TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

TARU LINDELL

PACKAGING SOLUTIONS FOR BIODEGRADABLE TISSUE ENGINEERING PRODUCTS

Master's Thesis

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Examiners and topic approved in Faculty council of the Faculty of Engineering Sciences meeting on 9. 9. 2015

ABSTRACT

TARU LINDELL: Packaging solutions for biodegradable tissue engineering products
Tampere University of Technology
Master of Science Thesis, 89 pages, 17 Appendix pages
March 2016
Master's Degree Program in Materials Science
Major: Biomaterials
Examiners: Professor Minna Kellomäki, DrTech Anne-Marie Haaparanta

Keywords: packaging, biodegradable, medical device, tissue engineering, regulation, sterilization

Tissue engineering is a rapidly evolving multidisciplinary field that develops biological substitutes to restore, maintain, or improve damaged tissues. Biodegradable tissue engineering applications are often complex, multi-component composite structures that combine a biodegradable medical device with cells, tissues, or other biological factors. Materials used in tissue engineering applications are often sensitive and cannot withstand high temperatures, humidity, irradiation, and/or chemicals. These qualities pose special challenges to sterilization process and packaging design. The heavy legislation and regulations that apply to tissue engineering products affect the packaging solution from choosing the sterilization method to the design and testing of the final package. The stringent and ever evolving regulations also slow down the adoption of new manufacturing and packaging technologies in the healthcare industry.

This thesis is a literature review. The scope of this thesis was to examine suitable packaging solutions for biodegradable tissue engineering products. For background, the current relevant legislation and regulations in the European Union and in the United States were reviewed, and different aspects of packaging of medical devices were discussed. Also several traditional and novel sterilization methods, as well as aseptic processing were reviewed, and multiple packaging solutions that are or could be suitable for sterile biodegradable implantable medical devices were presented.

Due to the diverse nature of biodegradable tissue engineering products, it is impossible to suggest one universal packaging solution. Some general guidelines, however, can be suggested based on the common features of biodegradable tissue engineering products, usability studies, and sustainability considerations. An ideal packaging would be a double sterile barrier system consisting of a rigid tray in a flexible, clear pouch that would also serve as a moisture barrier. All the packaging components should be easy to open quickly in an operating room environment. The outer carton should be as small as possible, while still providing sufficient protection and information. The final materials for the packaging should be chosen based on whether the product will be sterilized in the final package or whether it will be aseptically processed.

TIIVISTELMÄ

TARU LINDELL: Biohajoavien kudosteknologisten tuotteiden pakkaaminen
Tampereen teknillinen yliopisto
Diplomityö, 89 sivua, 17 liitesivua
Maaliskuu 2016
Materiaalitekniikan diplomi-insinöörin tutkinto-ohjelma
Pääaine: Biomateriaalitekniikka
Tarkastajat: professori Minna Kellomäki, tekniikan tohtori Anne-Marie Haaparanta

Avainsanat: pakkaus, biohajoava, lääkinnällinen laite, kudosteknologia, säädökset, sterilointi

Kudosteknologia on nopeasti kasvava monitieteellinen ala, jonka puitteissa kehitetään vahingoittuneiden kudosten korjaamiseen ja ylläpitoon tarvittavia biologisia korvikkeita. Biohajoavat kudosteknologiset tuotteet ovat usein monikomponenttisia komposiittirakenteita, joissa yhdistetään lääkinnällinen laite ja soluja, kudoksia tai muita biologisia tekijöitä. Kudosteknologisten tuotteiden materiaalit ovat yleensä herkkiä, eivätkä ne kestä esimerkiksi korkeita lämpötiloja tai kosteutta. Nämä ominaisuudet asettavat erityisiä vaatimuksia myös tuotteiden pakkauksille, ja esimerkiksi sopivan sterilointimenetelmän löytäminen voi olla haastavaa. Kudosteknologia on voimakkaasti säädelty ja valvottu ala, ja kaikkien tuotteeseen, tuotantoon ja pakkaukseen liittyvien toimien ja materiaalien tulee täyttää kyseistä tuotetta koskevat viranomaisvaatimukset. Voimakkaasta säätelystä johtuen myös uusien teknologioiden käyttöönotto on yleisesti hidasta terveysteknologiayrityksissä.

Tämä diplomityö on kirjallisuuskatsaus. Työn tarkoituksena oli tutkia mahdollisia pakkausratkaisuja biohajoaville kudosteknologia tuotteille. Taustatiedoiksi käsiteltiin ajantasaista lääkinnällisten laitteiden ja kudosteknologiasovellusten lainsäädäntöä Euroopan Unionissa ja Yhdysvalloissa, sekä käytiin läpi erilaisia lääkinnällisten laitteiden pakkaamiseen liittyviä yleisiä seikkoja. Työssä esiteltiin useita steriileille biohajoaville kudosteknologisille tuotteille soveltuvia pakkausratkaisuja. Myös perinteisiä ja uusia sterilointi- ja desinfiointimenetelmiä käytiin läpi, sekä käsiteltiin aseptista pakkausta.

Johtuen biohajoavien kudosteknologisten tuotteiden erilaisista ominaisuuksista on mahdotonta ehdottaa yleistä pakkausratkaisua joka sopisi kaikille tuotteille. Joitakin yleisiä johtopäätöksiä voidaan kuitenkin vetää perustuen biohajoavien kudosteknologiasovellusten yleisiin piirteisiin, käyttäjätutkimuksiin, sekä huomioiden ekologiset näkökohdat. Ideaali pakkaus olisi kaksinkertainen steriilipakkaus, jossa läpinäkyvään pussiin on pakattu jäykkä tarjotin joka sisältää tuotteen. Pussi toimisi myös tuotteen kosteussuojana. Kaikki pakkauksen osat olisi helppo avata leikkaussaliolosuhteissa ja etiketit olisivat selkeitä ja helppolukuisia. Pakkauskartongin tulisi olla mahdollisimman pieni, mutta kuitenkin suojattava tuotetta riittävästi ja tarjottava tarpeellinen informaatio. Lopulliset pakkausmateriaalit tulisi valita sen perusteella steriloidaanko tuote lopullisessa pakkauksessaan, vai pakataanko se aseptisesti.

PREFACE

This thesis was written at Tampere University of Technology, the Department of Electronics and Communications Engineering, as part of my Master of Science Degree in Materials Science. It is a literature review, concentrating on the biodegradable tissue engineering products and the challenges regarding their packaging solutions.

I would like to thank Professor Minna Kellomäki and DrTech Anne-Marie Haaparanta for offering me the opportunity to write this thesis, and for acting as examiners of this work. I am especially grateful to Dr Haaparanta for instructions, valuable comments and overall support during this project. I would also like to thank my family and friends for support and understanding, and for always believing in me. A big thank you also for my fellow students who offered comments and suggestions along the way.

In Tampere March 20, 2016

Taru Lindell

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ABBREVIATIONS AND SYMBOLS

1D	1-dimensional
2D	2-dimensional
3D	3-dimensional
^{60}Co	Cobalt 60 isotope
^{137}Cs	Cesium 137 isotope
^{192}Ir	Iridium 192 isotope
λ	Wavelength
ABS	Acrylonitrile butadiene styrene
ADSC	Adipose-derived stem cell
AIMDD	Active Implantable Medical Devices Directive 90/385/EEC
AOS	Active oxygen species
ATMP	Advanced-therapy medicinal product
bFGF	Basic fibroblast growth factor
BFS	Blow-fill-seal technology
BLA	Biologics License Application
BMP	Bone morphogenetic protein
C	Coulomb, electric charge unit
CAD	Computer-aided design
CBER	Center for Biologics Evaluation and Research
CDRH	Center for Devices and Radiological Health
CE-mark	Conformiteé Européenne mark
CEN	European Committee for Standardization
CENELEC	European Committee for Electrotechnical Standardization
CFR	Code of Federal Regulations
ClO^-	Hypochlorite ion
ClO_2	Chlorine dioxide
CO_2	Carbon dioxide
COCIR	European Coordination Committee of the Radiological, Electromedical, and Healthcare IT Industry
CTT	Cell- and tissue therapy product
DNA	Deoxyribonucleic acid
e-Beam	High energy electrons
eCFR	Electronic Code of Federal Regulations
ECM	Extra cellular matrix
EDMA	European Diagnostic Manufacturers Association
EEC	European Economic Area
EFTA	European Free Trade Association
EGF	Epidermal growth factor
eMDR	Electronic Medical Device Reporting
ES	Embryotic stem cell
EtO	Ethylene oxide
ETSI	European Telecommuni8cations Standards Institute
EU	European Union
eV	Electron volt, energy unit
FD&C Act	Federal Food Drug & Cosmetic Act
FDA	U.S. Food and Drug Administration
FDAMA	Food and Drug Administration Modernization Act of 1997

FDASIA	The FDA Safety and Innovation Act
FEP	Fluorinated ethylene propylene
FFS	Form-fill-seal technology
FIHTA	Finnish Health Technology Federation (Terveysteknologian liitto ry)
Fimea	Finnish Medicines Agency
FR	Federal Register
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
GTP	Good Tissue Practice
Gy	Grey, absorbed radiation dose unit
H_2O_2	Hydrogen peroxide
HA	Hydroxyapatite
HCT/P	Human cells, tissues, and cellular and tissue-based product
HDPE	High density polyethylene
HEPA	High efficiency particulate air
HOCl	Hypochlorus acid
Hz	Herz, frequency unit
IDE	Investigational Device Exemption
IGF	Insulin-like growth factor
IMDRF	International Medical Device Regulation Forum
iPS	Induced pluripotent stem cell
IR	Infrared radiation
ISO	The International Organization for Standardization
IVD	In Vitro Diagnostics
IVDMD	In Vitro Diagnostics Medical Devices Directive 98/79/EC
J	Joule, energy unit
MDD	Medical Devices Directive 93/42/EEC
MDR	Medical Device Reporting
MDSC	Muscle-derived stem cell
MSC	Mesenchymal stem cell
NB	Notified Body
NO_2	Nitrogen dioxide
O_2	Oxygen
O_3	Ozone
OCP	Office of Combination Products
OCTGT	Office of Cellular, Tissue, and Gene Therapies
OPA	Ortho-phthalaldehyde
PCL	Poly(capro lactone)
PDGF	Platelet-derived growth factor
PDMS	Poly(dimethylsiloxane)
PDO	Polydioxanone
PE	Polyethylene
PEEK™	Polyether ether ketone
PEI	Polyetherimide
PET	Polyethylene terephthalate
PETG	Polyethylene terephthalate glykol
PGA	Poly(glycolic acid)
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHS Act	Public Health Services Act

PHV	Polyhydroxyvalerate
PI	Polyimide
PLA	Polylactide
PLGA	Poly(lactide-co-glycolide)
PMA	Premarket Approval
PMMA	Poly(methylmetacrylate)
PP	Polypropylene
ppm	Parts per million
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PVC	Poly(vinyl chloride)
PVDF	Polyvinylidene fluoride
QSR	Quality System Regulation
RABS	Restricted access barrier system
RDF	Request for Designation
RNA	Ribonucleic acid
rPET	Recycled polyethylene terephthalate
SAL	Sterility Assurance Level
$scCO_2$	Supercritical carbon dioxide
TCP	Tricalsiumphosphate
TGF	Transforming growth factor
TiO_2	Titanium dioxide
US	United States
UV	Ultraviolet radiation

1. INTRODUCTION

Tissue engineering is a multidisciplinary field that develops biological substitutes to restore, maintain, or improve tissue functions (Czernuszka et al, 2002). The most common tissue engineering strategy is to place cells on or within an implantable biodegradable matrix – a scaffold - that degrades at a controlled rate and is replaced in the body by the regenerating tissue (Dhandayuthapani et al, 2011). Scaffolds are classified and regulated as medical devices, and tissue engineering products containing, or consisting of, engineered cells or tissues are classified as advanced therapy medicinal products. The advanced therapies that include gene-, cellular-, and tissue therapies, as well as drug-device, biologic-device, or drug-biologic combination products, are most recent additions to the larger biopharmaceuticals group. Biopharmaceuticals are considered to be the key to the future pharmaceutical treatments, and currently as many as half of all medicinal products in development are biopharmaceuticals. (Fimea, 2014)

Due to the rapid evolving of the field at the moment, the regulations regarding advanced therapies are at different stages of development in different countries. Harmonized terminology and technical requirements are yet to be established, and the regulatory agencies across the globe are working to create a risk-based regulation system with some common features. (Kellathur & Lou, 2012) Advanced therapy products are often complex composite structures, thus they impose new challenges on packaging and sterilization technology (Crosby, 2008). The correct selection of a package is crucial: estimated 10% of all medical device recalls are due to packaging failures (Cleanroom Technology, 2014).

The packaging of a device generally has three main functions: protection, utility and communication. Protection is necessary for maintaining the package integrity for its lifespan from sterilization to shipping, storage, handling and use. (Bix & De La Fuente, 2009) All implantable medical devices must be sterilized prior to their surgical placement (Athanasiou et al, 1996). Sterility and its maintenance, as well as prevention of cross-infection, are the most critical factors in patient care. The packaging around a medical device allows the device to be sterilized, provides a microbial barrier, and maintains the sterility of the device. (Sterile Barrier Association, 2006) Biodegradable implantable devices also need to be protected from moisture due to their hydrolytically unstable nature (Bernkopf, 2007). A package may also have additional functions beside protection, such as serving as a measuring device, dispenser, stabilizing stand or a disposal bin. Utility has to do with the usability of a product: in many cases medical professionals base their decision on superior medical technology, but in case of two or more similar products, other factors emerge, usability of the package being one of them. Device packages are also a means to

communicate information through materials, graphics and shape. Communication involves passing important information for the safe and effective use of the device. Clear and easy product identification is especially crucial for medical devices that are used in hospital settings. (Bix & De La Fuente, 2009) The visual appearance of the package also has to reflect the requirement of cleanliness associated with medical devices (Cleanroom Technology, 2014). The device manufacturer also has to make sure that components of the package, like adhesives and inks, do not interfere with the product's safety and efficacy (Bix & De La Fuente, 2009). For sterile medical devices, the packaging materials need to be compatible with the chosen method of sterilization (Teixeira, 2014).

Sterilization process can affect the physical and mechanical properties of the device, thus affecting its performance *in vivo* (Athanasiou et al, 1996). Some materials may not withstand the initial sterilization process, and some are known to degrade with time after sterilization, which poses a significant risk to the sterility and integrity of the device (Simmons, 2012). The traditional end sterilization methods are often not suitable for heat and moisture sensitive biodegradable scaffolds and complex advanced therapy products. More gentle sterilization methods have been or are being developed, but many of them still lack the approval of authorities in the European Union or in the United States. Therefore, a wide variety of biodegradable medical devices and combination products are aseptically processed, although it is usually the last option due to the high manufacturing costs and less robust process control. (Lambert et al, 2011; ISO 13408-7, 2012)

This thesis is a literature review. The purpose of this work is to examine possible packaging solutions for sterile biodegradable tissue engineering products. For background, current regulations regarding medical devices and advanced therapies in the two largest markets, European Union and the United States are reviewed, and general aspects of medical device packaging are discussed. Also multiple existing packaging solutions that are suitable for sterile biodegradable implantable medical devices are presented, and several traditional and novel sterilization methods are reviewed.

2. TISSUE ENGINEERING

Injuries and different pathological conditions can result to a tissue or organ failure. Traditionally damaged tissues and organs have been repaired by transplantation, surgical methods, artificial prostheses or mechanical devices, or by drug therapy. These methods, however, do not offer satisfactory results neither in repair nor long-term recovery, especially in the case of major damages. Tissue engineering provides an alternative solution to the issue. (Nature Biotechnology, 2000) Tissue engineering is a multidisciplinary field that develops biological substitutes to restore, maintain, or improve tissue functions (Czernuszka et al, 2002). The general tissue engineering strategies can be divided into three groups (Dhandayuthapani et al, 2011):

1) Implantation of isolated cells or cell substitutes into the organism.
2) Delivering of tissue-inducing substances, such as growth factors.
3) Placing cells on or within different, natural or synthetic, implantable matrices.

The third strategy is most commonly associated with the concept of tissue engineering (Dhandayuthapani et al, 2011). Natural, synthetic, or semisynthetic functional constructs are implanted into the damaged site, where they are either fully functional from the moment of implantation, or they will grow into the required functionality. Relevant cells are grown in the laboratory, and supportive structures, scaffolds, are needed to act as substrates for cellular attachment and to guide the cell growth in favored orientations. (Czernuszka et al, 2002; Nature Biotechnology, 2000) In addition to medical applications, there are also non-therapeutic tissue engineering applications, including biosensors for detecting biological or chemical agents and tissue chips for toxicity testing in drug development. (National Institute of Biomedical Imaging and Bioengineering, 2015) The tissue engineering market is growing fast: the total global market for tissue engineering products is estimated to be $23 billion in 2015, expected to reach a 23% yearly rise, and reach $94 billion in 2022 (Industry Experts, 2015).

2.1 Scaffolds in tissue engineering

Tissues consist of cells, and usually a group of cells make and secrete their own support structure, which is called the extra-cellular matric (ECM). The matrix supports the cells and acts as a relay station for various signaling molecules. (National Institute of Biomedical Imaging and Bioengineering, 2015) In tissue engineering the necessary support structures are provided by scaffolds, that are commonly defined as three-dimensional (3D), solid and porous biomaterial structures that are designed to perform some or all of the following functions (Dhandayuthapani et al, 2011):

(a) Promote interactions between cells and the biomaterial, cell adhesion and ECM deposition.
(b) Permit sufficient transport of nutrients, gasses, and regulatory factors that enable cell survival, proliferation and differentiation.
(c) Degrade at a controllable rate that matches the regeneration rate of the tissue at the implantation site.
(d) Be biocompatible; provoke minimal inflammation and toxicity *in vivo.*

Tissue engineering scaffolds have been used to regenerate bone-, cartilage-, ligament-, skin-, vascular- and neural tissues, and skeletal muscles (Dhandayuthapani et al, 2011). By the definition of the European Council Directive 92/43/EEC, scaffolds are classified as medical devices. The regulations regarding medical devices in the European Union and in the United States are discussed in more detail in chapter 3, and packaging of medical devices is discussed in chapter 5.

The scaffold's architecture defines the ultimate shape of the engineered tissue, thus the scaffold microstructure is a crucial factor in a tissue engineering construct (Hutmacher, 2000). Typical scaffold designs include meshes, fibers, sponges, and foams (Dhandayuthapani et al, 2011). Since most tissues in the body are 3D structures, also most tissue engineering scaffolds are 3D constructs; 3D nanoporous hydrogels, or 3D microporous structures. Scaffold microstructure can also be a one-dimensional (1D) fiber structure or a 2-dimensional (2D) substrate. Generally, 2D structures, such as well plates and Petri dishes, are used for cell culture studies. (Haaparanta, 2015) High, typically over 90% porosity, optimal pore size that depends on the type of cells used, and pore interconnectivity are important factors in the migration of cells, nutrition and waste exchange inside the scaffold (Hutmacher, 2000; Haaparanta, 2015). The advantages and disadvantages of different scaffold microstructures are presented in table 1.

Table 1. Scaffold microstructures and their respective advantages and disadvantages. (Modified from Haaparanta, 2015)

Scaffold microstructure	**Advantages/disadvantages**
1D fibers	Cells cultured on individual fibers. / Length and diameter of the fibers may vary.
2D substrates	Assessment of material chemistry, mechanics and micro-scale patterns. / Impermeable.
3D nanoporous hydrogel	Cells on top of hydrogel. / Interacting with 2D substrate in nanoscale. Cells encapsulated inside 3D structure. / Have to degrade the surrounding structure to extend process.
3D microporous structures	Highly porous, cells are able to spread in three dimensions. Depending on the pore size cells can be aligned in 1D, or attach to multiple struts and spread in 3D.

In addition to an appropriate microstructure, the scaffold material should be biocompatible. In biodegradable applications, the scaffold should degrade at a controllable degradation rate, to allow the healing tissue to eventually replace the scaffold. Appropriate surface chemistry is important for cell attachment, proliferation, and differentiation, and the scaffold's mechanical properties have to match those of the tissue at the site of implantation. Tissue engineering scaffolds should also be easily manufactured into different shapes and sizes. (Hutmacher, 2000; Czernuszka et al, 2002)

The choice of the scaffold material depends on the intended use. The material can be ceramic, glass, natural polymer, synthetic biodegradable polymer and in some cases, synthetic, non-biodegradable polymer. Examples of different scaffold materials are listed in table 2. (Dhandayuthapani et al, 2011; Shastri, 2003)

Table 2. Examples of different materials used in tissue engineering scaffolds. (Dhandayuthapani et al, 2011; Shastri, 2003; Amini et al, 2011)

Natural polymers	
Proteins	Silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, myosin
Polysaccharides	Cellulose, amylose, starch, chitin, dextran, glycosaminoglycans, hyaluronic acid
Polynucleotides	Deooxyribonucleic acid (DNA), ribonucleic acid (RNA)
Synthetic polymers	
Biodegradable polymers	Polylactides (PLAs), Polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) copolymer, Polycaprolactone (PCL), polyhydroxyalkanoates (PHAs), Polydioxanone (PDO), Polyhydroxybutyrate (PHB), Polyhydroxyvalerate (PHV)
Non-degradable polymers	Polytetrafluoroethylene (PTFE, teflon), extended-PTFE (Gore-Tex®), Polyethylene (PE), Polypropylene (PP), Poly(methylmetacrylate) (PMMA), Poly(dimethylsiloxane) (PDMS).
Ceramics	
Natural	Hydroxyapatite (HA), Tricalsiumphosphate (TCP)
Composition glasses	Bioactive glass
Glass ceramics	Apatite-wollastonite glass ceramic

Synthetic polymers are often cheaper than natural ones; they can be fabricated in large uniform quantities in controlled conditions, and they have a long shelf life. Due to their processability to different shapes and sizes and the possibility to control the degradation rate, porosity, microstructure, and mechanical properties, biodegradable synthetic polymers are a popular choice of material for tissue engineering scaffolds. (Dhandayuthapani

et al, 2011). There is also a growing demand for composite scaffolds. A major advantage of composite structures is that they can be tailored to meet the required conditions. (Haaparanta, 2015) A composite is a structure that consists of two or more distinctly different materials, each of which contributes to enhance the properties of the final product. The major component in the structure is usually a matrix, and another component acts as a reinforcing filler. (Hull & Clyne, 1996) In tissue engineering scaffolds, the matrix material is usually a polymer or a ceramic. The filler material can be in various forms; particles, fibres, or textiles. In tissue engineering, the most common composites are polymer-polymer compositions, and polymer-ceramic-, or polymer-bioactive glass constructions. A specific composite structure, a hybrid, combines the advantages of both natural and synthetic biomaterials. (Hull & Clyne, 1996; Haaparanta, 2015) An example of a hybrid structure is a collagen rich acellular matrix that is prepared by removing cellular components from a tissue via mechanical or chemical manipulation, and coated with a biodegradable polymer to improve mechanical stability and to enhance hemocompatibility of the protein matrix. (Dhandayuthapani et al, 2011)

The chosen scaffold material or materials, and the intended use of the scaffold, define the suitable scaffold fabrication method. Most fabrication methods involve heat and/or pressure applied to the material, or dissolving it into an organic solvent and molding the material into its shape. The scaffold structure development is affected by the fabrication method, thus the requirements of the specific tissue must be considered when selecting the appropriate fabrication technique. For example solvent casting and freeze drying produce porous scaffold structures; micromolding and emulsification produce microgels; and nanofibre electrospinning and microfiber wetspinning produce biocompatible fibres with good mechanical properties. Complex tissues that are composed of multiple cell types, as well as patient specific scaffolds, can be manufactured by inkjet-printing or computer-aided design (CAD) data manipulation techniques. (Dhandayuthapani et al, 2011)

2.2 Cells in tissue engineering

Cells that are used in tissue engineering applications can be from three different sources: autologous cells from the patient; allogeneic cells from another human donor, who is not immunologically identical to the patient; and xenogeneic cells that are from a different species. The use of xenogeneic cells in tissue engineering is controversial due to potential animal pathogen transfer. However, there has been studies whether xenogeneic cells could be used to temporarily support a failing tissue until a donor organ becomes available, or the tissue repairs itself. Each of these source categories can be further defined by whether the cells are adult stem cells, embryotic stem cells that are able to both self renew and differentiate to different cell types, or a mixture of differentiated cells at different stages of maturation. (Griffith & Naughton, 2002) In tissue engineering applications, cells are generally loaded into scaffolds *in vitro*, incubated to ensure attachment to the scaffold,

and implanted into the target tissue site (Caplan, 2007). By the definition of the Regulation (EC) No 1394/2007 tissue engineering products containing, or consisting of, engineered cells or tissues are classified as advanced therapy medicinal products (ATMPs). The regulations regarding ATMPs and packaging options for ATMPs are discussed in chapter 4.

Adult bone marrow contains multipotent progenitor cells, referred to as mesenchymal stem cells (MSCs) or adult stem cells. MSCs are able to differentiate into specific end-stage cell types, thus MSCs can be used in tissue engineering for reforming mesenchymal tissues, such as bone, cartilage, muscle, bone marrow stroma, and dermis, as well as other connective tissues, when implanted into different tissue sites. MSCs also secrete a variety of bioactive macromolecules that are part of immune response, as well as structure regenerative microenvironments at tissue damage sites. MSC-like cells have also been isolated from adipose tissue (adipose-derived stem cells, ADSC) and muscle tissue (muscle-derived stem cells, MDSC), and they exhibit MSC-like differentiation properties and specific cell surface markers. The differentiation characteristics, yield, and purity, however, are different for stem cell preparations from different tissues. (Caplan, 2007)

Embryonic stem cells (ES) have the ability to grow indefinitely and maintain their pluripotency at the same time, thus lot of expectations were loaded on human ES cells to gain knowledge on decease mechanisms, to screen new drug substances, and to treat various diseases and injuries. However, ESs are derived from the inner cell mass of mammalian blastocysts, and the use of human embryos faces numerous ethical questions and concerns. Also, an effective use of ESs would require generation of disease specific or patient specific ES cells, which has proven to be difficult to obtain. To circumvent these issues, induced pluripotent stem cells (iPSs) have been introduced. (Takahashi et al, 2007) The iPSs are adult somatic cells, such as skin fibroblasts, that have been reprogrammed by the activation of a certain number of genes (transgenes). The iPSs enable bypassing of immune rejection issues and ethical concerns related to the use of ESs, thus making patient-specific cell therapies possible. The unlimited expansion potential makes the iPSs a valuable cell source for tissue engineering applications, although there are still issues to be addressed, for instance, the efficiency of deriving specific cells from iPSs varies for each cell line. (Wang et al, 2011)

The term “growth factor” is often broadly used to describe proteins that affect cell migration, proliferation, and differentiation, in other words, the critical signaling molecules that instruct cells during their development. Growth factors can be grouped into three overlapping categories: mitogens that stimulate cell division, growth factors that induce proliferation but have also other functions, and morphogens that control the generation of tissue form. (Lee et al, 2010) In tissue engineering growth factors can be used to induce differentiation and tissue growth *in vitro*, or cell migration into the damage site *in vivo*. Growth factors can also be used to modulate the interactions between cells. (Griffith & Naughton, 2002) In tissue engineering applications growth factors are either chemically

immobilized into or onto the scaffold, or physically encapsulated in the delivery system. Popular growth factors used in tissue engineering applications are for instance: bone morphogenetic protein (BMP), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF). (Lee et al, 2010)

Cell expansion and tissue reconstruction through *ex vivo* cultures are key processes when producing engineered tissues with sufficient functionality and structural integrity. Figure 1 presents a typical cycle of autograph and allograph cell- and tissue processing for therapeutic applications. (Kino-oka & Taya, 2009)

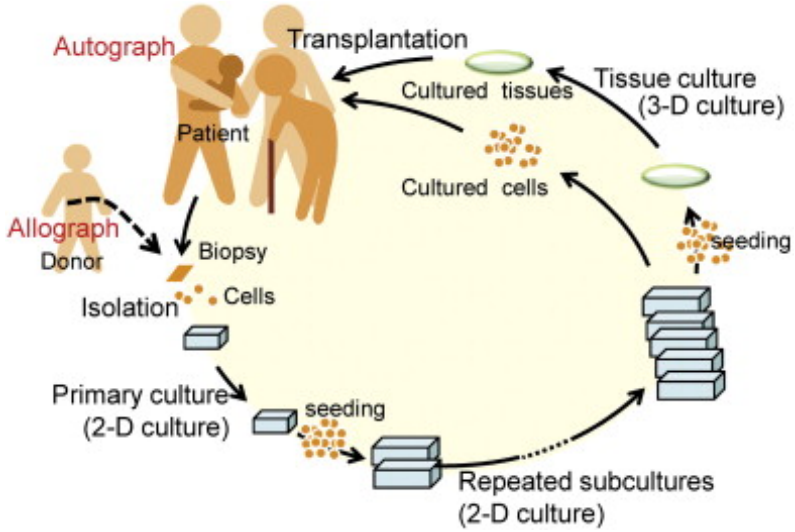

Figure 1. A schematic illustration of a typical cell- and tissue processing for therapeutic applications. (Kino-oka & Taya, 2009)

In typical cell- and tissue processing, cells are harvested by biopsy to prepare a starting cell population. The isolated cells are provided to a primary culture for acclimation, and following subcultures are repeated until a sufficient quantity of cells is obtained. Then, depending on the application, the cultured cells are either administered to the patient, or to tissue cultures for a reconstruction process to form biologically functional substitutes as final products. This kind of cell- and tissue processing can be defined as a low reproducibility tailor-made process that involves a number of manual procedures. Due to the direct use of un-sterilable products and the arduous culture operations, strict management against contamination and human error during manufacturing is essential. Innovative cell- and tissue processing techniques have been developed, but the major challenge is to upscale laboratory-scale designs into production-scale designs. (Kino-oka & Taya, 2009) The manufacturing of cultured tissues is still burdened by instability due to the qualitative fluctuation of cell sources as raw materials and the risk of biological contamination (Kino-oka, 2014).

3. MEDICAL DEVICES

Medical devices are devices that achieve their therapeutic effect via physical means, instead of a metabolic, pharmaceutical or immunological process, although the effect may be assisted by those processes. The technical revision on the European Council Directive 92/43/EEC on Medical Devices (MDD), Directive 2007/47/EC, defines a general medical device as any instrument, apparatus, appliance, software, material or other article, that is intended by its manufacturer to be used, alone or in combination or together with any accessories, for human beings for the purpose of diagnosis, prevention, monitoring, treatment, alleviation or compensation of a disease, injury or handicap; investigation, replacement, modification of the anatomy or a physiological process; or control of conception. (European Commission, 2015)

Medical devices can be classified in many different ways, but the classification based on the risk associated with misuse or failure is the most common one. The specific classification and regulatory requirements vary from country to country, but risk-based classification guides the appropriate level of manufacturing control and regulatory actions needed to ensure safe and effective products. (Bix & De La Fuente, 2009) The classification according to the European Council is discussed in chapter 3.2 and the classification according to the *U.S. Food and Drug Administration (FDA)* in chapter 3.3. The International Medical Device Regulators Forum (IMDRF) was established in 2011 on the grounds of former Global Harmonization Task Force to accelerate international harmonization of medical device regulatory convergence. It consists of a voluntary group of medical device regulators from different countries, and it provides a forum to discuss future directions in medical device regulatory harmonization. (IMDRF, 2015)

Medical devices is an extremely diverse group of devices. They vary greatly in size and complexity; from simple tongue depressors to Magnetic Resonance Imaging tunnels. Some medical devices are meant for mass markets, some are specialty items. Some are packed individually, and some in boxes of 1000s. Some medical devices are reprocessed, whereas others are disposable, and some are used for a lifetime. Risks regarding misuse and failure of the device are equally diverse, ranging from inconvenience to death. Medical devices field is constantly evolving which results in short life span of many products. (Bix & De La Fuente, 2009)

3.1 Medical devices regulation

Health-care related infections are a major concern in patient safety, even in well developed countries. Therefore manufacturing of medical devices and their sterile barrier systems are a highly regulated area, and traceability throughout the life cycle of the device

is essential. (Sterile Barrier Association, 2006) Figure 2 illustrates the life-span diagram of common stages of medical devices regulatory control.

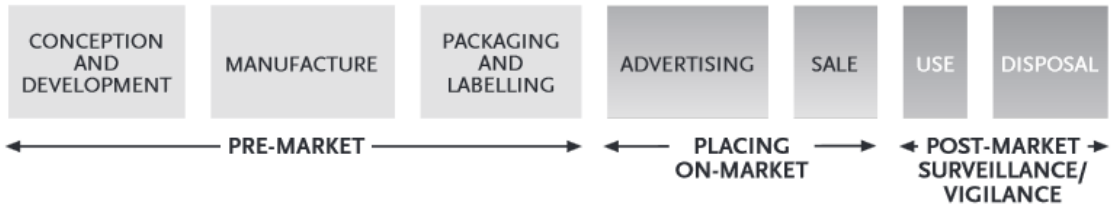

Figure 2. Common stages of government regulations on medical devices. (WHO, 2003)

Authorities in different countries have different systems for pre-market review, but they all apply risk management: medical devices must meet the safety and performance, quality system, and labeling requirements and the degree of regulatory requirements increase with the potential risks of the device. The evaluation of safety and effectiveness of medical devices is continued when they are placed in the market, for possible device failures or incidents related to misuse of the device. The actual terms of post market surveillance are also varying from country to country, but the two main activities are post-market surveillance studies and adverse event reporting. (WHO, 2003)

Quality system requirements can influence all phases in the life-span of a medical device. Applicable quality system requirements depend on the regulatory system of the country and the risk class of the device. International quality system standards are issued by the International Organization for Standardization (ISO). Quality system standards are generic management standards, therefore they are applicable to any organization. The two best known series of generic management system standards are *ISO 9000: Quality management*, for managing quality systems, and *ISO 14000: Environmental management*, for environmental management systems. The specific quality system standard for medical devices is *ISO 13485: Medical devices – Quality management systems – Requirements for regulatory purposes*. (WHO, 2003) According to ISO 13485, the manufacturer has to determine the applicable legal requirements, that is, to find out which acts, statutes, and regulations – current and the ones that are in preparation - are concerning their products and operations (Stålhberg, 2015). Although the ISO standards are widely recognized across the world, not all of them are recognized for example by the United States regulatory authorities. Medical devices regulation in the European Union (EU) and in the United States (US) are further discussed in the chapters 3.2 and 3.3.

Keeping up with legislation and regulation updates can be a major challenge to a medical device manufacturer. To find help with that task, many manufacturers participate in different medical devices societies, for example Eucomed Medical Technology, European Coordination Committee of the Radiological, Electromedical, and Healthcare IT Industry (COCIR), European Diagnostic Manufacturers Association (EDMA), and in Finland, the Finnish Health Technology Federation (Terveysteknologian liitto ry, FIHTA). Also Conforlex, a web service provided by FIHTA and Conforman Oy, offer information about

medical devices legislation internationally. Regarding regulations in the EU, the EU Law and Publications has a so called Bookshop –service, where it is possible to subscribe to a newsletter about new publications on medical devices. The most important source of information on the EU legislation, however, is the *Official Journal of the European Union*, the official publication channel for all documents regarding legislation in the EU. (Ståhlberg, 2015)

3.2 Medical devices regulation in the European Union

The core legal framework regarding medical devices in the EU consists of three *directives* (European Commission, 2015):

- Council Directive 93/42/EEC on Medical Devices (MDD) (1993)
- Council Directive 90/385/EEC on Active Implantable Medical Devices Directive (AIMDD) (1990)
- Council Directive 98/79/EC on In vitro Diagnostics Medical Devices (IVDMD) (1998)

European Council Directives are developed by the EU member states, and the member states are obligated to introduce them into their own laws. The directives replace individual national regulations. (Nolan, 2004) The directives also bind the countries that are not yet EU member states, but are applying to become a member, as well as potential future member states. The current EU member states in 2015, and the countries currently applying to become a member, are shown in table 3. Because a uniform legislation has been a prerequisite for extending the EU internal market area to all European Free Trade Association (EFTA) countries, the EU legislation also applies in the three countries that are not EU member states, but are part of the European Economic Area (EEC): Iceland, Lichtenstein, and Norway. (Ståhlberg, 2015)

Table 3. The current European Union member states (joining year in parenthesis) and countries that are currently applying to become a European Union member state (Ståhlberg, 2015).

Current European Union member states			
Austria (1995)	Estonia (2004)	Italy (1952)	Poland (2004)
Belgium (1952)	Finland (1995)	Latvia (2004)	Romania (2007)
Bulgaria (2007)	France (1952)	Lithuania (2004)	Slovakia (2004)
Croatia (2013)	Germany (1952)	Luxembourg (1952)	Slovenia (2004)
Cyprus (2004)	Greece (1981)	Malta (2004)	Spain (1986)
Czech Republic (2004)	Hungary (2004)	Netherlands (1952)	Sweden (1995)
Denmark (1973)	Ireland (1973)	Portugal (1986)	United Kingdom (1973)
Countries that are applying to become a European Union member state			
Albania	Iceland	Serbia	
Former Yugoslav Republic of Macedonia	Montenegro	Turkey	

The original directives have been amended several times, including the last technical revision brought about by the Directive 2007/47/EC. The latest consolidated versions of the directives are available at the European Commission website. In 2012 the European Commission adopted a *Proposal for a Regulation of the European Parliament and of the Council* on medical devices and *in vitro* diagnostic medical devices. When these regulations are officially adopted, they will replace the current medical devices directives. (European Commission, 2015) Changes to the directives have to be approved by the Council of Ministers and the European Parliament and thus, the process may take several years, especially if the proposed legislation is intended to create a more coordinated and centrally-acting body. (Kramer et al, 2014)

In addition to the legally binding European Council Directives there are non-binding guidance documents*: MEDDEVs*, *Consensus Statements*, and *Informative Documents*. The purpose of the guidance documents is to provide guidance in implementing the directives, and ensure a uniform application of the directives within the EU. Therefore the guidance documents are also expected to be followed, even though they are not legally binding. The three medical devices directives list the essential requirements for devices and their packaging that all medical devices sold in the EU must meet (European Commission, 2015). The AIMDD and IVDMD are not relevant to the subject of this thesis, thus they will not be further discussed.

It is the responsibility of the manufacturer of a device to conform to all of the sections of the essential requirements. Conformity assessment includes testing, inspection and certification; the procedure for each product is specified in the applicable legislation. The assessment is conducted by the manufacturer, unless the applicable legislation requires involvement of a *Notified Body (NB)*; a non-profit organization legally designated by an EU country. In that case, manufacturers are free to choose any NB from the list published and updated by the European Commission. (European Commission, 2015b) Each EU member state has a governmental *Competent Authority* that supervises the NBs and is responsible for the post-approval surveillance. The structure, personnel, functions, and funding of the Competent Authority varies between individual countries. (Kramer et al, 2014) The manufacturer, or an authorized representative, must compile a *Declaration of Conformity*, which should identify the product, the legislation according to which it is issued, the manufacturer, the NB, and reference to harmonized standards or other normative documents. *Harmonized standards* are European standards developed by a recognized European Standards Organization: the European Committee for Standardization (CEN), European Committee for Electrotechnical Standardization (CENELEC), or European Telecommunications Standards Institute (ETSI). They can be used to demonstrate that products, services, or processes comply with the relevant EU legislation. The list of current harmonized standards is published in the Official Journal of the European Union, and it is also available at the European Commission webpage. When a medical device complies with the essential requirements stated in the directives, it is marked with a *Conformité Européenne (CE) mark*, and can be legally placed into the market in any EU member country. (European Commission, 2015b) A flow chart of a general path for obtaining a CE mark is presented in figure 3.

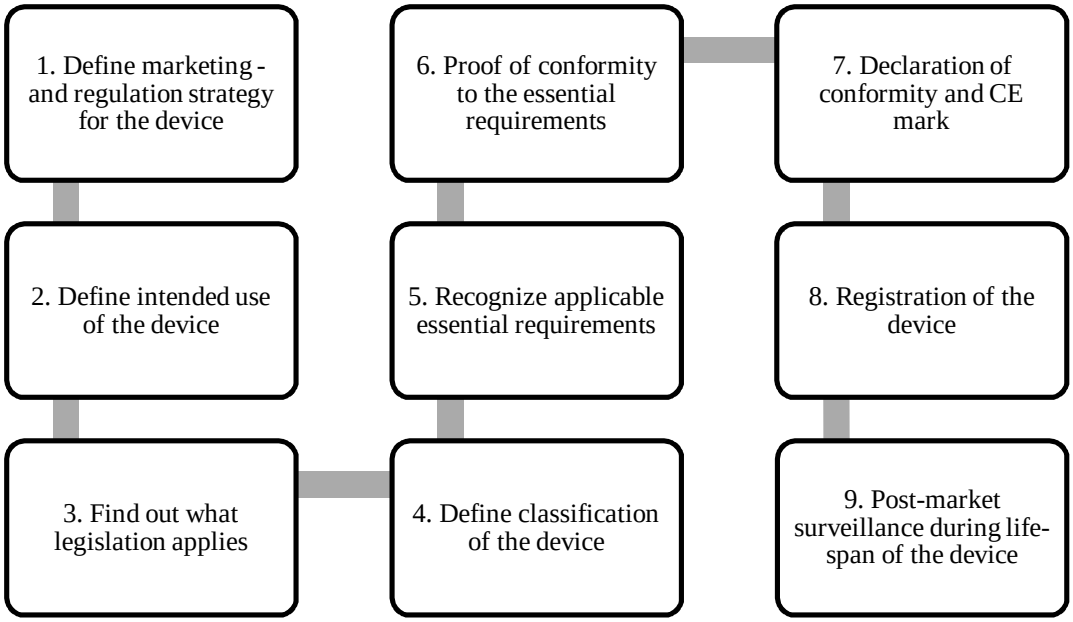

Figure 3. General steps that the manufacturer of a medical device has to take in order to obtain a CE mark. (Ståhlberg, 2015)

The MDD categorizes general medical devices in four classes: I, IIa, IIb and III. The classification is based on the duration of the use, invasiveness of the device, whether the device is reusable or intended for single use, whether it needs an outer power source to operate, and whether it is in contact with the central circulatory system or the central nervous system. Guidance document MEDDEV 2.4/1 rev. 9 contains guidelines relating to the application of the MDD. Summary of the classification criteria of general medical devices is presented in appendix A. The duration of the use of a medical device is divided in three categories: transient, short term and long term. According to the MDD transient use is intended to continue less than 60 minutes, short term use for maximum of 30 days, and long term use for more than 30 days.

As mentioned previously, all EU member states have a Competent Authority that supervises medical devices' compliance with the legislation and regulations. For example in Finland the Competent Authority is Valvira – the National Supervisory Authority for Welfare and Health. Valvira is the central ministry of Ministry of Social affairs and Health, and it is responsible for general guidance and supervision of functions under the 629/2010 Medical Devices Act, as well as other duties designated for Competent Authorities specified in the EU legislation. (Ståhlberg, 2015) Medical device legislation in Finland is listed in table 4.

Table 4. Legislation and regulations in Finland that conform to the Council Directive 93/42/EEC on Medical Devices. (Valvira, 2015)

629/2010	Medical Devices Act
Valvira 1/2010	Manufacturer's Incident report
Valvira 2/2010	Registration of Medical Devices
Valvira 3/2010	Clinical Investigations of Medical Devices
Valvira 4/2010	Healthcare professionals Incident Report
Valvira 1/2011	Conformity Assessment of Medical Devices
Valvira 2/2011	CE-marking of Medical Devices

As part of Finnish Government, the Ministry of Social affairs and Health can legislate acts regarding medical devices and provide guidance in their implementation. It also supervises medical devices sector in Finland, prepares and defines Finland's statements regarding medical devices sector in the EU, and is responsible for preparation and implementation of EU legislation in Finland. The Competent Authority also appoints and supervise NBs. Although manufacturers are free to choose any legally designated NB in the EU, it is often the easiest to choose one from the same EU member state where the manufacturer is located. In Finland, there are two NBs with wide range of qualifications: SGS Fimko Oy and VTT Expert Services Oy. (Ståhlberg, 2015)

The costs to obtain a CE certification depend on several things: which certification procedure applies to the product, whether the manufacturer does some or all of the conformity assessments themselves, and the level of support needed to prepare the required compliance documents, Technical File, user manuals, and product labeling. Manufacturers can ask and compare quotes from test laboratories, certification bodies, and consultants to get an estimated budget for their device. (Zuyderwijk, 2015)

3.3 Medical devices regulation in the United States

In the US, laws are developed via legislation, and they are vague mandates interpreted by the applicable federal agency, who writes regulations on how the law will be enforced (Nolan, 2004). In the medical devices field the applicable federal agency is the FDA, who's legal authority to regulate medical devices is based on the *Federal Food, Drug & Cosmetic Act (FD&C Act)*. The FD&C Act contains regulatory requirements defining FDA's level of control. (FDA, 2015) The Medical Device Amendments to the FD&C Act were enacted in 1976. These amendments, along with the Food and Drug Administration Modernization Act (FDAMA) of 1997 can be viewed as counterparts to the MDD directive in the EU. (van Drongelen et al, 2015)

To fulfill the regulatory requirements, FDA develops, publishes, and implements regulations. The *Federal Register (FR)* is the official daily publication for rules, proposed rules, notices of Federal agencies and organizations, executive orders, and other presidential documents. When rules are final, they are published annually in the *Code of Federal Regulations (CFR)*, which is a codification of the general and permanent rules. Most of the FDA's medical device regulations are in the Title 21 CFR Parts 800 – 1299. There is also a daily updated editorial compilation of the CFR material and FR amendments, the Electronic Code of Federal Regulations (eCFR), but it is not a legal official edition of the CFR. The FDA Safety and Innovation Act (FDASIA) that was signed into law in 2012, preserved the general structure of device evaluation, renewing user fees and performance targets through 2017. (FDA, 2015)

Companies who manufacture, repackage, relabel and/or import medical devices sold in the US are regulated by the FDA's Center for Devices and Radiological Health (CDRH) (FDA, 2015). The most important offices within the CDRH for manufacturers to know about, are the Division of Industry and Consumer Education (guidance), Office of Compliance (market surveillance, Establishment Inspection Reports, recalls), Office of Device Evaluation (pre-market notifications 510(k), pre-market approvals, Investigational Device Exemptions), Office of In Vitro Diagnostic (IVD) Device Evaluation and Safety (pre-market evaluation of IVD applications, post-market surveillance of IVDs), and Office of Surveillance and Biometrics (adverse effect reports). In addition to the federal authorities, there are local authorities in each state, and procedures between different states vary. Many, but not all, international standards are recognized in the US and are referred to as *Recognized Consensus Standards*. Recognized standards can be searched

in the database on the FDA's official webpage. Also national standardization organizations, for example Clinical and Laboratory Standards Institute, publish standards regarding medical devices. (Ståhlberg, 2015)

In the US, medical devices are classified into three classes based on the FD&C Act section 513 that establishes the risk-based classification system. Unlike in the EU, where detailed classification rules for medical devices are available, there are no detailed rules in the US, but the classification is done by the FDA. The regulatory classes are: Class I, Class II, and Class III. The classification is based on the level of control necessary to ensure a device's safety and effectiveness; Class I devices are subject to least regulatory control, and Class III devices are subject to the most stringent regulatory control. Table 5 presents the idea and descriptions behind the classifications. (FDA, 2015; van Drongelen et al, 2015)

Table 5. Classification descriptions for medical devices in the US. (FDA, 2015; van Drongelen et al, 2015)

Class I	Devices that are not intended for a use of substantial importance in supporting, sustaining, or preventing impairment of human health or life, and the devices do not present a potential unreasonable risk of illness or injury
Class II	Devices for which it is necessary to establish a performance standard, in order to ensure reasonable assurance of safety and efficacy
Class III	Devices for which there is not sufficient amount of information available to establish a performance standard. Devices that are intended to be for a use which is of substantial importance in supporting, sustaining, or preventing impairment of human health or life, devices that present a potential unreasonable risk of injury

The FDA has a database where device types and specific devices are assigned into one of these three device classes. A manufacturer can search the classification database, or go to one of the 18 medical specialty panels in which over 1700 distinct types of devices are described, and look for a predicate device for their product. If a product is completely new, the risk class cannot be established from these lists and the manufacturer has to contact the FDA for device classification. (van Drongelen et al, 2015) If an exact predicate device for a new product cannot be found, a manufacturer can submit a *513(g) Request for Information* to the FDA. The 513(g) submission should list the characteristics of the new device, and the manufacturer's rationale why it would fall into a specific class. The FDA then evaluates the information within 60 days and issues a ruling on the classification. (US Department of Health and Human Services, 2012)

The FDA can, on its own initiative or in response to a petition, reclassify a medical device based on new information. Reclassification based on new information is applicable to existing devices, and the new information must be publicly available valid scientific evidence. For novel devices, reclassification is done by the *de novo* procedure. By definition, a novel device is always classified as Class III device. For low or medium risk devices,

the manufacturer can submit a *de novo* request, which requires the FDA to reconsider the risk of the device and decide on its classification. (van Drongelen et al, 2015)

The classification regulations define the regulatory requirements for a general medical device type. The basic regulatory requirements, that manufacturers of medical devices sold in the US market must comply with, are presented in table 6. (FDA, 2015)

Table 6. The basic regulatory requirements for manufactures of medical devices in the United States. (FDA, 2015)

21 CFR Part 807	Establishment Registration
21 CFR Part 807	Medical Device Listing
21 CFR Part 807, Subpart E	Premarket Notification 510(k) *
21 CFR Part 814	Premarket Approval (PMA) *
21 CFR Part 812	Investigational Device Exemption (IDE)
21 CFR Part 820	Quality System Regulation (QRS)/Good Manufacturing Practices (GMP)
21 CFR Part 801	Labeling
21 CFR Part 803	Medical Device Reporting

* = Either Premarket Notification 510(k) unless exempt, or PMA, depending on the device. See table 7.

Medical device manufacturers and importers are required to register annually with the FDA. This process is called *Establishment Registration*. As part of the Establishment Registration process, foreign manufacturers must designate a US agent to represent them. The US agent assists the FDA in communication with the manufacturer, responds to questions concerning the manufacturer's devices, assists the FDA in scheduling inspections of the manufacturer's facilities, and receives information or documents from the FDA, in case the FDA is unable to contact the manufacturer directly. Manufacturers are also required to do a *Medical Device Listing*, in which they list their device with the FDA. Most medical devices sold in the US market need either a *Premarket Notification 510(k)* or a *Premarket Approval (PMA)*. This requirement is roughly based on the classification of the device, as presented in table 7. (FDA, 2015)

Table 7. Premarket Notification 510(k) or Premarket Approval needed, based on the device class. (FDA, 2015)

Class I	Most devices are Premarket Notification 510(k) exempt, but manufacturer must register device and company
Class II	Most devices require Premarket Notification 510(k)
Class III	Most devices require Premarket Approval

If a medical device requires a Premarket Notification, it cannot be commercially distributed before FDA authorization. There is no official form for the 510(k) application, but the requirements for a submission are described in 21 CFR Part 807, Subpart E. The 510(k) must demonstrate that the device is substantially equivalent to another safe and effective medical device that is already legally marketed in the US, and does not require a PMA. The FDA authorization is based on the information submitted by the applicant, and the decision is usually made within 90 days. (FDA, 2015) The differences between *de novo*-process, 513(g) Request for Information, and Premarket notification 510(k) processes are highlighted in table 8.

Table 8. The differences between de novo –process, 513(g) Request for information and Premarket notification 510(k).

De novo-process	513(g) Request for Information	Premarket notification 510(k)
Completely new device with no predicate devices, would automatically be classified as Class III: For low or medium risk devices, a *de novo*-request requires the FDA to reconsider the risk of the device and decide on its (re)classification.	No exact Class I or Class II predicate device can be found: 513(G) lists the characteristics of the new device, and the manufacturer's argument why it would fall into a specific class. The FDA evaluates the information and issues a ruling on the classification.	A Class I or Class II predicate device is found: Demonstrates that the new device is substantially equivalent to the predicate device that is already legally marketed in the US, and does not require a PMA. The FDA authorization is based on the information submitted by the applicant.

Medical devices that require a PMA are Class III high risk devices, or devices that are not found substantially equivalent to Class I and II predicate through the 510(k) process. The PMA process is the most stringent type of medical device marketing application, and includes a submission of technical-, non-clinical-, and clinical data. The PMA approval is based on the FDA's determination of sufficient scientific evidence of the safety and efficacy of the device. Formally, the FDA has 180 days to review the PMA and make a decision; however, in reality the review time is usually longer. (FDA, 2015)

An *Investigational Device Exemption (IDE)* allows the device to be used in a clinical study in order to collect safety and efficacy data. All clinical evaluations must have an approved IDE before the study is initiated, unless exempt by the FDA. An approved IDE allows the device to be legally shipped for the purpose of conducting investigations of the device, even if the device does not comply with other requirements for commercially distributed medical devices stated in the FD&C Act. The applicant also does not need to submit a PMA or Premarket Notification 510(k), register their establishment, or list their device, as long as the device is under investigation. They are also exempt from the quality system regulation, except for design controls (21 CFR 820.30). (FDA, 2015)

Quality System Regulation (QSR) and *Good Manufacturing Practice (GMP)* regulations include requirements for the methods and facilities for designing, purchasing, manufacturing, packaging, labeling, storing, installing and servicing of medical devices. Although widely recognized, international quality management standards ISO 9000, ISO 14000 and ISO 13485 are not recognized by the FDA, and the US definitions and qualifications for quality systems regarding medical devices can be found in the Title 21 CFR part 820. FDA does not certify quality systems, but it conducts random inspections for compliance with the Title 21 CFR part 820. Labeling regulations include the label of the device and all the informational material that accompanies the device. *Medical Device Reporting (MDR)* regulation contains mandatory requirements for manufacturers, importers and device user facilities to report device-related adverse events and problems to the FDA. From August 2015, the MDRs are accepted only in electronic format (eMDR). The reporting requirement includes death or serious injury caused or contributed by the device, as well as malfunctions that would likely to cause or contribute to a death or serious injury if they were to recur. (FDA, 2015) The current user fees for different medical devices applications can be found in the official FDA webpages. Examples of the fees related to the different application types in 2016 are presented in table 9.

Table 9. Examples of FDA user fees for medical devices application in 2016. (FDA, 2015b)

Application type	**Standard fee (US$)**	**Small business* fee (US$)**
De novo -process	No fee	No fee
513(g)	3,529	1,765
510(k)	5,228	2,614
PMA	261,388	65,347
Supplements to PMA	18,297 - 196,041	4,574 - 49,010
PMA annual report	9,149	2,287
Annual Establishment Registration	3,845	3,845

* Small businesses with an approved Small Business Deduction and gross sales of 30 million US$ or less are eligible to be waived on their first PMA

The regulatory process for medical devices in the US is illustrated in figure 4.

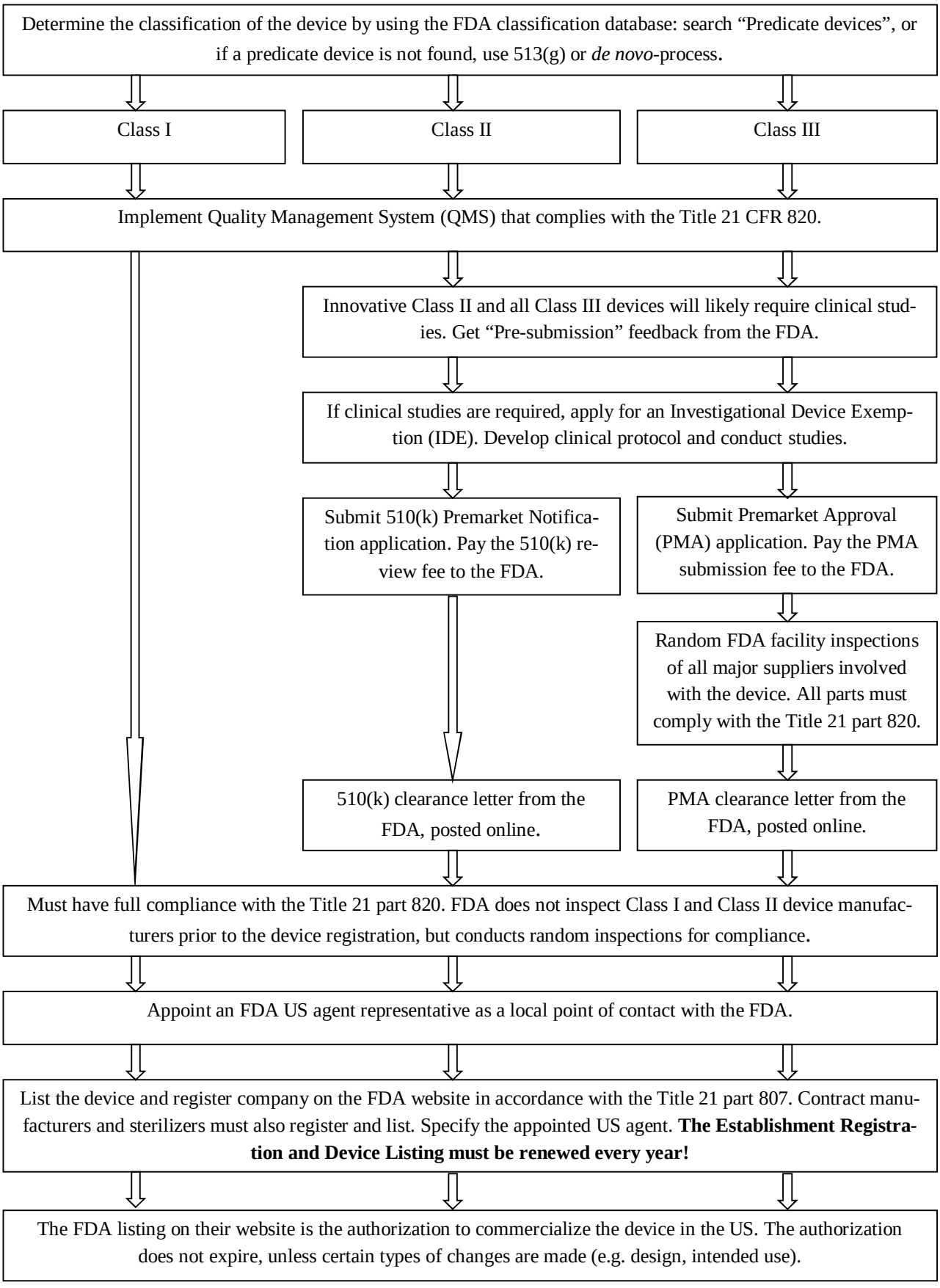

Figure 4. The regulatory process for medical devices in the United States. (Modified from Emergo Group, 2015)

4. CELL- AND TISSUE THERAPY PRODUCTS

Biopharmaceuticals include vaccinations, blood products, allergens, and products utilizing recombinant DNA techniques, such as insulins and antibodies, and is one of the fastest growing group of medicinal products. Recent additions to biopharmaceuticals group are the so called advanced therapies; gene-, cellular-, and tissue therapies. Biopharmaceuticals are considered to be the key to the future pharmaceutical treatments, and currently as many as half of all medicinal products in development are biopharmaceuticals. (Fimea, 2014)

4.1 Cell- and tissue therapy products regulation

Scientific and technological aspects of stem cell biology and tissue engineering are going through rapid developments, and the use of human cells and tissues for the treatment of various diseases and injuries has increased. The regulations are in different stages of development in different countries and the regulatory requirements are evolving, therefore there are no harmonized terminology and technical requirements in this field yet. Cell- and Tissue Therapy products (CTTs) are referred to by different terms depending on the regulatory agency. In the EU CTTs are regarded as *Advanced-Therapy Medicinal Products (ATMPs)*, and in the US CTTs are referred to as *Human Cells, Tissues, and Cellular and Tissue-based Products (HCT/Ps)*. The regulatory agencies across the globe are working to establish a risk-based system with some common features. Many regulatory controls regarding CTTs have been implemented in the last few years, and most regulatory agencies in different countries recognize CTTs as drugs or medicinal products. However, in most cases the current drug regulatory framework is not best suited for CTTs, hence separate frameworks have been established, or are currently being developed. (Kellathur & Lou, 2012)

4.2 Cell- and tissue therapy products regulation in the European Union

The EU has proposed a plan of action for the development of new biopharmaceuticals to ensure their quality, safety and efficacy. In the plan ATMPs are recognized as new product category that has to fulfill the same scientific and regulatory standards defined for other medicinal products. (Pacini, 2014) According to the definition in Regulation (EC) No 1394/2007, ATMPs contain or consist of engineered cells or tissues, and they are used in human for the purpose of regenerating, repairing, or replacing a tissue. ATMPs cannot be included in the same category with drugs or transplants because they contain viable human cells and their manufacture includes substantial manipulations. Also they may be applied for sites where they are not usually present, or to carry out biological functions in

which they do not usually participate. The ATMPs can be further divided into four different types of products: Gene Therapy Medicinal Products (GTMPs), Somatic Cell Medicinal Therapy Products (sCTMPs), Tissue Engineered Products (TEPs), and Combined Advanced Therapy Products (CATPs). CATPs, TEPs and sCTMPs can be grouped under a more general definition: Cell-based Medicinal Products (CBMPs). GTMPs differ from CBMPs because they do not contain living cells or tissue, and their medicinal effects are based on nucleic acids. (Pacini, 2014)

GTMPs contain genes that lead to a therapeutic, prophylactic, or diagnostic effect. In gene-therapy laboratory fabricated recombinant genes are inserted into the body to treat a variety of diseases, including cancer and genetic disorders. sCTMPs consist of cells or tissues that have been manipulated to change their original biological characteristics or that are intended to be used in different essential functions in the body. Somatic cell-therapy medicines can be used to cure, diagnose or prevent diseases. TEPs contain modified cells or tissues that are used to repair, regenerate or replace human tissues. A number of ATMPs combine biological materials and one or more medical devices, for example cells embedded in a biodegradable polymer scaffold (EMA, 2015). These products are called Combined Advanced-Therapy Medicinal Products (CATP) and they lie in the border of traditional pharmaceutical area and other fields, such as medical devices. Therefore they cannot be regulated as conventional drugs or medical devices, but they need adapted requirements. New technologies that utilize ATMPs include regenerative medicine, personalized treatments, and nanomedicines (European Commission, 2015c). Figure 5 presents a flow chart that helps to define in which category a biological medicinal product falls. (Pacini, 2014)

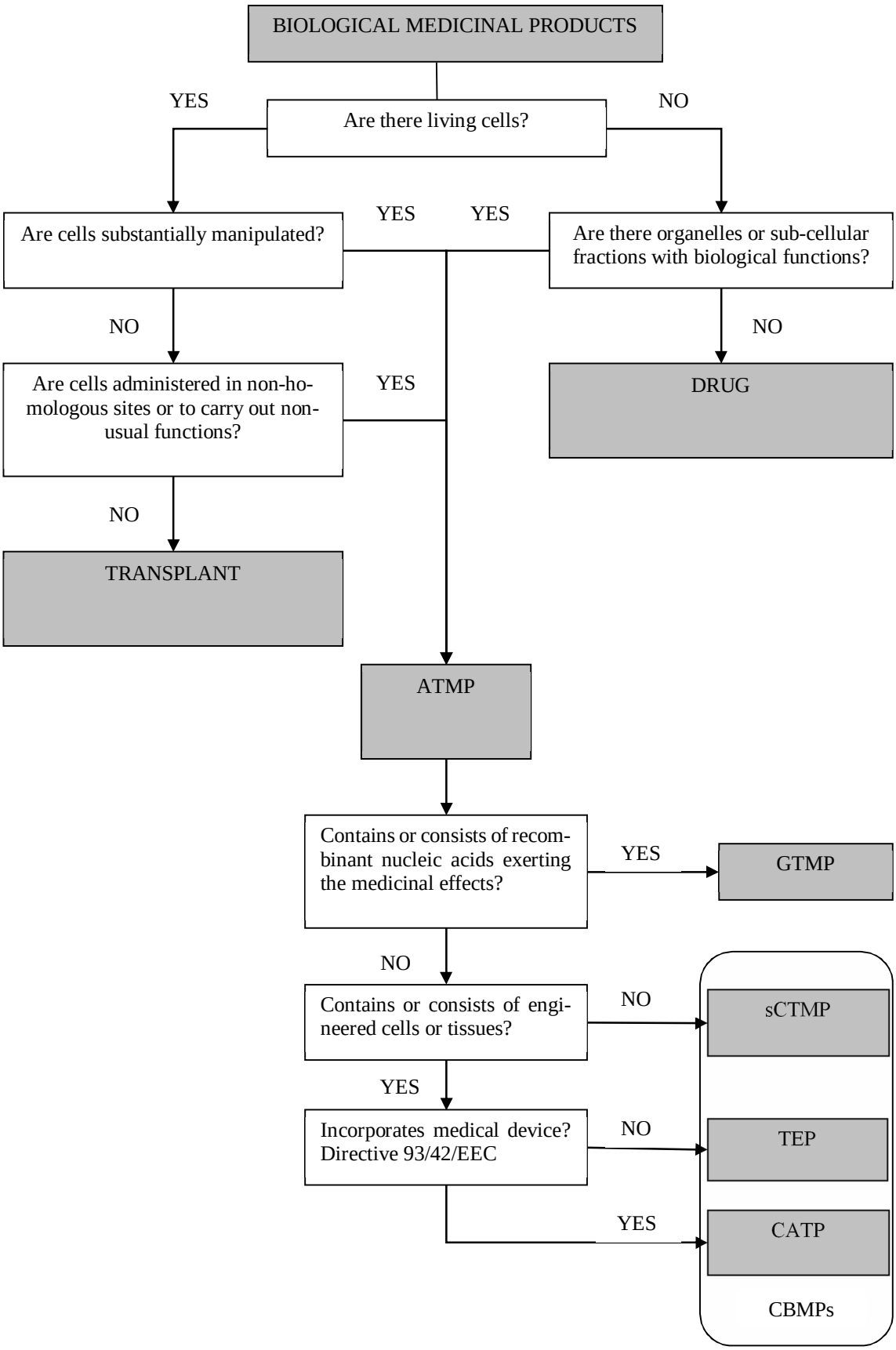

Figure 5. Flow chart for the definition of a biological medicinal product. (Modified from Pacini, 2014)

The legislative framework applicable for ATMPs in the EU is formed by the following directives and regulations:

- Regulation (EC) No 1394/2007
- Directive 2001/83/EC
- Regulation (EC) No 726/2004
- Directive 2009/120/EC

The overall regulation framework for the ATMPs is the regulation (EC) No 1394/2007. This regulation document is an amendment to both the directive 2001/83/EC that relates to medicinal products for human use, and to the regulation (EC) No 729/2004 that is related to the procedures for the authorization and supervision of medicines for human and veterinary use, and that also establishes the European Medicines Agency (EMA). The Committee for Advanced Therapies (CAT) was also established in accordance with the regulation (EC) No 1394/2007. CAT functions under EMA, and it is a multidisciplinary committee responsible for assessing the quality, safety and efficacy of ATMPs, as well as following the scientific developments in the field. The directive 2009/120/EC is also an amendment to the directive 2001/83/EC, with updated definitions and detailed requirements for gene-therapy medicinal products and somatic-therapy medicinal products. The directive 2009/120/EC contains detailed scientific and technical requirements for tissue engineered products and combined ATMPs. (EMA, 2015)

In addition to these legislation documents, the rules and regulations concerning the donation, procurement, testing, processing, storage and distribution of human cells and tissues are covered in the directive 2004/23/EC, also known as the *European Tissues and Cells Directive*. Companies developing ATMPs must also be aware of the legislation concerning different stages of the development process, including GMP and good clinical practice (GCP) requirements. Directive 2001/20/EC covers the implementation of GCP in the conduct of clinical trials. However, this directive is repealed by the regulation EU No 536/2014, which entered into force in June 2014, but will apply from May 28, 2016. All effective scientific guidelines, directives and other relevant information regarding ATMPs are published and available on the EMA website. (EMA, 2015)

As an example in Finland, the preparation of any ATMP based on cell or gene therapy or tissue modification, requires a national license from the Finnish Medicines Agency, Fimea. If it is uncertain whether the regulations concerning ATMPs are applicable to the product in question, the manufacturer must request a Fimea classification before submitting an application. The granting of the license requires that the quality and safety of the product meet the requirements, including the GMP requirements when applicable, specified for the particular product. The manufacturer must have procedures for monitoring adverse reactions and incidents, and all the materials used must be traceable. The application must also contain a risk assessment of the product based on known risk factors.

The information on which the product's safety assessment has been based (any non-clinical studies and clinical use of the product) must be included in the risk assessment. If the product has not been used on human before, additional safety studies may be needed. (Fimea, 2014b)

The first ATMP that successfully completed the entire development track from research through clinical testing to European approval, receiving European Marketing Authorization in 2009, was ChondroCelect® by TiGenix. ChondroCelect® is a cell-based medicinal product that is intended to be used in autologous chondrocyte implantation for the repair of single symptomatic cartilage defects in adults. (TiGenix, 2016)

4.3 Cell- and tissue therapy products regulation in the United States

In the US the regulation for cell- and tissue therapy products intended for human applications, referred to as HCT/Ps, consists of binding laws and regulations and non-binding guidance documents. Laws are passed by the congress and signed by the president, regulations are compiled by the FDA and approved by the executive branch, and guidance is the FDA's interpretation of the regulations, and is written and approved by the FDA. The laws concerning HCT/Ps are the FD&C Act and the Public Health Services (PHS) Act, and regulations for HCT/Ps are found under the 21 CFR Parts 1270 and 1271. The FDA supervises the entire life cycle of a medical product. Cell- and tissue products are regulated by the Office of Cellular, Tissue, and Gene Therapies (OCTGT) that functions under the Center for Biologics Evaluation and Research (CBER). (Oh, 2011) As for combination products that comprise of two or more regulated components, the FDA's Office of Combination Products (OCP) will assign one of the FDA's centers responsible for drugs, medical devices or biologics to act as the lead center, based on the primary mode of action of the combination product (van Drongelen et al, 2015).

The 21 CFR Part 1271.3 defines HCT/Ps to be articles that contain or consist of human cells or tissues, and that are intended for implantation, transplantation, infusion, or transfer to a human recipient. The FDA has implemented a risk-based approach to the regulation of HCT/Ps. The low risk products are regulated only under the section 361 of the PHS Act and 21 CFR Part 1271, and they do not require a premarket review. According to 21 CFR Part 1271.10 a product is of low risk if it fulfills the following criteria (FDA, 2014):

(a) It is minimally manipulated
(b) It is intended for homologous use only
(c) It does not combine cells or tissues with another article
(d) It does not have a systematic effect and it is not dependent on metabolic activity of living cells for its primary function, or

(e) If it has a systematic effect or it is dependent on metabolic activity, it is for autologous use, allogeneic use in first- or second degree relative, or it is for reproductive use

By the definition in 21 CFR Part 1271.3(f) minimal manipulation means such processing of structural tissue that does not alter the original relevant characteristics of the tissue, or such processing of cells or nonstructural tissues that does not alter the relevant biological characteristics of cells or tissues. If a product does not fit in these criteria, and the manufacturer does not qualify for any of the exceptions listed in 21 CFR 1271, the product is regulated as a drug, device, or biological product. In this case a premarket review is required, since the 351 of the PHS Act, and/or the FD&C Act, as well as applicable regulations including 21 CFR Part 1271 apply. (FDA, 2014)

According to Kellathur and Lou, the risk-based HCT/P regulation focuses on preventing the use of contaminated tissues with the potential of transmitting infectious deceases, such as HIV and hepatitis. On the other hand, the risk-based regulation aims to prevent contamination or damage to tissues caused by improper handling and processing. The regulation also aims to ensure that clinical safety and efficacy of the HCT/Ps are demonstrated. The regulatory framework for HCT/Ps is formed by the Good Tissue Practices (GTP) rule and GMP requirements. All HCT/Ps have to comply with GTP, and the higher risk HCT/Ps regulated under the section 351 of the PHS Act and/or FD&C Act are subjected to both GTP and GMP. The GMP requirements focus on the safety, purity and potency of the products, thus leading to high level of process control, consistency and product characterization. The GTP rule aims to prevent HCT/P contamination with disease agents, while ensuring their function and integrity. In addition, all facilities that process tissues or cells are required to register and list the establishment with the FDA. (Kellathur & Lou, 2012) A comparison of general regulatory requirements and marketing pathways for low and high risk single entity HCT/Ps, and medical devices are presented in table 10.

Table 10. Comparison of general FDA regulatory requirements and marketing pathways for single entity HCT/Ps, and medical devices. (Modified from Oh, 2011)

	Low risk HCT/P	High risk HCT/P	Medical devices
Applicable laws	361 PHS Act	361 PHS Act 351 PHS Act FD&C Act	FD&C Act
Applicable Regulations	21 CFR 1271	21 CFR 1271 21 CFR 600 21 CFR 300 21 CFR 200	21 CFR 800
Marketing pathway	No premarket review required	Biologics License Application (BLA)	510(k), PMA

The Biologics License Application (BLA) is regulated under the 21 CRF 600 – 680, and it is a permission to introduce, or deliver for introduction, a biologic product. It is an equivalent to PMA in medical devices. A BLA can be submitted by any legal person or entity who is involved in the manufacturing of the product, or an applicant who takes responsibility for compliance with product and establishment standards. The specified requirements for the applicant information, product/manufacturing information, pre-clinical studies, and labeling are found in the Form 356h. (FDA, 2010)

Combination products have become a large and growing segment of the medical device market. In 2011 they represented estimated over 30% of all new product submissions to the FDA. (Richter, 2011) The FDA defines combination products as products that comprise of two or more regulated components, such as: drug/device, biologic/device, drug/biologic, or drug/device/biologic, that are physically, chemically, or otherwise combined or mixed and produced as a single entity. Combination products can also be separate products that are packaged together in a single package or as a unit, as well as a drug, device, or biological product packaged separately but intended for use only with an approved, individually specified drug, device, or biological product where both are required to achieve the intended effect. The constituent parts of a combination product keep their regulatory status (for example as a device or drug) after they are combined. Also the GMP/QSR requirements that apply to each of the constituent parts continue to apply after the parts are combined to make a combination product. (Federal Register, 2013) The manufacturer must define the primary mode of action of the combination product, and submit a Request For Designation (RFD) to the FDA's OCP, which determines the primary mode of action. The primary mode of action dictates weather the foundational framework will

be medical device QSRs (21 CFR Part 820), drug GMPs (21 CFR Part 210/211), or biologic GMPs (21 CFR Part 600 – 680 and 21 CFR 1271). (Richter, 2011) While the potential of combination products in improving patient care and providing safer and more effective treatments has contributed to the rapid market growth, the regulatory framework continues to raise a variety challenges to the industry. The diversity in the types of combination products means that there is no general regulatory pathway that would apply to all. (Siew, 2014)

5. PACKAGING OF MEDICAL DEVICES

When deciding a suitable package for a medical device, certain questions need to be considered: What is the shape and mass of the product? What are the sterilization requirements? Are there any moisture retention or exclusion requirements? What is the intended use of the device and who are the end users? (Turner, 2011) Estimated 10% of all medical device recalls are due to packaging failures, and 31% of those are due to a hole in the packaging. Therefore, the correct selection of packaging is crucial. (Cleanroom Technology, 2014)

The packaging of a medical device generally has three main functions: protection, utility and communication. **Protection** means protecting the device from the environment and vice versa. Protection is necessary for maintaining package integrity for its lifespan from sterilization to shipping, storage, handling and use. (Bix & De La Fuente, 2009) If a device has a shelf life, the packaging has to maintain the device and ensure its functionality for the stated period of time. The term *shelf life* should not be confused with the term *useful life*. According to the FDA, useful life is the duration of actual use, or the number and duration of repeat uses of the device, before there is an impact to the device's ability to achieve its intended function. An *expiration date* means the termination of shelf life, after which the device may no longer function as intended. (Teixeira, 2014) Typical protection wanted from packaging is protection from shock, vibration, crushing, puncturing, tearing, bursting, splitting, humidity, and heat. One of the most vital protective functions of medical devices packaging is maintaining of the sterile barrier system. For sealed packages, seal integrity is an equally important characteristic. (Bix & De La Fuente, 2009) The packaging material supplier should provide information about materials' resistance to microbial access, mechanical properties, robustness and integrity, suitability for sterilization, and biocompatibility (Turner, 2011).

Utility is related to the usability of the packaging system. It is said that user interactions with the packaging of a medical device can be as important as interactions with the device itself. (Wiklund et al, 2010) In many cases medical professional base their decision on superior medical technology, but in case of two or more equal products, other factors emerge – packaging of the device being one of them. For many medical devices quick and easy opening and removal of contents without contamination is crucial. Packaging design plays the most important role in the opening function. In some cases, the package may also have additional functions beside protection, such as a measuring device, dispenser, stabilizing stand or a disposal bin. (Bix & De La Fuente, 2009; Crosby, 2008) A usability test with packaging and the enclosed materials helps to evaluate how users interact with the packaging and set up the medical device for use. Characteristics that can be assessed during a usability test are for example (Wiklund et al, 2010):

- Ease of lifting and carrying the package for short and long distances (package shape, weight, and handle design)
- Conspicuousness and comprehension of labels, instructions, warnings, and advertising
- Ease of opening the package (tearing open cardboard flaps, opening plastic bags, uncapping vials, peeling paper liners from plastic trays, removing seals) while maintaining sterility of the contents
- Ease of identifying and distinguishing between package contents
- Ease of identifying and distinguishing between packaging for similar devices or products (e.g. two different concentrations of the same product)
- Ease of removing package contents without damaging or contaminating them
- Ease of handling the package and components with one hand (if the other is used to perform another task)
- Package durability (i.e. resistance to damage during handling)
- Ease of storing the package (e.g. appropriateness of the overall size and shape of the package)
- Acceptance of the amount of material waste
- Visual appeal of the shape, graphics, and overall visual design of the package
- Legibility and conspicuousness of printed information, such as warnings and expiry dates, which might be essential to risk mitigation
- Ease of determining is the contents are damaged
- Ease of placing components back to the package

When deciding how to conduct the usability test, the end users of the product must be considered (Wiklund et al, 2010). The Institute of Packaging Professionals has conducted a survey among the Association of Perioperative Registered Nurses about preferred packaging of medical devices. The results are presented in figure 6. (Crosby, 2008)

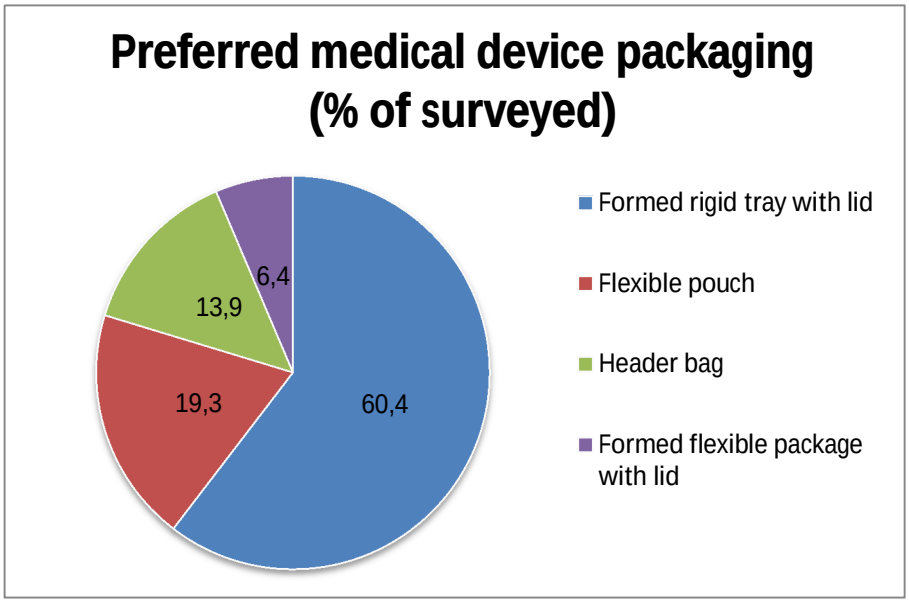

Figure 6. Preferred packaging of medical devices according to a survey among the Association of Perioperative Registered Nurses. (Crosby, 2008)

According to the survey, 84.4% preferred a double-barrier sterile system. Other important factors that came up were the ability to read the label, especially the sterility indicator and the expiration date, and the ability to quickly open the package during a surgical procedure. (Crosby, 2008)

A Voice of the Customer –survey and live nurses' panel was conducted at HealthPack in Luisville, KY, US in 2013. The surveyed nurses were from EU and the US. Also in this survey double sterile barrier packaging, consisting of a sealed tray within another tray or a pouch was a preferred packaging. Clear packaging was thought ideal, and easy opening is essential. All surveyed said they check the use before –dates, which should be easily observed. Instructions for use provided on CDs was not preferred, since there is often no access to a computer in the operating room. Recycling of materials is a growing interest also among nurses. (Allen, 2013b)

Secondary and primary medical device packages are also a means to **communicate** information through materials, graphics and shape. The level of packaging communication varies depending on the type of the medical device. Typically the communication role involves passing important information for the safe and effective use of the device; directions, warnings and product benefits. It may also include brand differentiation, motivation for purchase and so on. A very important function of package communication is product identification. This is crucial especially for medical devices that are used in hospital setting, where personnel may have only seconds to identify the correct device. (Bix & De La Fuente, 2009) With medical devices the requirement of cleanliness is a key element, and has to be reflected in the visual appearance of the device packaging as well (Cleanroom Technology, 2014).

The manufacturers of medical devices must provide essential information in several languages, which can affect the legibility, visibility and accuracy of the information. They also have to make sure that components of the package, like adhesives and inks, do not interfere with the product's safety and efficacy. If any of the components of the package migrate to the device, it is important that these unintended additives are nontoxic and do not affect the performance of the device. (Bix & De La Fuente, 2009) For sterile medical devices, the packaging materials also need to be compatible with the chosen method of sterilization. (Teixeira, 2014).

Environmental issues and waste reduction are also important factors to consider. Biodegradable and compostable bioplastics, such as PLA, have been used in food packaging industry for years. In their current form, however, they have some limitations with impact strength and heat deflection properties compared to traditional plastics, thus making them unsuitable packaging materials for sterile barrier systems. (Crosby, 2008)

5.1 Packaging of sterile implantable medical devices

The ISO 13485 standard defines that an implantable medical device is a device that is intended by surgical intervention to be totally or partially introduced into the human body or natural orifice, or that is intended to replace an epithelial surface or the surface of the eye. An implantable medical device is intended to remain at least 30 days after the procedure and it can only be removed by medical or surgical intervention. The ISO 13485 also states that a sterile medical device refers to a category of devices that are intended to meet the requirements for sterility.

Implantable medical devices can be for example prosthetics intended to replace a missing body part, or they can be used to deliver medication, monitor body functions, or provide support to organs and tissues. Implantable medical devices can be permanent, or they can be removed when no longer needed. (FDA, 2015) Implantable medical devices include a wide variety of different kinds of devices. Thus when choosing a suitable packaging for a sterile implantable medical device, the manufacturer has to consider the critical product characteristics, types of protection needed, type of sterilization process, and where, and how, the product is going to be dispensed. (Bix & De La Fuente, 2009). Part of this evaluation is the defining of environmental factors: characteristics that may have an impact on the device or its components during transport, storage, or use. Environmental factors include the conditions that can affect the device itself, the user of the device, or the patient in the intended use environment. Environmental conditions to be considered can be such as: temperature, humidity, moisture, atmospheric gas composition or pressure, energy, vibration, motion, lighting, and shock. (Teixeira, 2014)

Sterility and its maintenance, as well as prevention of cross-infection, are the most critical factors in patient care. The packaging around the medical device allows the device to be sterilized, provides a microbial barrier, and maintains the sterility of the device. (Sterile

Barrier Association, 2006) *A sterile barrier system* is the minimum packaging configuration that provides a microbial barrier, and allows aseptic presentation of the product at the point of use. A sterile barrier system differs from *protective packaging*, which is designed to prevent damage to the sterile barrier system and its contents. A *packaging system* is the combination of the sterile barrier system and the protective packaging. (Sterile Barrier Association, 2006) Inappropriate sealing compromises the package integrity, hence sealing of a sterile medical device package is critical. Seals can be weld or peelable. Weld seals are not intended to open, and they are produced by heat or ultrasonic. Peelable seals are designed to open, and they are achieved either with, or without heat (cold seals). Peelable cold seals cannot be resealed when opened, thus they can also be used as tamper evidence. (Bix & De La Fuente, 2009) There are over 100 standards and guidance documents relevant to sterile barrier systems, the full list is presented in appendix B.

According to the MDD, sterile barrier systems are considered to be *accessories* to medical devices. The MDD defines an accessory as "an article which, whilst not being a device, is intended specifically by its manufacturer to be used together with a device to enable it to be used in accordance with the use of the device intended by the manufacturer of the device". The directive also states that accessories are treated like medical devices in the requirement to comply with the essential requirements, however, according to MEDDEV 2.1/1 accessories do not usually follow the classification of the medical devices with which they are used. (European Commission, 2015) The essential requirements for devices that are delivered in sterile state according to the MDD Annex 1 are:

(a) Devices delivered in a sterile state must be designed, manufactured and packed in a non-reusable pack and/or according to appropriate procedures to ensure that they are sterile when placed on the market, and remain sterile under the storage and transport conditions laid down, until the protective packaging is damaged or opened
(b) Devices delivered in a sterile state must have been manufactured and sterilized by an appropriate, validated method
(c) Devices intended to be sterilized must be manufactured in appropriately controlled (e. g. environmental) conditions
(d) Packaging systems for non-sterile devices must keep the product without deterioration at the level of cleanliness stipulated and, if the devices are to be sterilized prior to use, minimize the risk of microbial contamination; the packaging system must be suitable taking account of the method of sterilization indicated by the manufacturer

The MDD Annex 1 also lists the information the manufacturer has to provide with the device. Each device must have with it enough information for safe and proper usage, considering the knowledge and training of the intended users. The information also has to identify the manufacturer of the device. If possible, the information needed for safe usage must be placed directly on the device itself, the packaging of each unit, or on the sales packaging. If the devices cannot be packaged individually, a leaflet with proper information has to be provided with a package of one or more devices. Class IIb and III

classified medical devices, and devices which intended purpose is not obvious, must contain instructions for use. The current regulations for the information the manufacturer of a medical device must provide in the label of the device and in the instructions for use are stated in the MDD Annex 1. The FDA labeling regulations can be found under 21 CFR Part 801. The terms *label* and *labeling* are similar, but according to the US FD&C Act, Section 201(k), there is a difference between them. The Act defines that the term label refers to written or printed information on the device or on its packaging, and labeling refers to the full collection of device related labels and documentation, including those that might not be part of the device or its packaging.

The MDD Annex 1, section 5, states that the devices must be designed, manufactured and packed in such a manner, that their characteristics and performances are not altered or adversely affected during their intended use as a result of transport and storage. The Annex 1, section 8, specifies the requirements for infection and microbial contamination control as it relates to packaging.

An essential standard to the development and validation of a packaging system for a sterile medical device is the *ISO 11607: Packaging for Terminally Sterilized Medical Devices*. ISO 11607 Part 1 contains the requirements and test methods for materials, preformed sterile barrier systems, sterile barrier systems and packaging systems, and ISO 11607 Part 2 contains the requirements for development and validation of processes for packaging medical devices that are terminally sterilized. (Nolan, 2004). The ISO 11607 is also an FDA's Recognized Consensus Standard (FDA, 2015). It is applicable in all facilities where medical devices are packaged in sterile barrier systems and sterilized, but it does not cover all the requirements concerning sterile barrier systems and packaging systems for aseptically packaged medical devices. (ISO 11607, 2006) The informative document ISO/TS 16775:2014 provides guidance for the application of the requirements in the ISO 11607-1 and ISO 11607-2 standards. Another important standard for terminally sterilized medical devices is the *EN-868 Packaging for terminally sterilized medical devices*. It provides specific requirements for many kinds of medical packaging, for example sterilization wraps, paper, paper bags, pouches and reels, paper for low temperature sterilization, adhesive coated paper, containers, nonwoven (uncoated) and nonwoven (adhesive-coated).

5.2 Packaging of sterile biodegradable implantable medical devices

Problems with traditional permanent implants – such as long term compatibility, possible migration, breakage, material reactions, and revision and removal surgeries - has led to development of biodegradable implants and devices. Non-permanent, biodegradable implants offer an alternative for patients, as they provide temporary support, and degrade in the body at a rate matching new tissue formation, thus removing the need for secondary

surgeries. Sutures, orthopedic fixation devices, tissue engineering scaffolds, dental implants, tissue staples, skin covering devices, and drug delivery devices are examples of commercially available biodegradable devices. (Amini et al, 2011; Bernkopf, 2007)

Biodegradable polymers can be of natural origin or synthetic, and they degrade through hydrolysis or enzymatic degradation. A variety of biodegradable polymer groups have been developed, such as poly(α-esters), polyphosphanezes, and polyurethanes. The most studied synthetic biodegradable polymers are poly(α-esters); PGA, PLA, and their copolymer PLGA. Also polydioxanone (PDO), polycaprolactone (PCL), polyhydroxybutyrate (PHB), and polyhydroxyvalerate (PHV) have been accepted for use in biodegradable medical devices. Also natural biodegradable polymers like chitosan, silk and starch, as well as biopolymers like collagen, elastin and hyaluronic acid have been used in developing biodegradable implants. (Amini et al, 2011)

Over the last decade, biodegradable metals have also emerged as an alternative to biodegradable polymers. Biodegradable metals degrade gradually in the body by corrosion, to eventually dissolve completely as the tissue they have been assisting has healed. The corrosion of a biodegradable metallic device generally proceeds by an electrochemical reaction with an electrolyte. Biodegradable metallic devices can be classified as pure metals, consisting of only one metallic element; biodegradable alloys, metals with varying microstructure and at least one alloying element; and biodegradable metal matrix composites, with all biodegradable components and the major component being a biodegradable metal. Metals that have been most investigated for biodegradable applications are magnesium and its alloys, and iron and its alloys. Also other metals, like wolfram, zinc and its alloys, and calcium- and strontium based biodegradable metals have been studied. The development of biodegradable metallic devices is still in the early stages and they are not yet commercially available, but they can potentially be adopted for applications like cardiovascular stents, bone implants, and wound closing devices in the future. (Zheng et al, 2014) Since biodegradable metals are only at early research stage, they are not further discussed in this thesis.

In addition to the packaging requirements for sterile implantable devices, discussed in the chapter 5.1, biodegradable implantable devices have special features that need to be considered when designing their package. Biodegradable devices are hydrolytically unstable, thus even a small amount of moisture can cause degradation during the storage of the raw materials, during the device manufacturing process, and during the storage after the device fabrication. (Bernkopf, 2007) Therefore, biodegradable devices have to be packaged as quickly as possible after fabrication, usually under an inert atmosphere or vacuum. Double bagging is generally used, and the bag material must be very resistant to water permeability – most often a polymer material or foil. Biodegradable devices are typically stored in a freezer to minimize the effect of moisture, but the packaged product should always be at room temperature when opened to avoid water condensation. The handling of the device at ambient conditions should be kept at minimum; however, studies have

shown that biodegradable polymeric devices can remain stable also at room temperature for over two years, as indicated by molecular weight retention, when packaged in desiccated moisture proof bags. The final packaging solution for a biodegradable implantable medical device should be an air-tight moisture proof container. (Middleton & Tipton, 2000) Biodegradable devices degrade fully in the body, therefore it is necessary that the complete product is sterilized before the application. It may sometimes be challenging to find a suitable sterilization method, because most biodegradable polymers are also sensitive to high temperatures. (Bernkopf, 2007)

5.3 Packaging of advanced tissue engineering products

Advanced tissue engineering products, combination devices, impose new challenges on packaging technology by adding complexity to the packaging system, and creating challenges with self-life stability, solvent loss, moisture and oxygen protection, and package testing. Also additional package qualifications may be needed, similar to pharmaceutical products. (Crosby, 2008) The practice of aseptic processing of solid combination devices has only recently been considered. Thus far, combination products with the device as the primary mode of action have most often been sterilized using end sterilization. However, the application of end sterilization to combination devices is also limited due to material compatibility issues. Therefore, as the combination product market continues to expand and evolve, so does the need for new applications of end sterilization solutions. (Lambert et al, 2011)

CTTs typically fall into one of the following categories (Crosby, 2008):

- Physically or chemically combined into one entity
- Co-packaged
- Separately provided cross-labeled products
- Separate investigational products with proposed cross-labeling

The key component of packaging of CTTs is the barrier protection. Moisture barrier protection is found in a few rigid plastic tray packaging applications. The best known technology is a clear poly-chloro-tri-fluoro-ethylene film, Aclar, laminated on PVC or polyethylene terephthalate (PET). The thickness of the Aclar structure depends on the targeted *moisture vapor transmission rate*, but it is usually between 0.015 – 0.075 millimeters. Aclar/PVC structures are common in pharmaceutical industry, whereas Aclar/PET structures are a growing area in combination products and biologics. Aclar laminate, however, has a limited draw depth, thus thermoformed trays with Aclar laminate can be no deeper than 2.54 centimeters (one inch). (Crosby, 2008)

Another moisture barrier option for CTTs is a co-extrusion that utilizes cyclic olefin copolymers as a core, covered with either PET or PP, a technology that is developed specifically for the combination product market. Due to the co-extrusion technology, the

draw depth is up to 12.5 centimeters allowing more variation in package size. The cyclic olefin copolymer core is typically 0.13 – 1.02 millimeters thick, depending on targeted moisture vapor transmission rate. Cyclic olefin copolymer is an amorphous material with good clarity properties, and it can be sterilized by irradiation and EtO. (Crosby, 2008) Moisture vapor and oxygen transmission rates of various packaging films are presented in the next chapter.

6. PACKAGING SOLUTIONS FOR STERILE BIODEGRADABLE IMPLANTABLE MEDICAL DEVICES

As discussed in the previous chapter, biodegradable medical devices are hydrolytically unstable, thus they need to be packaged in an air-tight, moisture proof container. The packaging material in contact with the device, the primary packaging, must be non-toxic. Biodegradable implants are delivered sterile; therefore they need to be either terminally sterilized or aseptically processed. Packaging has to provide a sterile barrier and protection of the product to ensure the product's integrity all the way to the end user. The secondary packaging that is not in direct contact with the product, offers further protection, a way to display the product as desired, as well as functions as a means of communication. The compatibility of the product and the packaging materials with the chosen sterilization method has to be confirmed. Different sterilization methods and aseptic processing are reviewed in chapter 7. Biodegradable devices are often packaged under an inert atmosphere or vacuum to remove air-containing moisture, and double packaging is generally favored. In this chapter multiple packaging solutions that are, or could be, suitable for sterile biodegradable tissue engineering products are presented.

Typical materials for sterile barrier systems are plastic films, coated or uncoated papers, and non-wovens. Key properties for sterile barriers are impermeability for films, or the microbial barrier for porous materials, biocompatibility, physical and chemical properties, compatibility with the manufacturing and sterilization processes, and stability after sterilization. Common sterile barrier packaging formats are pre-formed custom trays with die-cut lids and premade bags and pouches. (Sterile Barrier Association, 2013b)

Moisture barrier materials can be selected from plastics, metallized plastics, laminated metal foil/plastic composites, or welded metal foils. Plastic materials, however, do not usually have good barrier properties for both water and oxygen. Therefore composite films are commonly used. (Morgan Advanced Materials GmbH, 2014) The barrier film structure is typically a laminated multilayer. Functional layers enable property tailoring such as formability, mechanical strength, oxygen and water vapor barrier properties, as well as heat sealing. Multilayers also enable the use of thinner films without compromising security, thus giving cost benefits. (Cleanroom Technology, 2014) Aluminum foil is usually the best option for metal foil barrier packaging due to its ductility, availability, and cost. Two properties are typically reported for all barrier materials: Water Vapor Transmission Rate (WVTR; $g/m^2/24h$) and Oxygen Transport Rate (OTR; $cm^3/m^2/24h$). (Morgan Advanced Materials GmbH, 2014) Table 11 presents the OTR and WVTR values for several flexible packaging films.

Table 11. Oxygen and water vapor barrier properties of several flexible packaging films. (DuPont Teijin Films, 2001)

Film type	**Thickness (µm)**	**Oxygen transmission $cm^3/m^2/24h$ (100% O_2, 25 °C, 45% RH)**	**Water vapor transmission $g/m^2/24h$ (38 °C, 90% RH)**
Mylar® polyester	12	140	40
Metallized Mylar® (OD3)	12	0.5	<1
Polyvinylidene (PVdC) coated polyester	15	6	14
Cellulose (plain)	22	8-130 (depends on moisture)	3,500
Nitrocellulose coated cellulose	30	10	12
PVdC coated cellulose	28	8	5
LDPE	25	8,000	18
HDPE	25	3,000	9
Ethylene vinyl acetate	25	10,000	70
Propafilm™ C28	28	10	5
Propafilm™ CR	26	25	4
Propafilm™ MG	20	2,200	7
Propafoil™ (metal-lized)	25	100	1.5
Cast PP	25	4,200	12
Cast nylon	50	140+ (depends on moisture)	35
Cast nylon 66	30	80+ (depends on moisture)	180
Oriented nylon 6	15	45 (depends on moisture)	260
EVAL™ F	20	0.2 (depends on moisture)	75
EVAL™ E	20	1.8 (dependant)	29
PVC (plasticized)	20	2,000+ (depends on moisture)	200+
PVC (rigid)	20	260	60
PVdC (extruded)	20	3	5
PS (oriented)	25	2,500+	170
Nitrile barrier resin	20	16	120
Aluminium foil	9	0+ (depends on pinholes)	0+

In addition to the films listed in table 11, for example biaxially-oriented polyvinyl alcohol (BOPVA) and biaxially-oriented PP (BOPP) have good barrier properties against oxygen and moisture, and therefore they are widely used to food- and pharmaceutical packaging materials. For both BOPVA and BOPP the transmission rates are dependent on moisture and temperature. (Mo et al, 2014) Recently new ultra-high barrier films that utilize nanotechnology have emerged. Tera-Barrier Film Pte, Ltd has developed a novel transparent micro-pore-plugging film that claims to offer 10 times better moisture barrier than traditional transparent barrier films. Traditional films commonly have pinholes, cracks and grain boundaries that affect their barrier performance. The ultra-high barrier film plugs the defects at nanoscale with reactive and non-reactive nanoparticles that also react with and retain moisture and oxygen. Traditionally used aluminum foil also has excellent barrier properties, but compared to the ultra-high barrier film it has some disadvantages: it has an energy-intensive manufacturing process, it is not transparent, it is not stretchable,

and it cannot be use for packaging that has to go through metal detection or radio-frequency identification integration, for instance. (Lingle, 2014)

6.1 Bags and pouches

Bags and vented flexible packaging are frequently used for packaging of medical devices, primarily due to their low costs and suitability for high profile items (figure 7).

Figure 7. Above left: SteriVent® vented bag by SteriPak. Above right: QSEAL® pouches by QTS. Below left: Header bags by SteriPak. Below right: Laminated foil pouch by Schott.

Vented bags offer a clean, strong packaging. Vented bags are made of film, and they have small windows with breathable patches made of paper or Tyvek®, that enable gas sterilization. Tyvek® is a thin nonwoven olefin fibre fabric developed by DuPont. It is breathable, waterproof, and tear resistant. Header bags are also made of plastic, but instead of breathable patches, there is a peelable paper on Tyvek® strip on top of the bag. Header bags are also suitable for aseptic presentation, whereas vented bags are not. Flexible non-formed pouches can be flat or gusset pouches and they are usually used for single use items, such as catheters and tubing. Flat pouches are made from two webs and sealed along the perimeter. Flat pouches with the right material combination can be sterilized by all commercial sterilization methods. If non-permeable materials are used, sterilization options are limited to radiation or under controlled conditions, steam. In gusset pouches one web is gusseted on the sides or the bottom to make the pouch more suitable for higher

profile products. Flexible pouches provide a sterile barrier, the ability to withstand sterilization, and when properly designed, easy opening function. Different peel pouches can be run on form-fill-seal machines. (Bix & De La Fuente, 2009) The product should be immobilized inside the pouch to avoid its free movement and piercing or other damage to the pouch material or seal. Pouches are used for packaging of many different kinds of items, but due to the lower physical protection qualities, they are generally best suited for flat, low bulk products. (Turner, 2011)

Moisture sensitive items are generally packaged in barrier bags. There are two primary technologies that are used for moisture barrier bags: barriers of aluminum foil, and aluminized polymer. Foil/polymer is the oldest barrier technology, where a thin sheet of aluminum foil is laminated to nylon or Tyvek®. Aluminized polymer is a newer technology in which aluminum vapor is deposited onto polyester. The metal layer is so thin, that multiple layers of aluminized polyester can be laminated together, thus voids in one layer are covered by another layer. (Beamer, 1997) Examples of moisture barrier pouches are presented in figure 8.

Figure 8. Above left: Amcor high barrier packaging for medical devices. Above right: 3M™ Moisture Barrier Bag Dri-Shield 2700 with a humidity indicator card and desiccant. Below: Barrier pouches by Oliver-Tolas Healthcare Packaging for packaging of sensitive medical, pharmaceutical, biological or combination devices.

Common film structures for pharmaceutical and biotech packaging are for example: aluminum foil – PE (foil thickness ≥ 25 µm); OPA – aluminum foil – PE; Polyesther film – aluminum foil – PE; paper – aluminum foil – PE; aluminum foil – heat seal lacquer. (Flexifoil Packaging PVT LTD, 2012) Amcor is also offering a metallized heavy gauge PP film laminate structure for medical devices and orthopedic devices packaging. The metallized PP film laminate provides excellent moisture and UV barrier, yet is more resistant to flex-cracking than aluminum foil laminates and has better chemical resistance

due to the film facing sealant. (Amcor Limited, 2016) Also clear plastics without metal layers can be used as moisture barrier in some cases, but they offer limited protection and are only suitable for very short term dry storage or clean room situations. Even barrier bags are not completely moisture proof and over time moisture vapor will leak into the bag. Desiccants can be inserted into the bag to reduce the effects of humidity. Also humidity indicator cards with moisture sensitive color-changing chemical spots can be used to indicate the relative humidity inside the barrier bag. (Beamer, 1997)

6.2 Blisters and strip packs

Blisters are commonly used in pharmaceutical industry for single-dose packaging of tablets and capsules. Blisters packs are also suitable for heavier items, or products with edges or corners. Blisters are designed to restrain the product and they have an added advantage of presenting the product for the end users in a desired orientation. For example orthopedic implants are typically packed in blisters. (Turner, 2011)

Blister packs compose of a cavity or pocket made from a formable film, commonly a thermoformed plastic or cold formed aluminium film, and typically an aluminium foil lidding seal. Blister packs can be divided into three types based on the material and principle of forming: cold formed aluminium/aluminium blister packs, thermoformed aluminium/plastic blister packs, and aluminium/plastic/aluminium blister packs. Examples of the three blister pack types are shown in figure 9. (Jornen Machinery Co., Ltd., 2015)

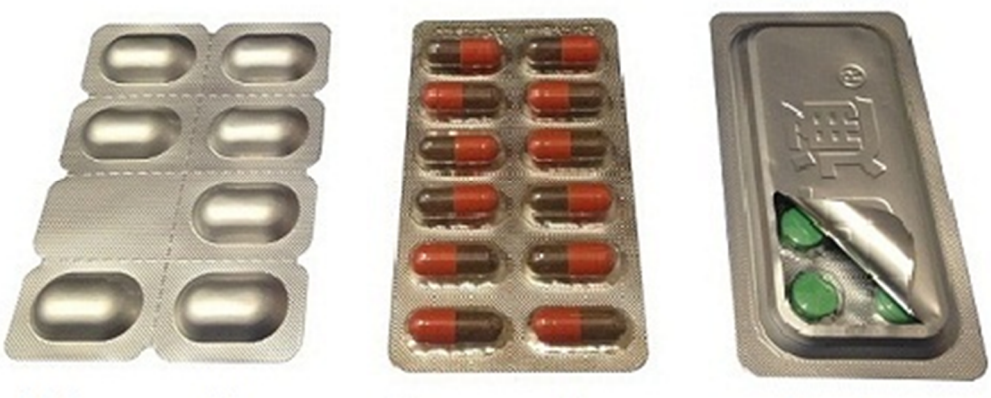

Figure 9. The three types of blister packs. Left: Cold formed aluminium/aluminium blister pack. Middle: Thermoformed aluminium/plastic blister pack. Right: Aluminium/plastic/aluminium blister pack. (Jornen Machinery, Co., Ltd., 2015)

The three different blister pack types have different protective properties. The general advantages and disadvantages of each type are presented in table 12 (Jornen Machinery Co., Ltd., 2015).

Table 12. Advantages and disadvantages of different types of blister packs. (Jornen Machinery Co., Ltd., 2015)

	Advantages	**Disadvantages**
Aluminium/aluminium blister pack	Nearly complete barrier against moisture, gases, and light. Allows extended product expiration date.	Slow speed and high cost production. Packaging inspection system complicated due to opaque of the package. Higher material costs.
Aluminium/plastic blister pack	Plastic usually PVC; easy to process, low cost. Transparent plastic enables visual product inspection.	PVC is a poor barrier against moisture and gases. Not suitable for light sensitive items.
Aluminium/plastic/ aluminium blister pack	Easy and low cost processing. Transparent plastic enables visual product inspection. Topical aluminium foil provides nearly complete barrier against moisture, gases, and light.	The use of topical aluminium foil increases production costs.

Aluminium/plastic blister packs are generally not suitable for biodegradable applications due to the lack of moisture barrier properties. Aluminium/aluminium blister packs and aluminium/plastic/aluminium blister packs have excellent barrier properties, and thus they are commonly used for packaging of sensitive, hygroscopic pharmaceutical and biopharmaceutical products. Blister pack manufacturers can have a variety of different lidding options available that can provide several opening features for different end-user purposes, such as push-thru, peelable, tear-open, and lock-tight. (Winpak, 2014)
Strip packs differ from blister packs in a way that a strip pack does not have thermoformed or cold formed cavities, but the pack is formed around the packaged item. An example of a strip pack is presented in figure 10. (Jornen Machinery Co., Ltd., 2015)

Figure10. An example of a strip pack. (Jornen Machinery Co., Ltd., 2015)

Strip packs are usually made of aluminium/PE laminated film, thus they have the same moisture, gas, and light barrier properties as cold formed aluminium films. The production of strip packs is typically slower than that of thermoformed blister packs. The packaging area of each item is larger in strip packs than in blister packs, because there are no pre-formed cavities, thus the strip packs are larger in size than blister packs. (Jornen Machinery Co., Ltd., 2015)

6.3 Tubes and vials

A dual tube offers an alternative to conventional blister packs and pouches in packaging of sterile biodegradable medical devices. For example SoTube™ by Selenium Medical has a so called NoTouch configuration, which allows the implant to be presented without physical contact. SoTube™ complies with the ISO 11607 and EN 868 standards for sterile medical device packaging. The materials used in the tube are biocompatible, and tubes are available in different sizes (figure 11). (Selenium Medical, 2016)

Figure 11. SoTube™ by Selenium Medical in an alternative for blisters and pouches in sterile medical device packaging.

SoTubes™ are transparent, and they have been color coded to make recognition of different implants easier. Also the sleeve of the tube is fully personalizable. SoTubes™ are designed to be used in the operating theater. They are also available with the packaging service in clean room class ISO 7 and ISO 5, or in separate components. (Selenium Medical, 2016)

Vials are available in various sizes and with a broad range of different geometrics, quality levels, and controlled surface chemistry. Vials are often made of Type 1 ultra-inert glass. Type 1 glass has excellent chemical resistance, neutrality and impermeability. Vials are commonly used for packaging of a wide range of injectables, as well as sensitive biotech drugs. (Schott AG, 2016) A vial by Schott AG, and a nest and a tub for vials packaging are presented in figure 12. Schott vials comply with European, US and Japan standards.

Figure 12. Left: A SCHOTT TopLyo® Type 1 glass vial with a hydrophobic coating, designed specifically for a lyophilisation process. Right: Vials can be packaged in a nest, which is then placed in a tub. Schott covers the tub with a Tyvek® inlay, and also seals it by a Tyvek® seal. The tub is further packed in a header bag and sterilized.

Cyclic olefin copolymer (COC) is an option for glass, having a transparent glass-like appearance, but being lighter and more break resistant than glass. COC has also excellent barrier properties against moisture, particle level is very low and there is no ion or heavy metal release. Schott AG for example uses COC for SCHOTT TopPac® prefillable syringes. (Schott AG, 2016)

6.4 Trays and lids

Thermoformed trays are an ideal packaging choice for irregularly shaped, high-profile devices and common for surgical procedure kits. Semi-rigid, structurally self-supporting trays provide good physical protection and are suitable for multicomponent kits. Flexible trays do not offer the same degree of physical protection as semi-rigid trays, but they are appropriate for low-cost devices and simple tray configurations. (Bix & De La Fuente, 2009) Typical tray styles are a tray with molded lid, a tray with heat sealed lid, and a standard tray with undercuts, shown in figure 13. An example of tray for a surgical procedure kit is presented in figure 14.

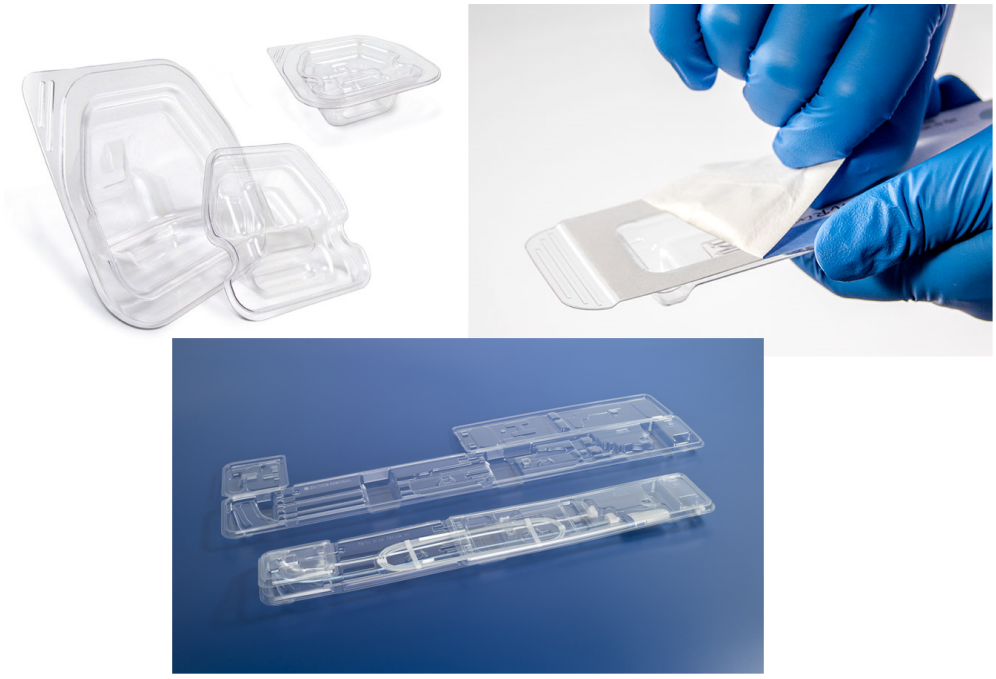

Figure 13. Typical tray styles. Above left: clear implant tray with molded lid by Brentwood Industries Inc. Above right: tray with heat sealed lid by Oliver-Tolas Healthcare Packaging. Below: standard long tray with undercuts by Prent Thermoforming.

Figure 14. A thermoformed tray for a surgical procedure kit by UFP Technologies.

A standard tray with undercuts and a tray with molded lid are designed to be used together with a pouch that provides a sterile barrier. These tray types do not have molded flanges for a heat sealed lid. Standard tray with undercuts and the tray with molded lid are usually used for long, narrow items such as catheters. Some kit trays are also designed using these styles. A tray with a heat sealed lid has an integral heal-seal flange molded around the perimeter of the tray. This type of tray can be used as a single sterile barrier, or it may be used with a pouch to provide a double sterile barrier. Typically the lid stock is covered with Tyvek®, but also coated papers, nonporous foil laminates and other flexible films can be used. (Life Science Outsourcing, 2016b)

Common materials used in thermoformed rigid trays are for example PET, polyethylene terephthalate glycol (PETG), acrylonitrile butadiene styrene (ABS), and high impact PS (HIPS). PET is a clear and rigid polymer and offers excellent gas and moisture barrier properties. PET is commonly used in food packaging and soft drink bottles. PETG is also clear and tough and easy to process. It is radiation tolerant and withstands many chemical sterilization methods without color defects. ABS is light, rigid and moldable, and it is also available in conductive and anti-static grades. HIPS is usually used for low cost, high volume applications. (UFP Technologies, 2016)

The use of universal packaging has become more prevalent during the recent years due to remarkable cost savings. In universal packaging the goal is to combine as many components as possible into one tray. That way, the number of both design and process validations can be reduced, and fewer sealing tools and lid and carton dies are needed. The small clamshell in figure 15 presents an example of a universal packaging that can be used in packaging of approximately 300 different implant screws. (Allen, 2013)

Figure 15. A universal packaging PETG clamshell by Barger that can be used in packaging of approximately 300 different implant screws. The Barger clamshell packaging features no sharp edges and is designed to be packaged in a pouch.

6.5 Applicators

In case the product is in the form of granules, paste, powder, or a combination of powder and liquid, for instance, applicators are an option for packaging. For example BonAlive® Biomaterials has applicators for their sterile bioactive glass granules and BonAlive® putty, figure 16.

Figure 16. Left: Applicator for BonAlive® putty. Right: Applicator for BonAlive® bioactive glass granules on a thermoformed tray. Both by BonAlive® Biomaterials.

The applicators can be designed in different sizes for different granule and unit sizes. BonAlive® applicators are intended to be further packaged in a sterile barrier pouch with a thermoformed tray. (BonAlive® Biomaterials, 2015)

MEDMIX offers unfilled applicators for mixing and delivering multicomponent biomaterials for various clinical applications, and Nordson MEDICAL has graft delivery systems for premixing bone craft materials with intravenous fluids, figure 17.

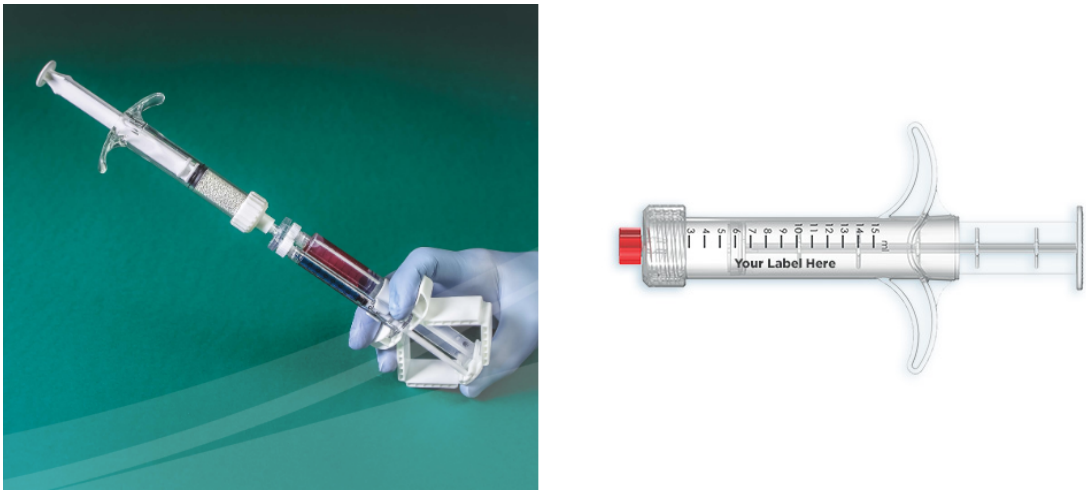

Figure 17. Right: Applicator system for mixing and delivering biomaterials by MEDMIX. Left: OsteoXpress™ Graft delivery device for premixing bone graft materials with intravenous fluids by Nordson MEDICAL.

6.6 Double packaging

Medical devices for sterile use are often double packaged: the inner package which is intended for the sterile field, is placed inside the outer package. Both provide a sterile barrier. As mentioned before, operating room nurses generally prefer double packaging

because it allows for error and facilitates presentation into the sterile field. (Buntz, 2010) Trays are often used in double sterile barrier packaging. Then the inner and outer trays are designed to nest together, as seen in figure 18.

Figure 18. Double trays with an inner and outer tray for double sterile barrier packaging of spinal implant products by Orthofix. The material is PETG sealed with coated Tyvek®.

The product is placed in the inner tray, and the lid is heat sealed in place. The sealed inner tray is then placed in the outer tray and the lid is again heat sealed to the outer tray. This type of packaging is typically used for orthopedic implants as well as surgical instruments. (Life Science Outsourcing, 2016b) Also double blister packs are commonly used in double sterile barrier packaging, as in figure 19.

Figure 19. Double Tyvek® peel blister pack for double sterile barrier packaging of sterile implants by KLS Martin Group.

6.7 Cartons and boxes

Folding cartons and boxes provide secondary protection for individually packaged sterile medical devices, as well as multiple unit packs. For multiple unit packs, the carton can be designed as a dispenser. The majority of folding cartons used for medical device packaging are custom designed to fit the products, and to enhance the product presentation by incorporating the specialized features of the product. The shipping container is a corrugated box with a sufficient strength to be used for shipping, sterilization, and storage. The inner package has to fit well with the outer box to avoid damage. The most commonly used shipping container is the Regular Slotted Container, and it can be used with inner-

packing materials, such as corrugated inserts, bubble wrap, and rigid or soft foam inserts to further protect the product. (Life Science Outsourcing, 2016)

The secondary packaging solutions field is also constantly evolving. Manufacturers now have a choice of secondary outer packaging from stock medical device packaging options (figure 20) to a carton design that is able to play a high-definition video with sound (figure 21).

Figure 20. A turnkey, or "stock" outer carton for medical device packaging by Barger.

Figure 21. A carton design with the ability to play a high-definition video with sound by Rondo-Pak.

Stock medical device packaging cartons enable introducing new products to market more quickly and with lower costs than custom designed packaging. They will also usually require fewer internal resources and lower capital investment from the manufacturer than custom packaging. (Barger, 2015) Rondo-Pak's multimedia carton represents the other end of carton designs: Rondo-Pak has introduced a carton that melds print and digital content for point-of-use or point-of-purchase messaging. The multimedia carton by Rondo-Pak integrates a thin, lightweight, high-definition video screen into a standard or custom carton. The video screen can face inwards or outwards. These multi-media cartons

can benefit product launches, they can be utilized in physician training kits, patient educations kits, clinical trials and sales demonstrations, for instance. The video may contain instructions for use, additional product information, or for example frequently asked questions – in multiple languages. The electronic components are free from lead- , arsenic- , and other hazardous materials, and can be removed for disposal. The graphic image on the printed carton also contains a digital watermark that enables a consumer to scan it with a smartphone to connect directly to web-based digital collateral. (McTigue Pierce, 2014)

6.8 Packaging systems

As defined in chapter 5.1, a packaging system is the combination of a sterile barrier system and protective packaging. The configuration of a packaging system depends on the product, what kind of protection is needed, the intended end users, the costs, and ecological considerations, for instance. Sterile medical device packaging system often consist of a double sterile barrier and a protective packaging. Examples of packaging systems are presented in figure 22.

Figure 22. Top left: Packaging system for stabilization and fracture fixation screw by Wright. Top right: Packaging system for a dental implant by Zimmer Dental Inc. Below left: Packaging system for Bio-Gide® collagen membrane by Geistlich Biomaterials. Below right: Packaging system for Bio-Oss® bone substitute for regenerative dentistry by Geistlich Biomaterials.

Many medical device companies are nowadays considering how the packaging affects the carbon footprint of the product, and for some it has already become part of the design

criteria. A double packaging, for instance, can more than double the amount of material needed to package the device. Many medical device makers are already using recycled PET (rPET) to manufacture secondary packaging in double barrier systems. (Allen, 2013; Buntz, 2010) Other ways to reduce the use of packaging materials are down gauging (weight reduction), reducing the package density, redesigning the package, or switching to another packaging type that requires less use of fossil fuel, produces less CO_2 during the manufacturing process, and produces less solid waste – such as pouches (Buntz, 2010). Smaller shipping cartons reduce the need for packaging material, pallet slip sheets and shrink wrap, as well as storage space (Gibson, 2011).

A significant shift towards more sustainable packaging systems in medical device sector is yet to happen. Sterilization requirements, safety regulations and biocompatibility are all partly to blame. However, the customers', governments', and other stakeholders' increasing interest in the materials and chemicals used in the product and packaging is forcing also the medical device industry rethink its strategy. Sustainability coalition has defined sustainable packaging with eight key criteria (Gibson, 2011):

A packaging should:

- Be beneficial, safe, and healthy to individuals and communities through its entire life cycle
- Meet market criteria for performance and cost
- Be sourced, manufactured, transported, and recycled using renewable energy
- Optimize the use of renewable or recycled source material
- Be manufactured using clean production technologies and best practices
- Be made from materials healthy in all probable end-of-life scenarios
- Be physically designed to optimize materials and energy
- Be effectively recovered and utilized in biological or industrial close-loop systems

It is difficult for most manufacturers to meet all the eight key criteria. More important would be that manufacturers recognized the whole system perspective; the view of the whole lifecycle and entire supply chain. Medical device manufacturers should no longer think moving towards more environmentally friendly packaging as a cost, but as an opportunity to find cost reductions from reduction in materials used, to lowered transportation costs. (Gibson, 2011)

7. STERILIZATION AND ASEPTIC PROCESSING

All implantable medical devices must be sterilized prior to their surgical placement (Athanasiou et al, 1996). Sterilization of a product means destroying all microbial life, including highly resistant bacterial endospores, by using a physical or chemical procedure. Although these procedures are not absolute, a product is considered to be sterile when all the parameters of a validated sterilization process have been met. (Patel, 2003) The effectiveness of a sterilization process is quantified by the probability of a non-sterile unit, and is described by the term Sterility Assurance Level (SAL). All implantable medical devices are required to be sterilized by a process that achieves a SAL of 10^{-6}, meaning one non-sterile unit in 1,000,000 units. End sterilization, also known as terminal sterilization, is a process where the product is sterilized within its sterile barrier system. End sterilization techniques are very efficient, they have a robust process control and they provide a high assurance of sterility. (Lambert et al, 2011) The most commonly used end sterilization methods for medical devices are based on heat, irradiation, chemicals or a combination of these techniques (Athanasiou et al, 1996). These methods, categorized by the means of the sterilizing agent, are discussed in the next chapters.

The FDA has its own system for categorizing sterilization methods in three groups: traditional, non-traditional and novel non-traditional. According to the FDA, traditional sterilization methods have a long history of safe and effective use demonstrated by validation, clearances, and literature. As opposed to the traditional methods, non-traditional sterilization methods do not have a long usage history, and while there is some published data and FDA evaluated information on validation of these methods, no FDA-recognized standards exist yet. Novel non-traditional sterilization methods category includes newly developed methods, which do have neither FDA-recognized standards nor FDA inspectional history, and there is little to no published information on validation. The novel non-traditional methods have no history of comprehensive FDA evaluation of sterilization validation data, and the FDA has not determined the method to be adequate to provide assurance of safe and effective use. (Lerouge, 2012) The sterilization methods currently placed in these categories are presented in table 13 (FDA, 2008; Lerouge, 2012).

Table 13. FDA categories for sterilization methods currently used for medical devices. (FDA, 2008)

Method	Sterilization effect based on
Traditional	
1. Dry heat	Heat
2. Moist heat	Heat
3. Gamma irradiation	Radiation
4. Electron beam (e-beam)	Radiation
5. Ethylene oxide gas (EtO)	Chemical
Non-traditional	
1. Hydrogen peroxide gas plasma	Chemical
2. Ozone	Chemical
Novel non-traditional	
1. Chlorine dioxide	Chemical
2. Ethylene oxide in-a-bag (diffusion or injection method)	Chemical
3. High intensity – or pulsed light	Radiation
4. Microwave radiation	Radiation
5. Sound waves	Radiation
6. Ultraviolet (UV) light	Radiation
7. Vaporized chemical sterilant systems, e.g. hydrogen peroxide and peracetic acid	Chemical

Sterilization process can affect the physical and mechanical properties of the device, thus affecting its performance *in vivo* (Athanasiou et al, 1996). Sterilization methods may also have an impact on cell proliferation in the sterilized scaffold (Noah et al, 2002). Also the integrity of the packaging materials of the device may be affected by sterilization. Some packaging materials may not withstand the initial sterilization process, and some are known to degrade with time after sterilization, which poses a significant risk to the sterility and integrity of the device. (Simmons, 2012) Due to these facts, it is crucial to select a suitable sterilization method (Athanasiou et al, 1996). The *AAMI TIR17:2008: Compatibility of materials subject to sterilization* - standard provides guidance in the qualification of polymers, ceramics, and metals in health care products that are sterilized by irradiation,

ethylene oxide (EtO), dry heat, moist heat, hydrogen peroxide (H_2O_2), and ozone. Besides the compatibility and stability of the device and packaging materials, the selection of the sterilization method is based on the ability to achieve the desired sterility assurance level. Secondary factors to consider are for example costs, availability, and knowledge of use and impact on similar products. When selecting a suitable sterilization method, the use of standards and guidance documents is recommended, especially if new materials or novel sterilization methods are considered. (Lambert et al, 2011) A summary of the sterilization methods reviewed in the following chapters is presented in the appendix C.

7.1 Heat sterilization

Dry heat sterilization technique use temperatures between 160 – 190 °C for a minimum of two hours. The microorganisms are destroyed through heat absorption by conduction. (Patel, 2003; Athanasiou et al, 1996) Due to the high temperatures and long sterilization time, dry heat sterilization should be used only for materials that might be damaged by moist heat, or that are impenetrable by moist heat (McKeen, 2012). **Moist heat** sterilization techniques use temperatures between 121 – 148 °C enhanced with high moisture and high pressure. The length of the sterilization cycle depends on the temperature and the size of the load and ranges between 10 – 60 minutes. Pressure greater than normal atmospheric pressure is needed to increase the temperature of the steam. Microorganisms are destroyed by heat and the process is accelerated by moisture; any living organism is killed when exposed to saturated steam at 120 °C for over 15 minutes. (Patel, 2003) Moist heat sterilization is commonly used whenever possible on all critical and semi-critical heat and moisture resistant devices. Steam sterilizers are also used in health care facilities to decontaminate biological waste. (McKeen, 2012) Both dry- and moist heat sterilization methods are easy to use, cheap, and there are no toxic residues left in the product (Athanasiou et al, 1996).

Many biodegradable polymers, for example PLA, PGA and PLGA, are susceptible to hydrolysis and deformation in high temperatures, thus moist and dry heat sterilization is not suitable for this type of materials (Bernkopf, 2007). Also many natural polymers, such as collagen, are temperature sensitive, thus heat is not a suitable sterilization method for them (Parenteau-Bareil et al, 2010; Noah et al, 2002).The packaging and seal materials of the device must also withstand the heat and the high humidity. If moist heat is used for sterilization, the packaging materials must be porous for steam to pass through to the device. The high temperatures used in this method will deform many plastics, such as poly(vinyl chloride) (PVC), polystyrene (PS), polyethylene (PE), and some synthetic fabrics. Materials that can tolerate varying temperatures are for example nylon, polypropylene, oriented polyester, polycarbonate and some grades of high-density PE (HDPE). If sterilization temperatures are carefully controlled between 121 °C – 123 °C, also packages containing Tyvek® may be safely steam sterilized. (Bix & De La Fuente, 2009)

Flash sterilization, also called immediate-use sterilization, is used for sterilizing cleaned patient care items that cannot be packaged, sterilized and stored before use. Flash sterilization may also be used if there is insufficient time to sterilize an item by the preferred packaging method. The sterilization conditions for an unwrapped item are three minutes at 132 °C. The sterilization equipment is often located near operating rooms to handle urgent needs. Precautions have to be taken for burn injuries, and the sterilized instruments can be cooled by air, or immersing in a sterile liquid. Flash sterilization is not recommended for implantable devices. (McKeen, 2012)

7.2 Ionizing radiation sterilization

Irradiation sterilization has become an increasingly popular sterilization method (Lásló, 2007). The temperature changes in irradiated materials are minimal; therefore irradiation may be a suitable sterilization method for many temperature sensitive materials, particularly health care products. Approximately 40 – 50% of disposable medical products are radiation sterilized. (Dziedzic-Goclawska et al, 2008) The main sources of ionizing radiation are X-rays, beta particles, gamma rays and high energy electrons (e-beam) (Drobny, 2012, Patel, 2003). X-rays are mainly used for diagnostic purposes in medicine and in certain analytical methods like X-ray microscopy, whereas gamma rays and e-beam are commonly used for sterilization (Drobny, 2012). Irradiation can affect some polymers' mechanical and chemical properties, therefore material compatibility has to be considered. In general, polymers that contain aromatic ring structures are more resistant to radiation effects, compared to aliphatic polymers. Also stabilizers, color correction tints or radical scavengers can be used to reduce radiation effects on polymers. (Sastri, 2010) As for the sterilization of tissue grafts, the efficacy, and degree of radiation induced damage of ionizing radiation is dependent on the sterilization conditions; dose, temperature, presence or absence of oxygen, as well as the physical state of the samples, such as the presence or absence of water (Dziedzic-Goclawska et al, 2008).

Gamma rays are electromagnetic radiation that is emitted from excited atomic nuclei of unstable atoms (Drobny, 2012). Figure 23 presents the electromagnetic spectrum and gamma rays' position in it.

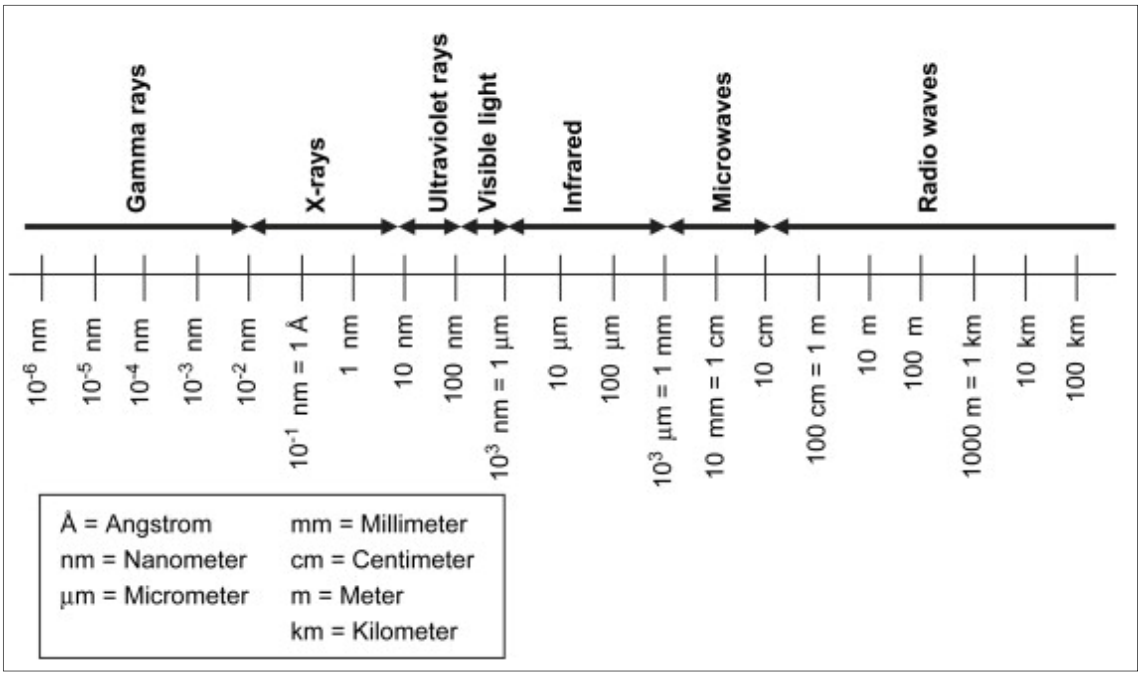

Figure 23. The electromagnetic spectrum. (Modified from Sastri, 2010)

Gamma rays penetrate into the matter deeper than beta particles, causing electron disruption, ionization, on the way. In living cells this ionization causes damage to the DNA and other cellular structures, eventually either killing the organism or making it incapable to reproduce. The most suitable gamma irradiation sources for medical and industrial applications are cobalt 60 (^{60}Co), cesium 137 (^{137}Cs) and iridium 192 (^{192}Ir). (Drobny, 2012) The radiation dose is usually 25 – 35 kilograys (kGy), depending on the product's bioburden (Hammad, 2008; Dziedzic-Goclawska et al. 2008). Gamma irradiation can be used for sterilization of various materials and devices due to its high penetrating power. The sterilization process leaves no radioactive residue in the materials. The process itself is continuous, fully automated and only one process variable, exposure time or dose, needs to be controlled. (Hammad, 2008)

Ionizing radiation can have a significant effect on polymers, particularly doses above 35 kGy may induce degradation of the polymer chain, resulting in reduced molecular weight, altered mechanical properties, and degradation profile (Bernkopf, 2007). Some polymers may also discolor or embrittle due to ionizing radiation. (Athanasiou et al, 1996; Bix & De La Fuente, 2009) Even low-dose gamma irradiation may alter molecular structure and significantly decrease mechanical and enzymatic resistance of collagen, causing accelerated degradation (Parenteau-Bareil et al, 2010; Noah et al, 2002). Some studies also show decrease in cell proliferation in gamma irradiated cells (Noah et al, 2002). To prevent heating to the critical temperature during the sterilization process, and thus the effect to the molecular weight of the polymer, the product can be cooled to 0 °C or less during irradiation by placing commercially available cooling agents in the irradiation container. Many biodegradable product manufacturers use gamma irradiation in controlled temperatures as the preferred sterilization method. (Bernkopf, 2007)

Since radiation sterilization does not require gas porous materials, for example aluminium pouches (Bernkopf, 2007) and all-plastic film packaging are suitable for this sterilization method. Also Tyvek® is suitable for both gamma and e-beam radiation sterilization due to its high-performance properties and radiation resistance. Although radiation sterilization can affect other packaging materials, for instance some papers may yellow. (Athanasiou et al, 1996; Bix & De La Fuente, 2009)

Ultraviolet (UV) radiation is the part of the electromagnetic spectrum between visible light and X-rays (figure 23). The specific wavelength area of the UV spectrum between 240 – 280 nm, also known as UVC irradiation, has a strong germicidal effect, with the peak effect at 265 nm. UV irradiation kills microorganisms by penetrating through cell membranes and damaging the DNA or RNA, thus making microorganisms unable to reproduce. The energy dosage of UV radiation needed to kill 99% of the microorganisms varies from 2500 µJ/cm^2 (*Bacillus megaterium* species bacteria) to 440 000 µJ/cm^2 (Tobacco mosaic virus). UV sterilization is used in various applications, such as food, water and air purification, medical sanitation and sterile work facilities. (McKeen, 2012)

UV irradiation does not leave toxic residues in sterilized items, and it does not alter the chemical composition, taste, odor, or pH of the product. (McKeen, 2012) However, also UV radiation has been reported to degrade some polymers, such as PE and polypropylene (PP) (Fischbach et al, 2001). Polymers such as polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), fluorinated ethylene propylene (FEP), and polyether ether ketone (PEEK^{TM}) have a good resistance to UV irradiation. The only plastics with excellent resistance are the imides, polyimide (PI) and polyetherimide (PEI). (Cole-Parmer Instrument Company LLC, 2006) Glass containers are essentially UV opaque, but quartz is transmissive in both visible and UV light (Xenon Corporation, 2015).

Fischbach et al. studied the effects of UV irradiation on spin-cast films made of biodegradable poly(D,L-lacti acid)-poly(ethylene glycol)-monomethyl ether diblock copolymers (Me.PEG-PLA). They found that after 2 hours' exposure to UV irradiation - which is sufficient for sterilization - cell adhesion to polymer films, polymer film topography, and chemical composition were maintained, as compared to non-irradiated films. Fischbach et al. emphasize that the sterilization time must be carefully controlled, however, since 5-24 hours' exposure lead to dramatic changes in polymer properties and biological interactions.

Wekhof et al. (2001) showed that **pulsed UV light** can provide effective sterilization without any UVC component, utilizing only UVA and UVB light. The sterilization effect in pulsed UV light is based on a targeted and momentous overheating that ruptures the micro-organisms. This method is fast, it has low operating costs, and items can be exposed through clear packaging. However, the treated media must be transparent to UV light and shadow effects must be avoided. According to Wekhof et al., pulsed UV light can be used for sterilization of clear packaging, medical tools, and surfaces, for example. In general,

pulsed UV light is suitable for all applications that can utilize continuous UV mercury vapor arc lamps. Material compatibility depends on the exposure dose, and generally materials with known UV transmission characteristics are good candidates for packaging materials. The FDA has approved the method for sterilization of food in 1999. (Xenon Corporation, 2015)

Infrared radiation (IR, figure 23) has been used to inactivate bacteria, spores, yeasts and molds in liquid and solid food products. IR is also inactivates enzymes. The efficacy of IR sterilization depends on various factors: IR power level, sample materials, sample depth, moisture content of the sample, temperature of the sample, peak wavelength, bandwidth of the IR heating source, and types of microorganisms present. The advantages of the IR sterilization are short cycle time, low energy consumption, no toxic residuals in the product and it has no environmental effects. IR technology may provide an alternative sterilization method for heat resistant devices, but there are no FDA cleared systems yet for sterilization of health care products. (McKeen, 2012)

Microwaves are radio frequency waves that are usually used at the frequency of 2450 megahertz (MHz) (figure 23). Microwaves produce an alternating electrical field that affects to water molecules and creates friction between them. The intermolecular friction generates heat. It has been reported that microwaves are an effective microbicide, but it is still debatable whether the sterilizing effect of microwaves is based on the produced heat, or on a non-thermal effect. In medicine microwaves are used for disinfecting for example contact lenses, dental instruments, urinary catheters, and milk. Due to the production of heat during the process, only materials that do not melt are compatible with this method. FDA has not cleared the use of microwaves in sterilization of medical devices. (McKeen, 2012)

Another radiation source is **high energy electrons (e-beam)**. E-beam is created in a vacuum by a heated cathode. The electrons are emitted from the cathode and then accelerated in an electrostatic field that is applied between the cathode and anode. The energy gain of the electrons is expressed in electron volts (eV) and it is proportional to the acceleration voltage. When an e-beam enters a material the energy of the accelerated electrons decreases significantly due to the large number of interactions between the electrons and the material. The accelerated electrons transfer their energy to the material through two types of interactions: in collision of electrons which results in material ionization and excitation, and in interaction with atomic nuclei that leads to the emission of X-ray photons. (Drobny, 2012)

E-beam sterilization has a much faster throughput rates than isotope radiation sources, delivery of the same dose of radiation lasts a few hours with gamma rays, but only minutes with e-beam. E-beam sterilization is cost effective and it can be integrated into an in-line process. The process does not involve volatiles or toxic chemicals, thus irradiated materials do not need to be aerated after the process. Also the absence of radioactive materials

makes the e-beam process often more acceptable within the public. The energy used for sterilization is typically 10 MeV which results to penetration of 38 mm in one-sided irradiation. (Chmielewski et al, 2008) Due to the poor penetration capability, larger e-beam sterilized items have to be irradiated from multiple sides to ensure complete sterilization (Massey, 2004). E-beam irradiation, similar to gamma irradiation, induces collagen degradation resulting in loss of mechanical and enzymatic resistance (Parenteau-Bareil et al, 2010). Cooling cannot be used in e-beam sterilization process. The presence of cooling agents in the irradiation container may lead to radiation shading during sterilization with accelerated electrons; radiation absorbs because of the high density of the material, thus leading to incomplete radiation of the neighboring product. Similar effect has not been observed with gamma irradiation. (Bernkopf, 2007)

A comparison between gamma- and e-beam radiation sources is presented in Table 14.

Table 14. Comparison of sources of gamma- and e-beam radiation. (Modified from Drobny, 2012)

Characteristics	**Source of radiation**	
	Gamma rays	**Electron beams**
Power source	Radioactive isotope	Electricity
Power activity	Half-life 5.27 years	Electrical on-off
Properties	Photons (1.25 MeV) $\lambda = 1.0 \times 10^{-3}$ nm	Electrons $m = 9.1 \times 10^{-31}$ kg
Charge	None	1.60×10^{-9} C
Equipment	Easy to operate and maintain	Complicate to operate and maintain
Emission	Isotopic, cannot be controlled	Unidirectional; can be scanned and bent by magnets
Penetration	Exponential attenuation	Finite range, depends on energy
Source Attenuation	Continuous attenuation, requires regular addition of source	No attenuation
Shielding	Continuous operation requires more shielding	Can be switched on and off, less demanding on shielding
Dose rate	10 kGy/h 2.8×10^{-3} kGy/s	360 000 kGy/h 100 kGy/s

Beta particles are free electrons that are transmitted from a linear accelerator through a high voltage electron beam. These accelerated electrons penetrate into matter until they are stopped by collisions with other atoms that are ionized in the impact. The sterilization

effect is based on the secondary electrons produced by the collisions and the sterilization efficiency depends on the density and thickness of the object, as well as the energy of the accelerated electrons. (Patel, 2003) Beta radiation is used in industrial applications, such as gauging. For example plastic film thickness or paper thickness can be monitored in process with beta particles. Also different types of coating thicknesses can be measured. Medical applications include radiotherapy with different beta radiation sources for cancer treatment, and treatment of other medical conditions, such as arthritis. (World Nuclear Association, 2014)

Oliveira et al. (2005) have studied the effects of beta radiation on different starch-based biomaterials. According to them the advances in electron beam technology has made beta radiation a worthy competitor to traditional gamma irradiation. The penetration power of beta radiation is lower than in gamma rays, which is less damaging to materials' mechanical bulk properties for example in biodegradable polymers. With starch-based biomaterials, beta radiation was found to increase hydrophilicity in studied polymers, but did not significantly affect the mechanical properties. Beta radiation is less damageable to collagen than gamma irradiation, but the applicability finally depends on the type of the collagen based biomaterial produced (Parenteau-Bareil et al, 2010).

Atmospheric plasma created by electrical discharge of a gas has been investigated during recent years as a potential physical agent for sterilization and biological decontamination**.** **Low temperature radio frequency (RF) plasma** sources at atmospheric pressure have been developed for practical applications for industry. As the process occurs in atmospheric pressure, processing and treatments can be implemented continuously, without the costly vacuum equipment. Hong et al. (2009) studied inactivation efficiency of low temperature plasma below 70 °C, based on helium gas mixed with different concentrations of oxygen. They used electrical power supply with a frequency of 13-56 MHz for the generation of low temperature plasma at atmospheric pressure. According to Hong et al., low temperature RF plasma method destroys microbes by disrupting cell envelopes, thus releasing cellular components and leading to loss of cell viability. They also concluded that the method is fast, and it can potentially be used to destroy bacterial cells, including spores, on contaminated surfaces.

7.3 Chemical sterilization

Ethylene oxide (EtO) gas is a chemical that kills microorganisms and spores. The sterilization effect is a combination of humidity, EtO gas, temperature and time. EtO sterilization process does not require very high temperatures, usually in the range of 50 – 60 °C, but moisture is needed to facilitate microbial kill. Therefore products must be exposed to a humid environment before EtO exposure. Humidity and EtO gas are driven into the products using vacuum cycles. The process is time consuming, the total sterilization cycle times vary from 6 hours to several days. (Lambert et al, 2011) The sterilization effect is

temperature dependent and based on a chemical reaction: alkylation of nucleic acid complexes, proteins and enzymes. (Massey, 2004) To reach the effect, EtO gas must be directly in contact with microorganisms on the items. EtO gas is highly flammable and explosive in air, thus explosion proof, environment controlled sterilization chamber is needed. (Patel, 2003) Due to its flammability, EtO is often diluted with fluorocarbon gases or carbon dioxide (Massey, 2004; Rutala & Weber, 2013). EtO sterilization can leave toxic residues to the sterilized items, therefore sterilized items need to be aerated after sterilization (Patel, 2003). A complete removal of residual traces of EtO is difficult to achieve in products with large surface area, such as meshes, warps, and wovens (Bernkopf, 2007). Despite these down sides, EtO sterilization covers about 50% of the industrial end sterilization market (Lambert et al, 2011).

As EtO is chemically highly reactive, it can act as plasticizer for biodegradable polymers causing changes in the polymer structure. In addition, the processing temperature is still too high for many biodegradable polymers. (Bernkopf, 2007) However, EtO seems to be less damageable to for example collagen than gamma irradiation, and it has been the most used method for sterilization of collagen scaffolds (Parenteau-Bareil et al, 2010; Noah et al, 2002). EtO sterilization doesn't seem to have a significant effect on cell proliferation (Noah et al, 2002). As for the packaging materials, the higher temperatures, pressures and humidity during the EtO sterilization process can cause package stress and loss of seal integrity that appears in the form of seal fatigue or sterilizer creep. Packaging materials suitable for EtO sterilization should be porous, moisture and EtO tolerant, have hot adhesive strength and be broadly compatible with chemicals and products. (Bix & De La Fuente, 2009)

Ozone sterilization is an alternative to EtO gas sterilization. Ozone gas is a strong oxidizer with a characteristic odor, and it chemically alters and inactivates various chemical and microbiological contaminants. Ozone is produced from energized oxygen that splits into two monoatomic molecules which then collide with oxygen molecules (O_2) to form ozone (O_3). Ozone can be produced artificially by the action of high-voltage discharge in air or oxygen and it has been used for decades to decontaminate water, and to sterilize air and food products. Compared to EtO, ozone is safer to use. Human nose can detect very low ozone levels, approximately 0.003 parts per million (ppm), so technical staff can be aware of the presence of the gas before it poses a hazard. (Lerouge, 2012) Ozone sterilization can be used for moisture and heat sensitive devices. There is no published data yet available about material compatibility, penetrability and organic material resistance, and only limited data on microbicidal efficacy. The FDA has however cleared the method for metal and plastic instruments, including some instruments with lumens. (Rutala & Weber, 2013)

Hydrogen peroxide (H_2O_2) is a known antimicrobial agent that destroys microorganisms and inactivates resistant spores by oxidizing key cellular components (Patel, 2003; Sultana, 2007). H_2O_2 does not have odor, irritation, or disposal issues. It does not have a

blood coagulating effect and it does not fix tissues to surfaces. H_2O_2 may enhance removal of organic matter and organisms. There are some functional and cosmetic material compatibility issues, mainly with metals like brass, zinc, copper, and nickel/silver plating. H_2O_2 causes serious eye damage with contact, so operators must follow safety precautions. (Rutala & Weber, 2013) H_2O_2 is also utilized in vaporized H_2O_2, and H_2O_2 gas plasma systems, as described in the next chapters.

Vaporized hydrogen peroxide is safe for both the environment and the operator. It leaves no toxic residues so aeration of the products after sterilization is not needed. The sterilization process is fast; the cycle time is about 55 minutes. (Rutala & Weber, 2013) During the process vaporized H_2O_2 is injected into the sterilization chamber via series of pulses. The operating temperature is 30 – 40 °C and the method is suitable for many heat and moisture sensitive metal and nonmetal devices. (Patel, 2003; Rutala & Weber, 2013). Material compatibility, however, has to be considered; liquids, linen, powders or any cellulose materials are not compatible with this method, and it also requires a synthetic packaging (e.g. PP). There are also restrictions based on the internal diameter and length of the sterilized devices. Materials compatibility data for this method is still limited as is the data of clinical use and comparative microbicidal efficacy. (Rutala & Weber, 2013) A commercially available vaporized H_2O_2 system by the STERIS Corporation, the *Amsco® V-PRO® maX Low Temperature Sterilization System*, received an FDA approval in 2011 (Schneider, 2013).

Hydrogen peroxide gas plasma uses H_2O_2 that is activated to form a reactive plasma or vapor. Plasma is ionized gas consisting of ions and electrons and it can be distinguished from solid, liquid and gas, thus often referred to as the fourth state of matter. (Patel, 2003) H_2O_2 plasma sterilization is suitable for heat and moisture sensitive items, since it uses operating temperatures below 50 °C and the process happens in a low moisture environment. The cycle time is from 30 minutes to 4 hours, depending on the size of the system (Patel, 2003; Massey, 2004; Rutala & Weber, 2013). The method is non-toxic and it has negligible environmental effect; the by-products are water vapor and oxygen, thus there is no need for aeration or ventilation of the products after the sterilization process. The process is simple to install, operate, and monitor. (Lerouge, 2012; Rutala & Weber, 2013) *STERRAD® 100NX® Sterilizer* in an FDA approved, commercially available sterilizer by Advanced Sterilization Products, that uses low temperature H_2O_2 gas plasma technology (Schneider, 2013).

Due to the limited penetration ability, H_2O_2 gas plasma sterilization is considered to be a surface sterilization method (Bernkopf, 2007). Poor penetration limits the size of each sterilization load and impairs the sterilization efficiency for some devices, especially those with a small diameter/length ratio. In addition the *STERRAD®* sterilization systems are fairly costly to purchase and the cost of H_2O_2 ampoules is also quite high. (Lerouge, 2012) Finally, the *STERRAD®* sterilization system is incompatible with several materials: it cannot be used for liquids, powders or strong peroxide absorbers. (Patel, 2003;

Massey, 2004) Paper, cotton, cellulose and linen absorb H_2O_2 thus also being incompatible with this method. Polymers, for example polyacetal and nylons, can be damaged by oxidative species produced in the sterilization process. Biomedical devices sterilized by H_2O_2 plasma are practically assured to experience some surface modification during the process. H_2O_2 is a strong oxidizer and plasma is known for its ability to modify surfaces through etching, deposition, and other surface modification reactions depending on the design and plasma parameters. The surface reactions may affect polymeric surface wettability and adhesion properties. (Lerouge, 2012) This method also requires synthetic packaging (e.g. polypropylene wraps, polyolefin pouches) and a special container tray (Rutala & Weber, 2013).

Peracetic acid liquid sterilization is a low temperature chemical sterilization system. The sterilizing effect of peracetic acid is based on disruption of bonds in proteins and enzymes. It may also affect cell walls and oxidize essential enzymes thus interfering cell membrane transportation and impairing essential biochemical pathways. Peracetic acid kills also spores at low concentrations. Peracetic acid is water soluble, it leaves no residues in the sterilized items after rinsing, and it has no harmful health or environmental effects. (Sultana, 2007) By-products of peracetic acid sterilization are acetic acid, oxygen and water (Rutala & Weber, 2013).

The items sterilized with peracetic acid must be immersible, quite small in size, and able to withstand the 55 °C processing temperature. (Sultana, 2007) With *The Steris system 1®* that uses *The Steris 20™ Sterilant Concentrate* the sterilization time is 12 minutes, followed by repeated rinses with sterile water. The standard cycle is completed in less than 30 minutes. (Patel, 2003) Immersing in a low concentration peracetic acid is the most common method to sterilize acellular collagen (Parenteau-Bareil et al, 2010). Peracetic acid may enhance removal of organic material and endotoxin, and it does not cause blood coagulation or fix tissues to surfaces. The limitations of this method include potential material compatibility issues, and the method being a point-of-use system, which means that the sterilized items cannot be stored sterile. Peracetic acid causes serious skin and eye damage with contact, so personnel must follow safety precautions. (Rutala & Weber, 2013)

Formaldehyde steam is considered to be a low temperature sterilization method, although the operation temperature is approximately 70 – 75 °C. Formaldehyde destroys microorganisms by coagulation of proteins in cells. In the process formalin is vaporized into formaldehyde gas, formaldehyde concentration being 8 – 16 mg/L. The gas is then lead into the sterilization chamber. The sterilization cycle has multiple stages, including initial vacuum to remove air from the sterilization chamber, steam admission to the chamber, and pulses of formaldehyde gas followed by steam. Formaldehyde is removed from the chamber and sterilized items by repeated alternate evacuations and flushing with steam and air. Reliable sterilization is achieved when using high concentration of gas, temperatures between 60 – 80 °C, and 75 – 100% relative humidity. The cycle time is

shorter than in EtO sterilization, for example, and the operating costs are relatively low. Formaldehyde steam has some limitations regarding penetration power, and the process temperature is still too high especially for many biodegradable polymers. In addition, formaldehyde is a mutagen and potential carcinogen. (Rutala et al, 2008)

Carbon dioxide (CO_2) is used for precision cleaning and disinfecting of medical instruments and devices. It can be utilized in cleaning of polymer and silicone surfaces before bonding, coating, or assembly for use in cleanrooms, biomedical devices and aerospace applications. (Markarian, 2013) CO_2 is a well-known bactericide and number of studies has demonstrated its effectiveness on various micro-organisms with several different treatment parameters, including temperature, time, pressure and use of additives. **Supercritical carbon dioxide** ($scCO_2$) is one of the newer sterilization methods that have been reported that utilizes CO_2. $ScCO_2$ has been found to be compatible with various biological materials, as well as biodegradable 3D scaffolds, and it has formed no toxic residues in treated materials. (Simmons, 2012; Bernhardt et al, 2015) The sterilizing effect of $scCO_2$ is believed to be based on the ability of $scCO_2$ to diffuse readily into the cells and alter the pH within the cell. In the presence of water, CO_2 will react to produce carbonic acid, which further reduces the pH within the cell. Thus even a small amount of water enhances the sterilizing effect of $scCO_2$. In the studies, the $scCO_2$ sterilization process has been found to be sensitive to three factors: 1) the chemical nature of the cell wall, and cell shape (the surface-to-volume ratio, for example) 2) efficiency of contact between the $scCO_2$ phase and the solid cell phase, and 3) exposure to water. (Dillow et al, 1999) $ScCO_2$ is non-toxic and non-reactive, it has high penetration ability, and it is easy to remove from sterilized items by depressurization. Due to its non-reactive nature, $scCO_2$ does not alter the morphology, structure, or mechanical properties of sensitive polymers. (Bernhardt et al, 2015)

Bernhardt et al. (2015) studied $scCO_2$ sterilization at low temperatures on sensitive biomaterials, such as polysaccharide-based hydrogels and collagen-based scaffolds. They showed that the $scCO_2$ procedure under addition of 0.25% water, 0.15% hydrogen peroxide, and 0.5% acetic anhydride was effective against a wide variety of microorganisms and bacterial spores, and it was less compromising to the mechanical properties of the studied biomaterials compared to gamma irradiation, EtO, and steam sterilization. They also compared cytocompatibility of the studied hydrogels and scaffolds after $scCO_2$, gamma -, EtO -, and steam sterilization, and found that cell viability and proliferation of human mesenchymal stem cells were not compromised by the $scCO_2$ treatment. According to Bernhardt et al, $scCO_2$ is a very effective, cytocompatible, and gentle sterilization method, and thus a promising alternative especially for biomaterials. The application of scCO2 technology, in combination with peracetic acid as additive, for the sterilization of various allograft tissues has been successfully commercialized by NovaSterilis. (Bernhardt et al, 2015)

Nitrogen dioxide (NO_2) gas has shown to induce single strand breaks in microbial DNA, thus disrupting cellular functions in a wide range of microorganisms and bacterial spores. NO_2 has lower oxidation potential than H_2O_2 and O_3. Due to that, NO_2 is compatible with most polymers used in the manufacturing of medical devices, and also with many biomolecules that are not compatible with other sterilization methods. Also many common packaging materials, such as nonwoven PP wraps, Tyvek/film peel pouches, and Tyvek/plastic trays, are compatible with the NO_2 sterilization process, however paper and other cellulosic materials are not. The sterilization process does not require heat, but it is impacted by humidity; increasing humidity enhances spore inactivation, which is thought to be related to the hydration of the spore coat (Schneider, 2013) NO_2 sterilization process operates in room temperature. The low boiling point of NO_2, 21 °C, reduces the sterilant condensation on surfaces, thus leading to rapid aeration time at the end of sterilization cycle. The possible condensed or absorbed NO_2 evaporates faster than for example H_2O_2. NO_2 sterilization is an emerging low temperature sterilization method. (Markarian, 2013)

Commercially available *RTS Series NO_2 Sterilizers* by Noxilizer use NO_2 gas in room temperature process that takes about two to three hours to complete. Noxilizer's RTS series sterilizers can be installed as an in-house system, and integrated in-line with the production. They are effective at low humidity levels, which allows processing of most moisture sensitive materials. Example applications are sterilization of drug delivery applications, cartridge delivery systems, orthopedic implants, prostheses, surface sterilization with no penetration of closure system and sterilization of moisture and heat sensitive items. The RTS series sterilizers are CE–marked under the MDD, and they are classified as a non-traditional sterilization method by the FDA. (Noxilizer, 2016)

Another recently developed sterilization method uses reactive species, such as **active oxygen species** (AOS) or free radicals generated by a cold plasma apparatus. The active oxygen species are generated by photochemical reaction from oxygen using UV irradiation. The inactivation effect of atomic oxygen generated in a low-pressure oxygen plasma apparatus has been studied, and it has been confirmed that atomic oxygen exposure promotes the inactivation of microorganisms. The use of AOS has several advantages; low processing temperature, dry processing, no residual effects, nontoxic, and low initial and operational costs. AOS have a very short lifetime, so there will not be high residual concentrations of AOS in sterilized items. (Yoshino et al, 2015)

7.4 Disinfectants

Disinfection is a chemical procedure that eliminates practically all recognized pathogenic microorganisms, but not necessarily all microbial forms, such as bacterial endospores. Three levels of disinfection can be distinguished: high, intermediate, and low. High level disinfectants eliminate all organisms, except high level of bacterial spores. Intermediate level disinfection kills most viruses and bacteria (including mycobacteria), and low level

disinfectant only destroys limited spectrum of viruses and bacteria. (McKeen, 2012) Disinfection is usually faster and less expensive than sterilization, but whether a product should be sterilized or disinfected depends on its intended use. Critical objects, such as implanted medical devices, must always be sterilized before use. Less critical objects, such as items that touch only non-intact skin or mucous membranes can be disinfected. (Patel, 2003) Other aspects that affect the choice of a suitable disinfection method are activity spectrum, compatibility with surfaces and disinfected devices, exposure time, costs, and harmful effects on the users, patients or the environment. (Gunaydin et al, 2014) The presence or organic matter may interfere with the antimicrobial activity of some disinfectants. The interference occurs either by a chemical reaction between the disinfectant and the organic matter, leading to a complex that is less germicidal, or by organic matter acting as a physical barrier, protecting microorganisms from the disinfectant. Thus it is important to thoroughly clean the surfaces before disinfection. (Rutala et al, 2008) This chapter presents a brief overview of several disinfection methods.

Alcohol in a health care setting usually refers to either ethyl alcohol or isopropyl alcohol. Alcohols destroy bacteria by penetrating the cell wall and denaturizing proteins and enzymes. The optimal bactericidal concentration is 60 – 90% alcohol solution in water (volume/volume), and the effectiveness drops sharply when diluted below 50%. Although ethyl alcohol and isopropyl alcohol are rapidly bactericidal against vegetative forms of bacteria, fungicidal and virucidal, they do not destroy bacterial spores. That's why they are not recommended for sterilizing medical and surgical materials. Alcohols may also damage plastics, elastomers, and coatings. In health care settings alcohols are used mainly to disinfect for example thermometers, scissors, stethoscopes, small surfaces, or medication preparation areas. (McKeen, 2012) Ethanol immersion with the combination of fungicide and antibiotic is used in laboratory to sterilize collagen scaffolds which have been physically cross-linked (Parenteau-Bareil et al, 2010). The FDA has not cleared any liquid sterilant or high level disinfectant that has alcohol as the main ingredient (McKeen, 2012).

Chlorine has been used as a disinfectant to treat drinking water since a 100 years. It is by far the most used method for disinfecting water, and its antimicrobial effectiveness has been widely assessed. Free available chlorine is an effective biocide against a wide variety of bacteria, viruses, fungi, and algae. Regardless of the original source of the available chlorine, the biocidal effect is based on hypochlorus acid (HOCl). HOCl is a weak acid, which tends to dissociate in water at increasing pH. (Clasen & Edmondson, 2006) The dissociated form of HOCl, the hypochlorite ion (ClO^-), is less microbicidal than HOCl, thus the disinfection activity of chlorine decreases with an increase of pH. In addition to chlorine, also many chlorine compounds – hypochlorites, chlorine dioxide, sodium dichloroisocyanurate, and Chloramine-T - are commonly used to disinfect drinking water. (McKeen, 2012) All chlorine products have some level of toxicity, hence making them

effective microbicides. However, when chlorinated water is ingested, the available chlorine is reduced by saliva and stomach fluid into harmless chloride ion salts. (Clasen & Edmondson, 2006)

Hypochlorites are the most widely used chlorine disinfectants, for example as household bleaches, and they are available in both liquid and solid form (McKeen, 2012). Hypochlorites have a wide spectrum of antimicrobial activity. They are also able to remove biofilms, and dried or fixed organisms from surfaces. Hypochlorites do not leave toxic residues to the sterilized products, they affect fast and they are inexpensive. It should be noted that hypochlorites release toxic chlorine gas if mixed with ammonia or acids. (McKeen, 2012)

Chlorine dioxide (ClO_2) is an oxidizing liquid agent that is effective against bacteria, viruses, yeasts, and molds. ClO_2 reacts with several cellular structures, including the cell membrane. The sterilizing activity is fast even in relatively low concentrations (1 – 30 mg/L). (McKeen, 2012) In the sterilization process, a compound of dilute chlorine gas is converted with sodium chlorite to form chlorine dioxide gas. The sterilized devices are exposed to this gas in the sterilization chamber. The processing time with ClO_2 is quite long; it takes 6 hours of contact time to achieve sterilization. The antimicrobial activity is reduced in the presence of organic matter. ClO_2 is corrosive, so this sterilization method may have some material compatibility issues. ClO_2 must also be generated onsite. (Patel, 2003) ClO_2 is used for sterilizing drinking water, as a sanitation agent in food and beverage processing, and in health care facilities to decontaminate rooms and isolators, and to sterilize products and components. (McKeen, 2012)

Sodium dichloroisocyanurate is the sodium salt of a chlorinated hydroxytriazine, and like other forms of chlorine, it produces hypochlorus acid, a well-known oxidizing agent. Whereas hypochlorites release all of their chlorine as free available chlorine, sodium dichloroisocyanurate releases approximately 50%, and the balance remains as reservoir chlorine in the form of chlorinated isocyanurates, to be released as the equilibrium is disturbed. Until recently, isocyanurates were mainly used for disinfection of swimming pool water, in the food industry, and in different cleaning and sanitizing applications, such as baby bottles and contact lenses. (Clasen & Edmondson, 2006) Nowadays they are also used for disinfecting drinking water – primarily in emergencies, but also in household point-of-use water treatment. Studies have shown that sodium dichloroisocyanurate and sodium cyanurate have low acute oral toxicity, and sodium cyanurate does not induce any carcinogenic, teratogenic, or genotoxic effects. (WHO, 2008)

Chloramine-T (N-chloro-4-methylbenzenesulfonamide sodium salt) is an organic compound, generated by chlorinating benzene sulfonamide or *para*-toulene sulfonamide, and it is a potential alternative to the use of chlorine (Sanli-Yurudu et al, 2007). Chloramine-T inhibits bacterial growth by disturbing bacterial metabolism. Its molecular structure is similar to *para*-aminobenzoic acid, which is an intermediate in bacterial metabolism.

Chloramine-T is available in tablets and powder, and it has to be dissolved before use. Chloramine-T is mainly used for sterilizing surfaces, where it is sprayed on and allowed to stand for 15 minutes or more before wiping off. (McKeen, 2012) Chloramine-T is a stable solution that is safe for humans, non-cytotoxic, and biodegradable, but it has a minor corrosive effect on common industrial materials, such as stainless steel, aluminum, and various polymers (Sanli-Yurudu et al, 2007).

Superoxidized water has become a widely used disinfectant during the recent years. Superoxidized water is generated by applying an electric current on salty water. After electrolysis the water contains hypochlorus acid, hypochlorite ions, dissolved oxygen, ozone, and superoxide radicals. Superoxidized water has a broad antimicrobial activity; it is effective against bacteria, fungi, viruses and parasites. Superoxidized water does not harm tissues, and as non-toxic it is safe for the users, as well as the environment. The usage costs are low. Although the antimicrobial activity of superoxidized water is very promising for surfaces and water disinfection systems, there is still lack of information about this disinfectant. (Gunaydin, 2014) There may be material compatibility issues, thus the user needs to check with the manufacturer for the compatibility with the disinfectant. The FDA has cleared superoxidized water as a high level disinfectant. (Rutala et al, 2008)

Formalin is water-based solution consisting of 37 weight-% of formaldehyde. Formaldehyde destroys microorganisms by alkylating the amino- and sulfhydral groups of proteins, and ring nitrogen atoms in purine bases. Formalin is effective against many bacteria, viruses, fungi and spores, but requires a long contact time. Due to the irritating fumes and pungent odor, the use of formaldehyde is limited especially in health care settings. Formaldehyde is also a potential carcinogen, so the handling time and employee exposure have to be monitored. Formaldehyde is used for example as an embalming agent, to preserve anatomic specimens, and to prepare viral vaccines. (Rutala et al, 2008)

Glutaraldehyde is a saturated di-aldehyde that is widely used as a high level disinfectant and chemical sterilant. Glutaraldehyde's aqueous solutions are acidic, and generally not effective against bacterial spores. For glutaraldehyde solution to become sporicidal it has to be activated, that is, made alkaline. The biocidal effectiveness of glutaraldehyde is based on the alkylation of sulfhydryl-, hydroxyl-, carboxyl-, and amino groups in microorganisms, leading to the alteration of DNA, RNA, and protein synthesis. Glutaraldehyde solutions are widely used in health care facilities due to their biocidal properties, activity in the presence of organic matter, and non-corrosiveness. Glutaraldehyde is mostly used for disinfecting of medical equipment. It is non-corrosive to metal and does not damage lenses, rubber, or plastic. Acute or chronic exposure to glutaraldehyde can cause skin irritation, mucous membrane irritation, or pulmonary symptoms, for instance. Thus employees' glutaraldehyde exposure should be monitored. The FDA has cleared glutaraldehyde sterilants that contain 2.4 – 3.4% glutaraldehyde to be used undiluted. A glutaralde-

hyde-phenol/phenate concentrate that contains 1.12% glutaraldehyde and 1.93% phenol/phenate at its use concentration has been cleared by the FDA as a high level disinfectant. (Rutala et al, 2008)

Iodophors are combinations of iodine and a solubilizing agent or carrier. The formed complex acts as a sustained-release reservoir of iodine that releases small amounts of free iodine in aqueous solution. The best known iodophor is povidone-iodine, a combination of polyvinylpyrrolidone and iodine. Iodine penetrates the cell wall of microorganisms rapidly and the microbicidal effect is believed to be based on disruption of protein and nucleic acid structure and synthesis. Iodophors are used as antiseptics on skin and tissues, and for disinfecting medical equipment. Iodophors formulated as antiseptics contain less free iodine than those formulated as disinfectant, therefore antiseptic iodophors should not be used for disinfecting hard surfaces. Iodine and iodine-based antiseptics should not be used for silicone materials. (Rutala et al, 2008)

Ortho-Phthalaldehyde (OPA) contains 0.55% 1,2-benzenedicarboxaldehyde. OPA's microbial affect seems to be similar to glutaraldehyde – interaction with amino acids, proteins, and microorganisms. OPA has a lipofilic aromatic nature that is likely to assist the penetration through the outer layers of mycobacteria and gram-negative bacteria. OPA has several advantages over glutaraldehyde: it is not a known irritant and does not require exposure monitoring, it has barely perceptible odor, and it does not need activation. OPA has also excellent material compatibility. (Rutala et al, 2008) Disadvantages is that OPA stains proteins grey, including unprotected skin, as well as clothing, and environmental surfaces. Thus it needs to be handled with care. It also has a slow sporicidal activity and the use is more expensive than glutaraldehyde. (McKeen, 2012) OPA can be neutralized with glycine before disposal through the sanitary sewer system (Rutala et al, 2008).

Quaternary ammonium compounds are widely used as disinfectants. They are good cleaning agents, but high water hardness and some materials, such as cotton and gauge pads, can lessen the anti-microbial activity because of insoluble precipitates, or absorption of active ingredients, respectively. The bactericidal effect is based on inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane. Quaternary ammonium compounds are used for environmental sanitation of non-critical surfaces, such as floors, furniture, and walls. Certain registered quaternary ammonium compounds can be used for disinfecting less-critical medical equipment. (Rutala et al, 2008)

Phenol has long been used in hospital disinfection, but during the last decades the studies have concentrated in numerous phenol derivatives, **phenolics**, and their antimicrobial properties. Phenolics are originated when a functional group, alkyl-, phenyl-, benzyl-, or halogen group, replaces one of the hydrogen atoms on the aromatic ring. Two most common phenolics are *ortho*-phenylphenol and *ortho*-benzyl-*para*-chlorophenol. In high concentrations phenol acts as a protoplasmic poison; it penetrates and disrupts the cell wall

and precipitates the proteins. In low concentrations phenol and higher molecular weight phenolics inactivate essential enzyme systems and cause essential metabolites to leak from the cell wall. Phenolics are absorbed by porous materials, and residual disinfectant can cause irritation. Many phenolics are used as disinfectants for environmental surfaces and noncritical medical devices. (Rutala et al, 2008)

Surfacine is an antimicrobial agent that can be used for animate or inanimate surfaces. Surfacine incorporates a water-insoluble antimicrobial drug compound, silver iodide, with a surface immobilized coating, a modified polyhexamethylene biguanide. Microbial contact with the surface leads to transfer of silver from the coating to the organism, and microorganisms contacting the coating accumulate silver until the toxicity threshold is exceeded. (McKeen, 2012)

Photocatalysis offers another alternative to the traditional surface disinfection methods. The disinfection abilities of photocatalytic oxidation on titanium coated surfaces are being studied for use in many different applications. The biocidal activity of titanium thin films attached to solid surfaces has been shown in several studies; organic compounds can be oxidized to CO_2 by hydroxyl radicals generated on titanium dioxide (TiO_2) surfaces. The bactericidal effect of TiO_2 photocatalysis is believed to be a combination of cell membrane damage and further oxidative attack of internal cellular components that leads to cell death. Photocatalytic reactions do not require high temperatures, and the irradiation source can be various types of visible light, UV-light or fluorescent light, depending on the intended use. The method is safe, nontoxic, ecofriendly, and relatively inexpensive, as well as applicable to many different applications. Photocatalysis has been used in air and water disinfection, and it is studied for use in food industry, laboratory applications, and medical applications, such as biomedical implant surfaces. (Nath et al, 2012)

Formic acid has shown to be a potential sterilization agent for collagen (Parenteau-Bareil et al, 2010). Formic acid is also used in combination with hydrogen peroxide; the combination forms a fast acting sterilant against spore-forming bacteria. ASP® has developed a system that utilizes the combination in their Endoclens systems, which are designed for point-of-use sterilization of endoscopes. Endoclens has a fully automated system and rapid cycle time, less than 30 minutes. (Rutala & Weber, 2001)

7.5 Aseptic processing

If a medical device is not compatible with any end sterilization technique, and the materials cannot be changed for more compatible ones, the manufacturer of the device may have to aseptically process the device (Lambert et al, 2011). Aseptic processing is a general term for a group of technologies that enable sterilized components and products to be manufactured under conditions that mitigate contamination risk (Agalloco & Agers, 2010). Aseptic packaging is usually the last option due to the high manufacturing costs

and less robust process control. End sterilized products provide superior patient safety compared to aseptic processing, therefore the regulatory bodies prefer end sterilization whenever possible. (Lambert et al, 2011) Traditionally aseptically processed products have been liquids, powders, or suspensions that cannot be terminally sterilized. Nowadays, along with the development of different kinds of implantable medical devices, a wider variety of devices are aseptically processed. Examples of medical devices and combination products that typically require aseptic processing are: biodegradable implants, artificial and/or non-viable biologically based matrixes, hermetically sealed electromechanical devices, and combination products that include viable cells, such as implants coated with drug and/or biologically derived substances. (ISO 13408-7, 2012)

As opposed to end sterilization, aseptic processes are designed to exclude microbial contamination during the manufacturing process. The final sterile product is achieved over several process steps, thus process control over all the possible microbial contamination sources is much more difficult than in end sterilization. (Lambert et al, 2011) In aseptic processing either the entire product is sterilized and then introduced into a sterilized package, or the product components are sterilized separately, then further processed and assembled, and the final product is packed into a sterilized package. During aseptic processing, the handling of sterile containers and devices or their components is performed in a controlled environment, in which the air, materials, equipment, and personnel are regulated to control microbial and particulate contamination levels. Subsequent sterility testing is expected to verify the sterility of the product. (Teixeira, 2014) *The ISO 13408: Aseptic processing of healthcare products* -suite of standards cover the aseptic processing of health care products. The standards outline the requirements for aseptic processes, as well as validation and routine control of the manufacturing process. The ISO 13408 standard series is recognized by the FDA (FDA, 2015). *The ISO 14644: Cleanrooms and associated controlled environments* contains the classification criteria for cleanrooms, as well as specifications for testing and monitoring, test methods, design, construction, and operation, and classification of airborne molecular contamination. The ISO 14644 is also recognized by the FDA (FDA, 2015)

In aseptic processing, personnel are the main source of contamination. The most effective way to eliminate personnel-borne contamination is to isolate personnel from the product by barrier systems, or to eliminate the need for personnel in the process entirely by automation. These methods are called advanced aseptic processing methods. (Baseman, 1998) In advanced aseptic processing direct intervention with open product containers or product contact surfaces by operators wearing conventional cleanroom garments is not required and never permitted. *Restricted Access Barrier Systems (RABSs)* and *isolators* are used to create a protective physical structure between personnel and the product. The terms RABS and isolator are often used interchangeably, but they are slightly different systems. (Agalloco & Agers, 2010) A RABS is in most cases “open”, so the air flows freely out of the RABS into the surrounding room. An open RABS also permits open

door interventions during the process. A RABS is decontaminated manually by aseptically gowned personnel using high-level disinfection with sporicidal materials. (Kania, 2008) An isolator is either sealed or supplied with High Efficiency Particulate Air (HEPA) -filtered air. It is a fully isolated unit that can operate at positive, neutral or negative pressure with respective to the surrounding environment. An isolator has an automated decontamination system that uses vaporous or gaseous sporicidal agents. An isolator can be used for aseptic processing as well as containment of potent compounds, or simultaneously for both asepsis and containment. (Agalloco & Agers, 2010; Kania, 2008)

Blow-fill-seal (BFS) and form-fill-seal (FFS) systems have been extensively used in aseptic packaging, but the incorporation of RABS and isolators have further improved these technologies (Agalloco & Agers, 2010). In the BFS/FFS systems, a polymer container is formed, filled and sealed in one continuous automated process. The primary advantage of these systems is reducing human intervention. BFS/FFS technologies are very common in pharmaceutical industry, where they are used in packaging of liquids in both small containers, such as ophthalmic drug ampoules, as well as in larger containers, such as saline solutions. Recently, the BFS/FFS technology has expanded into injectables and biologics, for example vaccines and monoclonal antibodies. The BFS/FFS systems have been successfully used for aseptic processing of heat sensitive materials that cannot withstand end sterilization, for more than 30 years. (Markarian, 2014, Patel et al, 2006) With newer designs, BFS/FFS technologies can be used for the aseptic filling of small number of bags at slower speed, ideal for bulk or clinical subdivision or for niche products (Agalloco & Agers, 2010).

Another approach to improve contamination control in aseptic processing is the use of robots and automation that eliminate the use of personnel by replacing manual activities with machines. So far, robots and automation have mostly been utilized in secondary packaging operations, although there are robots developed for handling activities, such as component supply on a routine bases, in isolators or cleanrooms. Also different automated bottling and capping systems, as well as aseptic sampling systems are available. Healthcare product manufacturers are typically slow to adopt new technologies, thus aseptic processes are usually more advanced in other aseptic industries. (Agalloco & Agers, 2010)

In cell and tissue processing the sub-culturing for cell expansion is the core process. During the manufacturing, strict control against contamination and human error is mandatory due to the un-sterilizable products and the complexity of culture techniques. An automated processing system would enhance safety, security and cost saving in the processing of cells and tissues for therapeutic applications. The criteria for such facility design, however, has not been clear to date, even though there is a growing need for capable, scalable manufacturing technologies. The major challenge in the development of these technologies has been the transmission from laboratory-scale design to production-scale design of biological products that are reproducible, safe, and clinically effective. The products

would also need to be economically acceptable and competitive in order for the upscaling to be successful. For example Kino-oka (2014) has been developing a novel bioreactor design as well as a facility design for aseptic cell processing. (Kino-oka, 2014)

8. CONCLUSIONS

Packaging of sterile tissue engineering products is a complex issue. Medical devices are a highly regulated area, and all manufacturing and packaging activities have to comply with the relevant legislation and regulations. Especially in the fairly new field of advanced therapy products the regulations in different countries are at different stages of development, and globally harmonized terminology and technical requirements are yet to be established.

Advanced tissue engineering products can contain several components, each with different properties. This imposes new challenges on packaging technology by adding complexity to the packaging system and creating challenges with self-life stability. The manufacturer of the device has to define what kind of protection from the environmental factors the product requires. These factors include the conditions that can affect the device itself, the user of the device, or the patient in the intended use environment. Environmental conditions to be considered may be for example: temperature, humidity, moisture, atmospheric gas composition or pressure, energy, vibration, motion, lighting, and shock.

Two common features for all biodegradable tissue engineering products are that they are always delivered sterile, and they are susceptible to hydrolytic degradation. Therefore, the sterile and moisture barrier properties of packaging materials of biodegradable tissue engineering products are of most importance. A sterile barrier maintains the sterility of the product all the way to the end user. Typical materials for sterile barrier systems are plastic films, coated or uncoated papers and non-wovens, and common packaging formats are pre-formed trays and premade bags and pouches. Biodegradable products have to be packaged as quickly as possible after fabrication, usually under an inert atmosphere or vacuum. A moisture barrier packaging protects the product from hydrolytic degradation which would alter the mechanical properties and affect the performance of the product *in vivo*. The packaging material is most often a foil or a laminated polymer film. Recently developed ultra-high barrier films that utilize nanotechnology enable extremely thin, transparent packaging with superior moisture and oxygen barrier properties.

Due to the multi-component structures of advanced tissue engineering products, it may be challenging to find a suitable end sterilization method. Sterilization may cause immediate or delayed effects to the sterilized products, or it may affect the cell proliferation in the sterilized scaffold. Also the packaging materials must be compatible with the chosen end sterilization method. Novel, gentler sterilization technologies for sensitive materials are being developed, but for the most of them there is still very limited data available about material compatibilities and delayed effects. Sterilization methods that use supercritical carbon dioxide or nitrogen dioxide, however, have shown promising results in

compatibility with many moisture and heat sensitive materials. Sterilizers that use nitrogen dioxide are also already commercially available. In case these low temperature and low moisture sterilization methods are still not compatible with the product, aseptic processing might be the only option for fabrication, although it is more costly and less robust than end sterilization.

When designing a packaging of a product, user acceptance is an essential consideration. In an often hectic operating room environment it is crucial that the device packaging is easy to open and the contents can be removed without contamination. The label must be easy to read. A clear packaging is often thought ideal. Healthcare professionals generally favor trays and flexible pouches as packaging, and double sterile barrier is highly preferred. It is apparent, that packaging has an impact on which product will be chosen if several similar products are available.

The major shift towards sustainable packaging systems in the healthcare industry is yet to happen, but the interest towards recycling and sustainability is definitely increasing. Many manufacturers are starting to recognize the whole life cycle perspective of products, and acknowledge the effect of packaging to the carbon footprint of the product. The favored double packaging more than doubles the amount of material needed for packaging, thus significantly increasing the amount of waste. If the double packaging is essential, other ways to reduce the use of packaging materials are down gauging, reduction of the package density, or redesigning the package. The thin ultra-high barrier nanofilms may also help reduce the use of packaging materials. It may also be worth to consider if switching to another packaging type that produces less waste, requires less use of fossil fuels, and produces less CO_2 during the manufacturing process is possible. Smaller shipping cartons further reduce the need for packaging materials, pallet slip sheets and shrink wraps, as well as storage space. While reducing material, transportation and storage costs, “green packaging” may potentially be a valuable marketing asset in the future.

The scope of this thesis was to examine possible packaging solutions for biodegradable tissue engineering products. Based on the studied materials, an ideal packaging for a biodegradable tissue engineering product would be a double sterile barrier system consisting of a rigid tray in a flexible, clear pouch that would also serve as a moisture barrier. The labeling should be clear and easy to read, and both the pouch and the tray should be easy to open quickly in an operating room environment. The outer carton should be as small as possible, while still providing sufficient protection and the required information. The final materials for the packaging should be chosen based on whether the product will be sterilized in the final package or aseptically processed. However, due to the diverse nature of biodegradable tissue engineering products, suggesting one universal packaging solution suitable for all biodegradable tissue engineering products is impossible. While the special features of each product have to be considered, these results can offer some general guidelines for which aspects to consider when designing a final package for a biodegradable tissue engineering product.

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APPENDIX A: CLASSIFICATION OF MEDICAL DEVICES ACCORDING TO THE DIRECTIVE 93/42/EEC

Modified from: Frumento, 2011.

Device type	Class I	Class IIa	Class IIb	Class III
Category 1: Noninvasive devices	All non-invasive devices unless they are listed in any other higher class.	Devices for channeling or storing blood, body liquids or tissues, or liquids or gases for infusion, administration or introduction into the body. Devices for storing organs, parts of organs, or tissues. Devices that may be connected to an active medical device in Class IIa or a higher class. Devices for modifying the biological or chemical composition of blood through filtration, centrifugation or exchanges of gas or heat.	Devices for modifying the biological or chemical composition of blood, other body liquids or other liquids for infusion into the body, that do not use filtration, centrifugation, or exchanges of gas or heat.	N/A
Category 2: Non-invasive devices in contact with injured skin	Devices used as a mechanical barrier, for compression or for absorption of exudates.	Devices for managing the micro-environment of a wound.	Devices used principally with wounds which have breached the dermis and can only heal by secondary intent.	N/A
Category 3: Invasive devices	All invasive devices other than surgically invasive devices, that are not in connection to an active medical device and are for transient use. Devices for short-term use if they are used in the oral cavity as far as the pharynx, in an ear canal up to the ear drum, or in a nasal cavity.	Devices for short-term use. Devices for long term use in the oral cavity as far as the pharynx, in an ear canal up to the ear drum, or in a nasal cavity, and are not liable to be absorbed by the mucous membrane. All invasive devices other than surgically invasive devices, intended for connection to an active medical device in Class IIa or a higher class.	Devices for long-term use.	Devices for transient or short term use for diagnosing, monitoring or correcting a defect of the heart or of the central circulatory system through direct contact with these parts of the body. Devices for short term use in direct contact with the central nervous system. Devices for short-term use to have a biological effect or to be wholly or mainly absorbed.
Category 4: Surgically invasive devices	Reusable surgical instruments	Devices for transient use unless they are listed in a higher class. Devices for short-term use unless otherwise specified.	Devices for transient use or short term use to supply energy in the form of ionising radiation.	Devices for transient use or short term use intended specifically to diagnose, monitor or correct a defect of the heart or of the central circulatory system through

Device type	Class I	Class IIa	Class IIb	Class III
		Devices for short-term use to undergo chemical change in the body or to administer medicines, and the devices are placed in the teeth.	Devices for transient use to have a biological effect or to be wholly or mainly absorbed. Devices for transient use to administer medicines by means of a delivery system, if this is done in a manner that is potentially hazardous considering the mode of application. Devices for short-term use to undergo chemical change in the body, or to administer medicines.	direct contact with these parts of the body. Devices for short term use in direct contact with the central nervous system. Devices for short-term use and to have a biological effect or to be wholly or mainly absorbed.
Category 5: **Implantable devices and long term surgically invasive devices**	N/A	Devices to be placed in the teeth.	All implantable devices and long term surgically invasive devices are in Class IIb unless otherwise specified. Devices to undergo chemical change in the body or to administer medicines if the devices are placed in the teeth.	Devices used in direct contact with the heart, the central circulatory system or the central nervous system. Devices to have a biological effect or to be wholly or mainly absorbed. Devices to undergo chemical change in the body or to administer medicines.
Category 6: **Active devices**	N/A	All active therapeutic devices to administer or exchange energy are in Class IIa unless otherwise specified. Active devices for diagnosis if they are intended to supply energy which will be absorbed by the human body, except for devices used to illuminate the patient's body in the visible spectrum. Active devices for diagnosis if they are intended to image in vivo distribution of radiopharmaceuticals. Active devices to allow direct diagnosis or monitoring of vital physiological processes and not specified otherwise. All active devices to administer and/or remove medicines, body liquids or other	All active therapeutic devices to administer or exchange energy to or from the human body in a potentially hazardous way, considering the nature, the density and site of application of the energy. Active devices to control or monitor the performance of active therapeutic devices in Class IIb, or to influence directly the performance of such devices. Active devices for monitoring of vital physiological parameters, where the nature of variations is such that it could result in immediate danger to the patient, such as variations in cardiac performance, respiration, activity, or of the central nervous system.	N/A

Device type	Class I	Class IIa	Class IIb	Class III
		substances to or from the body unless otherwise specified.	Active devices to emit ionising radiation for diagnostic and therapeutic interventional radiology, including devices which control or monitor such devices, or which directly influence their performance. All active devices to administer and/or remove medicines, body liquids, or other substances to or from the body in a manner that is potentially hazardous, considering the nature of the substances involved, the part of the body concerned and the mode of application.	
Category 7: **Special cases**	N/A	All devices to be used specifically for disinfecting medical devices. Non-active devices specifically for recording of X-ray diagnostic images.	All devices used for contraception or the prevention of the transmission of sexually transmitted diseases unless otherwise specified. All devices to be used specifically for disinfecting, cleaning, rinsing or hydrating contact lenses.	All devices incorporating as an integral part a substance which, if used separately, can be considered to be a medicinal product, as defined in Article 1 of Directive 65/65/EEC, and which is liable to act on the human body with action ancillary to that of the devices. Devices used for contraception or the prevention of the transmission of sexually transmitted diseases that are implantable or long term invasive devices. All devices manufactured utilising animal tissues or derivatives rendered non-viable. Breast implants, shoulder, knee and hip implants. Active implantable devices

APPENDIX B: STANDARDS AND GUIDANCE DOCUMENTS RELEVANT TO STERILE BARRIER SYSTEMS

The list of relevant standards is compiled by Sterile Barrier Association. (Sterile Barrier Association, 2013)

ASTM D4169	Standard Practice for Performance Testing of Shipping Containers and Systems. Guidance, testing.
ASTM D4332	Standard Practice for Conditioning Containers, Packages, or Packaging Components for Testing. Guidance, testing.
ASTM D7386	Standard Practice for Performance Testing of Packages for single parcel Delivery Systems. Guidance, testing.
ASTM F17 - 13a	Standard Terminology Relating to Flexible Barrier Packaging. Guidance, packaging & materials.
ASTM F1980	Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices. Guidance, testing.
ASTM F2097	Standard Guide for Design and Evaluation of Primary Flexible Packaging for Medical Products. Guidance, testing.
ASTM F2475	Standard Guide for Biocompatibility Evaluation of Medical Device Packaging Materials. Guidance, biological evaluation.
ASTM F2559/F2559M	Standard Guide for Writing a Specification for sterilizable peel pouches.Guidance, packaging & materials
ASTM F2825	Standard practice for climatic stressing of Packaging systems for Single Parcel Delivery. Guidance, testing.
ASTM F99	Standard Guide for Writing a Specification for flexible barrier rollstock materials. Guidance, packaging & materials.
CEN/TR 13688	Packaging - Material recycling - Report on requirements for substances and materials to prevent a sustained impediment to recycling. Technical report, environment.
CEN/TR 13695-1	Packaging - Requirements for measuring and verifying the four heavy metals and other dangerous substances present in packaging and their release into the environment - Part 1: Requirements for

	measuring and verifying the four heavy metals present in packaging. Technical report, environment.
CEN/TR 13695-2	Packaging - Requirements for measuring and verifying the four heavy metals and other dangerous substances present in packaging, and their release into the environment - Part 2: Requirements for measuring and verifying dangerous substances present in packaging, and their release into the environment. Technical report, environment.
CEN/TR 13910	Packaging - Report on criteria and methodologies for life cycle analysis of packaging. Technical report, environment.
EN 13427	Packaging - Requirements for the use of European Standards in the field of packaging and packaging waste. Standard, environment.
EN 13428	Packaging - Requirements specific to manufacturing and composition - Prevention by source reduction. Standard, environment.
EN 13429	Packaging - Reuse. Standard, environment.
EN 13430	Packaging - Requirements for packaging recoverable by material recycling. Standard, environment.
EN 13431	Packaging - Requirements for packaging recoverable in the form of energy recovery, including specification of minimum inferior calorific value. Standard, environment.
EN 868-10	Packaging for terminally sterilized medical devices - Part 10: Adhesive coated nonwoven materials of polyolefines - Requirements and test methods. Standard, packaging and materials.
EN 868-2	Packaging for terminally sterilized medical devices - Part 2: Sterilization wrap - Requirements and test methods. Standard, packaging and materials.
EN 868-3	Packaging for terminally sterilized medical devices - Part 3: Paper for use in the manufacture of paper bags (specified in EN 868-4) and in the manufacture of pouches and reels (specified in EN 868-5) - Requirements and test methods. Standard, packaging and materials.
EN 868-4	Packaging for terminally sterilized medical devices - Part 4: Paper bags - Requirements and test methods. Standard, packaging and materials.

EN 868-5	Packaging for terminally sterilized medical devices - Part 5: Sealable pouches and reels of porous materials and plastic film construction — Requirements and test methods. Standard, packaging and materials.
EN 868-6	Packaging for terminally sterilized medical devices - Part 6: Paper for low temperature sterilization processes - Requirements and test methods. Standard, packaging and materials.
EN 868-7	Packaging for terminally sterilized medical devices - Part 7: Adhesive coated paper for low temperature sterilization processes - Requirements and test methods. Standard, packaging and materials.
EN 868-8	Packaging for terminally sterilized medical devices - Part 8: Re-usable sterilization containers for steam sterilizers conforming to EN 285 - Requirements and test methods. Standard, packaging and materials.
EN 868-9	Packaging for terminally sterilized medical devices - Part 9: Uncoated nonwoven materials of polyolefines - Requirements and test methods. Standard, packaging and materials.
EN 980	Symbols for use in the labelling of medical devices. Standard, labelling.
EN ISO 15223-1	Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements. Standard, labelling.
EN ISO 14937	Sterilization of health care products - General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. Standard sterilization.
EN ISO 15225	"Nomenclature - specification for a nomenclature system for medical devices for the purpose of regulatory data exchange". Standard, nomenclature.
EN ISO 17025	General requirements for the competence of testing and calibration laboratories. Standard, testing.
ISO 10993-1	Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process. Standard, biological evaluation.

ISO 10993-10	Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization. Standard, biological evaluation.
ISO 10993-11	Biological evaluation of medical devices - Part 11: Tests for systemic toxicity. Standard, biological evaluation.
ISO 10993-12	Biological evaluation of medical devices - Part 12: Sample preparation and reference materials. Standard, biological evaluation.
ISO 10993-13	Biological evaluation of medical devices - Part 13: Identification and quantification of degradation products from polymeric medical devices. Standard, biological evaluation.
ISO 10993-16	Biological evaluation of medical devices - Part 16: Toxicokinetic study design for degradation products and leachables. Standard, biological evaluation.
ISO 10993-17	Biological evaluation of medical devices - Part 17: Establishment of allowable limits for leachable substances. Standard, biological evaluation.
ISO 10993-18	Biological evaluation of medical devices - Part 18: Chemical characterization of materials. Standard, biological evaluation.
ISO 10993-2	Biological evaluation of medical devices - Part 2: Animal welfare requirements. Standard, biological evaluation.
ISO 10993-3	Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity. Standard, biological evaluation.
ISO 10993-5	Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity. Standard, biological evaluation.
ISO 10993-6	Biological evaluation of medical devices - Part 6: Tests for local effects after implantation. Standard, biological evaluation.
ISO 10993-7	Biological evaluation of medical devices - Part 7: Ethylene oxide sterilization residuals. Standard, biological evaluation.
ISO 10993-9	Biological evaluation of medical devices - Part 9: Framework for identification and quantification of potential degradation products. Standard, biological evaluation.

ISO 11607-1:2006 Packaging for terminally sterilized medical devices - Part 1: Requirements for materials, sterile barrier systems and packaging systems. Standard, packaging and materials.

ISO 11607-2:2006 Packaging for terminally sterilized medical devices - Part 2: Validation requirements for forming, sealing and assembling processes. Standard, packaging and materials.

ISO 11607-1:2006/Amd 1:2014 Packaging for terminally sterilized medical devices - Part 1: Requirements for materials, sterile barrier systems and packaging systems. Standard, packaging and materials.

ISO 11607-2:2006/Amd 1:2014 Packaging for terminally sterilized medical devices - Part 2: Validation requirements for forming, sealing and assembling processes. Standard, packaging and materials.

ISO 14006 Environmental management systems – Guidelines for incorporating eco-design. Standard, environment.

ISO 14971 Medical devices – Application of risk management to medical devices. Standard, risk management.

ISO 186 Paper and board - sampling to determine average quality. Standard, statistics.

ISO 18601 Packaging and the environment - General requirements for the use of ISO standards in the field of packaging and the environment. Standard, environment.

ISO 18602 Packaging and the environment – Optimization. Standard, environment.

ISO 18603 Packaging and the environment – Reuse. Standard, environment.

ISO 18604 Packaging and the environment - Material recycling. Standard, environment.

ISO 18605 Packaging and the environment - Energy recovery. Standard, environment.

ISO 18606 Packaging and the environment - Organic recycling. Standard, environment.

ISO 187 Paper, board and pulps - Standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples. Standard, testing.

ISO 2233	Packaging - Complete, filled transport packages and unit loads - Conditioning for testing. Standard, testing.
ISO 22442-1	Medical devices utilizing animal tissues and their derivatives - Part 1: Application of risk management. Standard, risk management.
ISO 2859-1	Sampling procedures for inspection by attributes - Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection. Standard, statistics.
ISO 4180	Packaging - complete, filled transport packages - general rules for the compilation of performance test schedules. Standard, testing.
ISO 9001	Quality management systems – Requirements. Standard, quality management.
ISO/TR 16218	Chemical recovery. Standard, environment.
ISO/TR 17098	Report on substances and materials which may impede recycling. Technical report, environment.
ISO/TS 10993-19	Part 19: Physico-chemical, morphological and topographical characterization of materials. Technical specification, biological evaluation.
ISO/TS 10993-20	Part 20: Principles and methods for immunotoxicology testing of medical devices. Technical specification, biological evaluation.
ISO/TS 16775:2014	Packaging for terminally sterilized medical devices - Guidance on the application of ISO 11607-1 and ISO 11607-2. Technical specification, packaging and materials.
ISO14040	Environmental management - Life cycle assessment - Principles and framework. Standard, environment.
ISO 13485	Medical devices – Quality management systems – Requirements for regulatory purposes. Standard, quality management.
ISO 18472	Sterilization of health care products - Biological and chemical indicators - Test equipment. Standard, indicators.
ISO 20857	Sterilization of health care products - Dry heat - Requirements for the development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.

ISO 25424	Sterilization of medical devices - Low temperature steam and formaldehyde - Requirements for development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.
ISO 11137-1	Sterilization of health care products - Radiation - Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.
ISO 11137-2	Sterilization of health care products - Radiation - Part 2: Establishing the sterilization dose. Standard, sterilization.
ISO 11137-3	Sterilization of health care products - Radiation - Part 3: Guidance on dosimetric aspects. Standard, sterilization.
ISO 11138-1	Sterilization of health care products - Biological indicators - Part 1: General requirements. Standard, indicators.
ISO 11138-2	Sterilization of health care products - Biological indicators - Part 2: Biological indicators for ethylene oxide sterilization processes. Standard, indicators.
ISO 11138-3	Sterilization of health care products - Biological indicators - Part 3: Biological indicators for moist heat sterilization processes. Standard, indicators.
ISO 11138-4	Sterilization of health care products - Biological indicators - Part 4: Biological indicators for dry heat sterilization processes. Standard, indicators.
ISO 11138-5	Sterilization of health care products - Biological indicators - Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes. Standard, indicators.
ISO/TS 11139	Sterilization of health care products – Vocabulary. Technical specification, vocabulary.
ISO 11140-1	Sterilization of health care products - Chemical indicators - Part 1: General requirements. Standard, indicators.
ISO 11140-3	Sterilization of health care products - Chemical indicators - Part 3: Class 2 indicator systems for use in the Bowie and Dick-type steam penetration test. Standard, indicators.

ISO 11140-4	Sterilization of health care products - Chemical indicators - Part 4: Class 2 indicators as an alternative to the Bowie and Dick-type test for detection of steam penetration. Standard, indicators.
ISO 11140-5	Sterilization of health care products - Chemical indicators - Part 5: Class 2 indicators for Bowie and Dick-type air removal tests. Standard, indicators.
ISO 11737-1	Sterilization of medical devices - Microbiological methods - Part 1: Determination of a population of microorganisms on products. Standard, bioburden.
ISO 11737-2	Sterilization of medical devices - Microbiological methods - Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process. Standard, sterility testing.
ISO/TS 13004	Sterilization of health care products - Radiation - Substantiation of selected sterilization dose: Method VDmaxSD. Technical specification, sterilization.
ISO 13408-1	Aseptic processing of health care products - Part 1: General requirements. Standard, aseptic processing.
ISO 13408-2	Aseptic processing of health care products - Part 2: Filtration. Standard, aseptic processing.
ISO 13408-6	Aseptic processing of health care products - Part 6: Isolator systems. Standard, aseptic processing.
ISO 13408-7	Aseptic processing of health care products - Part 7: Alternative processes for medical devices and combination products. Standard, aseptic processing.
ISO 14160	Sterilization of health care products - Liquid chemical sterilizing agents for single-use medical devices utilizing animal tissues and their derivatives - Requirements for characterization, development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.
ISO 14161	Sterilization of health care products - Biological indicators - Guidance for the selection, use and interpretation of results. Standard, indicators.
ISO 14937	Sterilization of health care products - General requirements for characterization of a sterilizing agent and the development, validation

	and routine control of a sterilization process for medical devices. Standard, sterilization.
ISO 15882	Sterilization of health care products - Chemical indicators - Guidance for selection, use and interpretation of results. Standard, indicators.
ISO 17664	Sterilization of medical devices - Information to be provided by the manufacturer for the processing of re-sterilizable medical devices. Standard, reprocessing.
ISO 17665-1	Sterilization of health care products - Moist heat - Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.
ISO/TS 17665-2	Sterilization of health care products - Moist heat - Part 2: Guidance on the application of ISO 17665-1. Technical specification, sterilization.
ISO/TS 17665-3	Sterilization of health care products - Moist heat - Part 3: Guidance on the designation of a medical device to a product family and processing category for steam sterilization. Technical specification, sterilization.
ISO 11135	Sterilization of health-care products - Ethylene oxide - Requirements for the development, validation and routine control of a sterilization process for medical devices. Standard, sterilization.

APPENDIX C: SUMMARY OF THE STERILIZATION AND DISINFECTION METHODS

Method	Advantages	Disadvantages	Suitability for biodegrable polymers		Compatible packaging materials
			Synthetic	Natural	
Dry heat	Easy, cheap, no toxic residues	High temperatures (160 – 190 °C) exceeds the melting temperature of most biodegradable polymers.	No	No	Nylon, polypropylene, oriented polyester, polycarbonate, some grades of high-density PE (HDPE).
Moist heat	Easy, cheap, no toxic residues	High temperatures (121 – 148 °C) and moisture: Exceeds the melting temperature of many biodegradable polymers, causes hydrolytic degradation.	No	No	Only porous materials, nylon, polypropylene, oriented polyester, polycarbonate, some grades of high-density PE (HDPE), Tyvek® (in 121 – 123 °C), medical grade paper.
Flash sterilization	Easy, cheap, no toxic residues	On site sterilization method for sterilizing cleaned patient care items that cannot be packaged, sterilized and stored before use. High temperature (132 °C) exceeds the melting temperature of most biodegradable polymers.	No	No	On site method, used for items that cannot be stored.
Gamma rays	High penetrating power, suitable for temperature sensitive materials, no radioactive residues, no aeration needed, continuous fully automated process	May affect mechanical properties of several polymers, cause discoloring and embrittlement. To prevent heating during sterilization process, the product has to be cooled to 0 °C. Requires shielding.	Limited	No	PE and PE copolymers, radiation tolerant grade PP, PS, PC, PET, PU, unplasticized PVC, aluminum pouches, Tyvek®.
UV irradiation	No toxic residues, does not alter chemical composition, taste, odor, or pH of the product.	Degrades some polymers (such as PE and PP). Poor penetration power.	Limited	Not enough data available	PTFE, PVDF, FEP, PEEKTM, PI, PEI. Glass is UV opaque.

Method	Advantages	Disadvantages	Suitability for biodegrable polymers		Compatible packaging materials
			Synthetic	Natural	
Pulsed UV light	Fast, low operating costs, items can be exposed through clear packaging, no toxic residues. Suitable for clear packaging, medical tools, and surfaces.	Treated media must be transparent to UV light and shadow effects must be avoided. FDA has approved the method only for treatment of food.	Limited	Not enough data available	Clear packaging materials. Same materials than for UV irradiation.
Infrared radiation	Short cycle time, low energy consumption, no toxic residues, no environmental effects	Generates heat, method not cleared by FDA, efficacy depends on IR power level, sample materials, sample depth, moisture content of the sample, temperature of the sample, peak wavelength, bandwidth of the IR heating source, and types of microorganisms present.	No	Not enough data available	Heat resistant materials.
Microwaves	No toxic residues.	Generates heat, method not cleared by FDA	No	Not enough data available	Heat resistant materials.
E-beam	Fast, cost effective, can be integrated into an in-line process, no volatile or toxic chemicals, no radioactive materials, no aeration needed after sterilization.	Poor penetrating power; larger items have to be irradiated from multiple sides. Cooling agents cannot be used due to shading effect.	Limited	Limited	
Beta particles	Might be suitable for natural polymers due to lower, less damaging penetration power.	Lower penetration power not efficient enough for all items. Not a lot of research information available.	Not enough data available	Yes	Not enough data available
Low temperature radio frequency (RF) plasma	Fast, no toxic residues, atmospheric pressure enables continuous processing.	Surface sterilization method, temperature (70 °C) too high for many biodegradable polymers.	Limited	Limited	Not enough data available
EtO gas	Low processing temperatures (50 – 60 °C).	Long process time, must have direct contact with sterilized items, requires moisture. Highly flammable and explosive in air. Leaves toxic residues, materials need to aerated after process. Difficult to remove all toxic residuals from porous 3D structures. Acts as a plasticizer to many biodegradable polymers.	Limited	Yes	Porous, moisture and EtO tolerant, hot adhesive strength, broadly compatible with chemicals.
Ozone	Alternative to EtO gas sterilization, safer to use than EtO. Can be used for heat and moisture sensitive items. FDA approved the method for metal and plastic instruments.	Only limited data of the method available.	Not enough data available	Not enough data available	Not enough data available
Hydrogen peroxide	Does not coagulate blood or fix tissues to surfaces. No odor, irritation or disposal issues.	Material compatibility issues with some metals, such as brass, zinc, copper, and nickel/silver plating. Causes serious eye damage in contact.	Not enough data available	Not enough data available	Synthetic materials.

Method	Advantages	Disadvantages	Suitability for biodegrable polymers		Compatible packaging materials
			Synthetic	Natural	
Vaporized hydrogen peroxide	Very fast, low temperature (30 – 40 °C), suitable for moisture sensitive materials, safe for operator and environment. No toxic residues.	Not compatible with liquids, linen, powders, and all cellulose materials, restrictions for the diameters of the sterilized product. Only limited data available.	Not enough data available	Not enough data available	Synthetic packaging materials (e.g. polypropylene)
Hydrogen peroxide gas plasma	Low temperature (<50 °C), suitable for moisture sensitive materials, fast, non-toxic, no environmental effects, process simple to install, operate and monitor.	Poor penetration power, restrictions for the diameters of the sterilized product. Relatively expensive. Not compatible with liquids, powders, strong peroxide absorbers (paper, cotton, cellulose), causes surface modification	Not enough data available	Not enough data available	Synthetic packaging materials (e.g. polypropylene, polyolefin). Requires a special container tray.
Peracetic acid	Very fast, Low temperature (55 °C), water soluble, no toxic residues, no environmental effects, does not coagulate blood or fix tissues to surfaces.	Sterilized items must be immersible, point-of-use system; items cannot be stored sterile, peracetic acid causes serious skin and eye damage in contact.	Not enough data available	Yes	Not enough data available
Formaldehyde steam	Low temperature sterilization method (70-75 °C), relatively low costs and short process time.	Temperature too high for many biodegradable polymers, requires humidity. Limited penetration power. Formaldehyde is a mutagen and a potential carcinogen.	No	Not enough data available	Not enough data available
Carbon dioxide	Non-toxic. Can be utilized in cleaning of polymer and silicone surfaces before bonding, coating, or assembly for use in cleanrooms, biomedical devices and aerospace applications.	Surface sterilization method.	Yes	Yes	Not enough data available
Supercritical carbon dioxide	Compatible with various biological materials. Low temperature, low pressure method. Leaves no toxic residues. Non-reactive, high penetration ability. Does not alter the morphology, structure, or mechanical properties of sensitive polymers.	Only limited data of the method available.	Yes	Yes	Not enough data available
Nitrogen dioxide	Compatible with most polymers used in medical devices, and with many biomolecules that are not compatible with other sterilization methods. Low temperature method (room temperature), rapid aeration.	Process requires some humidity.	Limited	Yes	Nonwoven PP wraps, Tyvek/film peel pouches, Tyvek/plastic trays. Not compatible with paper and other cellulosic materials.

Method	Advantages	Disadvantages	Suitability for biodegrable polymers		Compatible packaging materials
			Synthetic	Natural	
Active oxygen species	Low processing temperature, dry processing, no residual effects, nontoxic, low initial and operational costs.	Only limited data of the method available.	Not enough data available	Not enough data available	Not enough data available
Alcohols	Low cost, no harmful environmental effects. Effective skin disinfectant.	Do not destroy bacterial spores, may damage plastics, elastomers and coatings. Not recommended for medical and surgical materials. FDA has not cleared any liquid sterilant or high level disinfectant that has alcohol as the main ingredient. Only limited data of the method available.	Not enough data available	Yes (with fungicide and antibiotic)	Not enough data available
Chlorine	Effective water disinfectant.	All chlorine products have some level of toxicity.	Not enough data available	Not enough data available	Not enough data available
Hypochlorites	Wide spectrum of antimicrobial activity, remove biofilm, fast, no toxic residues, cost effective	Release toxic chlorine gas if mixed with ammonia or acids.	Not enough data available	Not enough data available	Not enough data available
Chlorine dioxide	Effective in low concentrations	Long processing time (hours), some material compatibility issues due to corrosive nature. Antimicrobial activity reduce in presence of organic matter. Point-of-use system. Corrosive.	Not enough data available	No	Not enough data available
Sodium dichloroisocyanurate	Low acute oral toxicity, and does not induce any carcinogenic, teratogenic, or genotoxic effects.	Mainly used for water disinfection.	Not enough data available	Not enough data available	Not enough data available
Chloramine-T	Potential alternative to chlorine. Stable solution, safe for humans, non-cytotoxic, and biodegradable	Mainly used for surface sterilization. . Has a minor corrosive effect on common industrial materials, such as stainless steel, aluminum, and various polymers.	Not enough data available	Not enough data available	Not enough data available
Superoxidized water	Non-toxic, safe for the users and the environment. Low costs.	Only limited data available, potential material compatibility issues.	Not enough data available	Not enough data available	Not enough data available
Formalin	Effective against many bacteria, viruses, fungi and spores.	Requires a long contact time. Irritating fumes and pungent odor, thus limited use. Potential carcinogen. Employees' exposure should be monitored.	Not enough data available	Not enough data available	Not enough data available
Glutaraldehyde	Non-corrosive, good biocidal properties and activity in the presence of organic matter.	Generally not effective against spores. Has to be activated before use. Can cause irritation and pulmonary	Not enough data available	Not enough data available	Not enough data available

Method	Advantages	Disadvantages	Suitability for biodegrable polymers		Compatible packaging materials
			Synthetic	Natural	
	Compatible with metals, glass, rubbers, some plastics.	symptoms. Employees' exposure should be monitored.			
Iodophors	Antiseptics on skin and tissues and disinfectant for medical devices.	Not compatible with silicone materials.	Not enough data available	Not enough data available	Not enough data available
Ortho-Phthalaldehyde	No irritation, no exposure monitoring. Does not need activation before use. Excellent material compatibility.	Stains proteins grey. Slow sporicidal activity, use is more expensive than glutaraldehyde.	Not enough data available	Not enough data available	Not enough data available
Quaternary ammonium compounds	Good cleaning agents.	Water hardness and some materials, such as cotton and gauge pads, can lessen the anti-microbial activity.	Not enough data available	Not enough data available	Not enough data available
Phenolics	Disinfectants for environmental surfaces and noncritical medical devices.	Absorbed by porous materials, residual disinfectant can cause irritation.	No	No	Not enough data available
Surfacine	Antimicrobial agent for surface disinfection.	Only limited data of the method available.	Not enough data available	Not enough data available	Not enough data available
Photocatalysis	Low temperatures, irradiation source can be various types of visible light, UV-light or fluorescent light, depending on the intended use. Safe, nontoxic, eco-friendly, and relatively inexpensive. Applicable to many different applications. Studied for use in medical applications.	Only limited data of the method available.	Not enough data available	Not enough data available	Not enough data available
Formic acid	Potential sterilization agent for collagen. In combination with hydrogen peroxide forms a fast acting sterilant against spore-forming bacteria.	Only limited data of the method available.	Not enough data available	Yes	Not enough data available