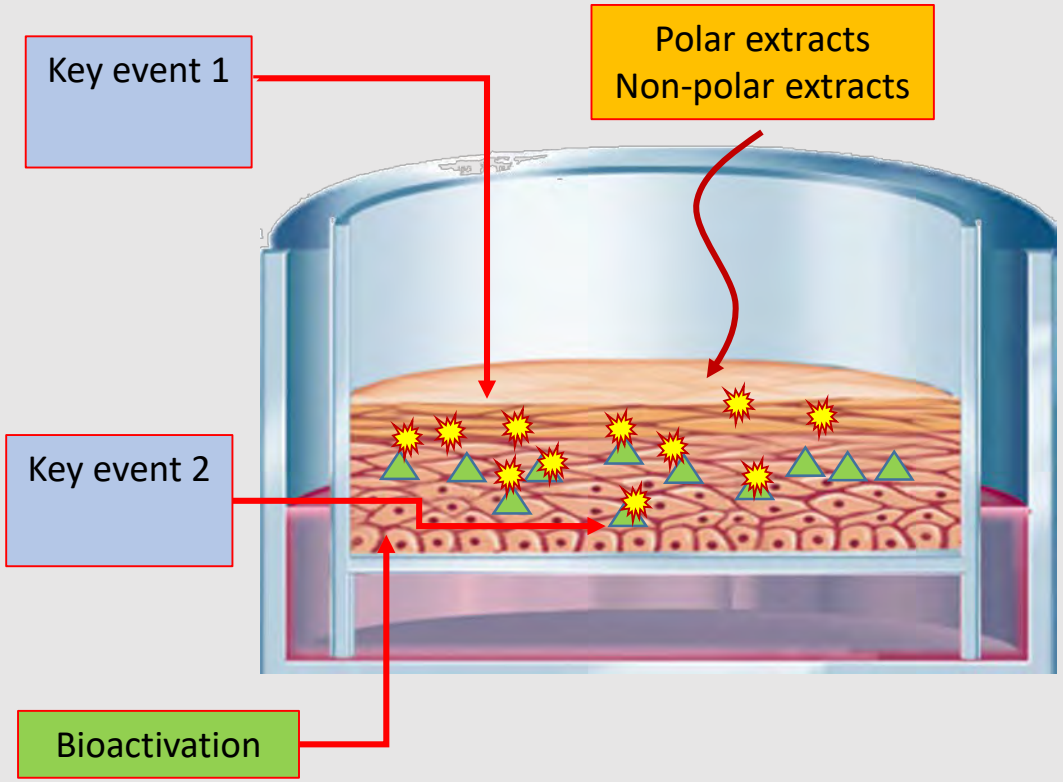
LLNA (1999)

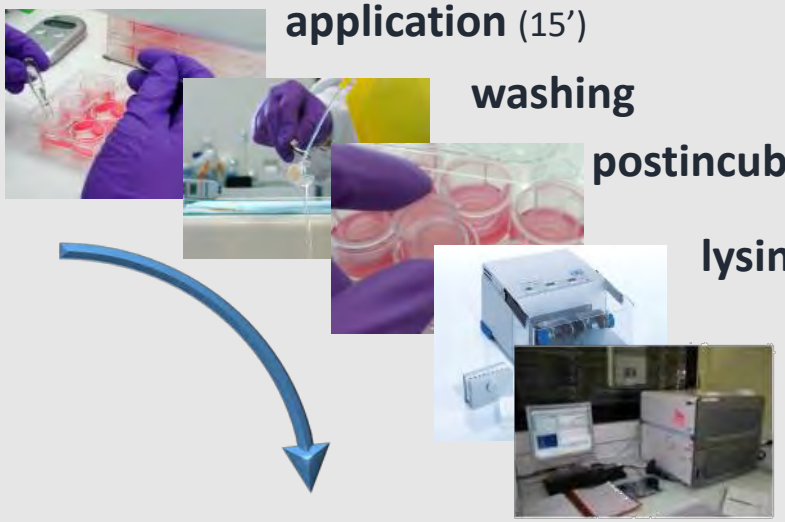
OECD 429

SENS-IS assay: TOXICOGENOMIC



ImmunoSearch





application (15')

washing

postincubation (6h)

lysing

RT-PCR quantification

62 biomarkers classified into 3 groups : irritation, ARE (17) and SENS-IS (21) genes

Validation assay by analysis of:
- negative control (Propylene Glycol)
- irritant control (5% SLS)
- two sensitizer controls (50% HCA, 1% TNBS)

Sensitization: positive if at least:
- **7/17 genes** in ARE genes group (REDOX group)

and/or

- **7/21genes** in SENS-IS genes group are significantly induced

Sensitization assays with RhEs could overcomes some limitations of 2D cell assays:

EPISKIN

➔ **complexe test systems, solubility, bioactivity**

Toxicology in Vitro 71 (2021) 105068

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Pre-validation of SENS-IS assay for *in vitro* skin sensitization of medical devices

C. Pellevoisin [a,*], F. Cottrez [b], J. Johansson [c], E. Pedersen [c], K. Coleman [d], H. Groux [b]

[a] EPISKIN, 4, rue Alexander Fleming, Lyon, France
[b] ImmunoSearch, Les Cyclades, Chemin de Campercousse, Grasse, France
[c] RISE Research Institutes of Sweden AB, Borås, Sweden
[d] Medtronic plc, Minneapolis, MN, USA

ABSTRACT

According to ISO 10993-1:2018, the skin sensitization potential of all medical devices must be evaluated, and for this endpoint ISO 10993-10:2010 recommends the use of *in vivo* assays. The goal of the present study was to determine if the *in vitro* SENS-IS assay could be a suitable alternative to the current *in vivo* assays. The SENS-IS assay uses the Episkin Large and SkinEthic RHE reconstructed human epidermis models to evaluate marker genes. In our study, the SENS-IS assay correctly identified 13 sensitizers spiked in a non-polar solvent. In a subsequent analysis six medical device silicone samples previously impregnated with sensitizers were extracted with polar and non-polar solvents. The SENS-IS assay correctly identified five of these extracts, while a sixth extract, which contained the weak sensitizer phenyl benzoate, was classified as negative. However, when this extract was concentrated, or a longer exposure time was used, the assay was able to detect phenyl benzoate. The SENS-IS assay was transferred to a naïve laboratory which correctly identified sensitizers in six blinded silicone samples, including the one containing phenyl benzoate. In light of these results, we conclude that the SENS-IS assay is able to correctly identify the presence of sensitizers in medical devices extracts.

1. Introduction

A systematic review of 20,107 human patch tests conducted in 2007 and updated in 2017, showed a 20.1% prevalence of allergic contact dermatitis (ACD) in the general population (Alinaghi et al., 2019). These findings highlighted the importance of this endpoint in toxicological risk assessment strategies for consumer goods, cosmetics and medical devices. Since up to 13% of the population in developed countries are sensitive to nickel, cobalt, or chromium, it is not surprising that immune responses to metallic implants are often reported in the literature (Haddad et al., 2019). In addition, skin-contacting medical devices that contain acrylates, such as glucose sensors and insulin pumps, may also be responsible for ACD (Herman et al., 2018; Uter et al., 2020). To minimize this risk, skin sensitization is one of the three biocompatibility tests that are required for all medical devices (ISO 10993-1, 2018); the others being irritation and cytotoxicity. Historically, the evaluation of skin sensitization potential has relied on these *in vivo* tests: The Buehler assay developed in 1965 (Buehler, 1965), the guinea pig maximization tests (GPMT) developed by Magnusson and Kligman in 1969 (Magnusson and Kligman, 1969) and the Local lymph node assay (LLNA) developed in 1999 by Kimber et al. (1986) The GPMT is considered to be more sensitive than the Buehler assay and is preferred for implanted medical devices. In 1999 the LLNA was evaluated and recommended in by the Interagency Coordinating Committee on the Validation of Alternative Methods as an alternative to guinea pig tests for assessing the potential of chemicals to cause ACD (National Institute of Environmental Health Sciences (NIEHS), 1999; Dearman et al., 1999). It reduces the number of animals required, eliminates the pain and distress of allergic responses, requires less time to perform and provides dose-response information facilitating potency classification. In 2009, the European Union's ban on the use of animal testing for cosmetic ingredients (Commission Directive 2009/130/EC of 12, 2009) heightened the need for alternatives to animal testing. While stand-alone *in vitro* methods for acute local toxicological endpoints such as skin corrosion and irritation are now widely used instead of animal tests, yet tests for more complex endpoints such as skin sensitization are harder to replace. In 2012 the Organization for Economic Co-operation and Development (OECD) published its adverse outcome pathway (AOP) for skin sensitization, which was an important milestone (OECD, 2012) (Fig. 1). An AOP is the sequence of events from chemical structure through the molecular initiating event to the *in vivo* outcome of interest. In the AOP for skin sensitization, the first KE is covalent binding of haptens to skin proteins, which is the molecular initiating event. This leads to keratinocyte activation, the second KE at the cellular level, that includes

* Corresponding author.
E-mail address: cpellevoisin@episkin.com (C. Pellevoisin).

https://doi.org/10.1016/j.tiv.2020.105068
Received 19 May 2020; Received in revised form 23 November 2020; Accepted 6 December 2020
Available online 8 December 2020
0887-2333/© 2020 Elsevier Ltd. All rights reserved.

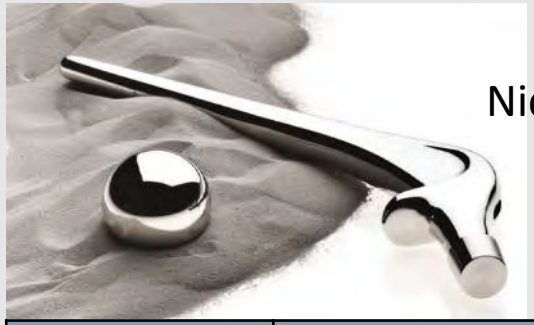
Pre-validation of SENS-IS assay for in vitro skin sensitization of medical devices

C. Pellevoisin [a,*], F. Cottrez [b], J. Johansson [c], E. Pedersen [c], K. Coleman [d], H. Groux [b]

- 15 chemicals spiked in solvents
- 7 materials spiked with or without sensitizers
- 2 laboratories
  - ImmunoSearch (France)
  - RISE Research Institutes (Sweden)

Application to Materials and MD

- ISO 10993-12 Extraction in polar (NaCl) an non polar (SO) ➔ SENS-IS assay



Nickel powder

Number of overexpressed genes	Saline	Sesame oil
IRRITATION	5	1
SENS-IS	7	2
ARE	3	2
IRRITATION	NEGATIVE	NEGATIVE
SENSITIZATION	POSITIVE	NEGATIVE

Ruber

Number of overexpressed genes	Saline	Sesame oil
IRRITATION	6	1
SENS-IS	0	1
ARE	4	8
IRRITATION	NEGATIVE	NEGATIVE
SENSITIZATION	NEGATIVE	POSITIVE

Latex gloves

Number of overexpressed genes	Saline	Sesame oil
IRRITATION	1	1
SENS-IS	1	0
ARE	4	2
IRRITATION	NEGATIVE	NEGATIVE
SENSITIZATION	NEGATIVE	NEGATIVE

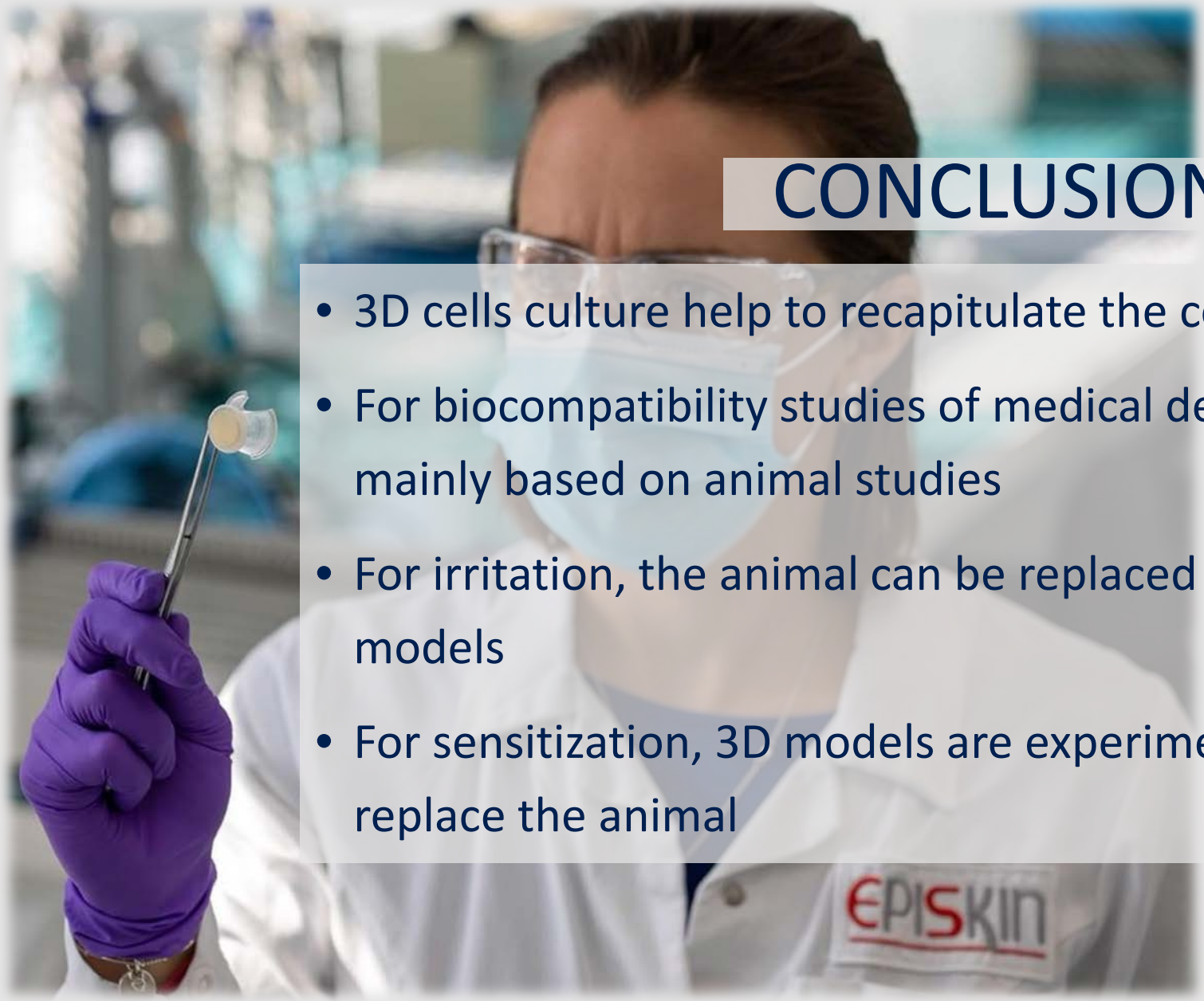
Adhesive patch of glucose sensor

Number of overexpressed genes	Saline	Sesame oil
IRRITATION	2	1
SENS-IS	0	1
ARE	7	0
IRRITATION	NEGATIVE	NEGATIVE
SENSITIZATION	POSITIVE	NEGATIVE

Positive control

(Silicone spiked with benzoquinone)

Number of overexpressed genes	Saline	Sesame oil
IRRITATION	8	7
SENS-IS	4	1
ARE	12	16
IRRITATION	NEGATIVE	NEGATIVE
SENSITIZATION	POSITIVE	POSITIVE



CONCLUSIONS

- 3D cells culture help to recapitulate the complexity of the human body
- For biocompatibility studies of medical devices, the biological assays are mainly based on animal studies
- For irritation, the animal can be replaced by in vitro methods with 3D models
- For sensitization, 3D models are experimental models of choice to replace the animal