Tampere University

Lilli Suominen

REGULATIONS AND REQUIREMENTS FOR EXTRACELLULAR MATRIX DERIVED INJECTABLE HYDROGELS IN EU

Bachelor's thesis
Faculty of Medicine and Health Technology
June 2023

ABSTRACT

Lilli Suominen: Regulations and requirements for extracellular matrix derived injectable hydrogels in EU
Bachelor's thesis
Tampere University
Biotechnology and biomedical engineering
June 2023

This thesis examines European Union regulations regarding an injectable hydrogel derived from the extracellular matrix. It is written as a literature review, where the main sources are EU regulations and the guidelines of the European Medicines Agency. There are no similar products on the market yet, so applying them correctly is important. At the beginning of the work, the necessary information about the product is first clarified, meaning what type of product it is, what is its intended use and what it's used for. This information can be used to determine the type of product, which determines which legislation should be applied. The purpose of the work is to find out how the product in question can be brought to the market and what should be taken into account on the market.

Hydrogels have attracted interest as new types of regenerative treatments. This is due to their nature, as they have similar properties to human soft tissues such as softness and biocompatibility. An injectable hydrogel derived from a natural extracellular matrix could be used to treat potential cardiac damage by delivering it to cardiac tissue via a catheter. In damaged cardiac tissue, it creates a healthy growth environment for cells, helping the cardiac tissue to regenerate. The product is intended to be manufactured by decellularizing the extracellular matrix of porcine cardiac tissue, leaving only the natural supporting tissue. This way, the matrix's own properties can be utilized.

Based on this work, the ECM derived injectable hydrogel is a class III medical device to which the medical device regulation applies. The regulatory framework for medical devices contains guidelines and requirements that define the required procedures, examinations and systems. A medical device needs a CE mark to operate on the EU market and obtaining it requires certain actions.

In order to enter the market, a product must be subject to a conformity assessment, which shows that the product meets the requirements set for it. Conformity assessment deals with product type inspection, conformity to requirements, production quality assurance and product-specific assessment. The manufacturer must also create a quality management system and design dossier and maintain them. The finished product must be registered in the Eudamed register maintained by the European Commission. In addition, the product must be subject to post-market surveillance that ensures its safety in the future.

Keywords: Injectable hydrogel, extracellular matrix, regulations, Medical device, EU

TIIVISTELMÄ

Lilli Suominen: Asetukset ja vaatimukset koskien solunulkoisesta matriisista johdettuja injektoitavia hydrogeelejä EU:n alueella
Kandidaatintutkielma
Tampereen yliopisto
Bioteknologian ja biolääketieteen tekniikan tutkinto-ohjelma
Kesäkuu 2023

Tässä opinnäytetyössä tarkastellaan Euroopan unionin asettamia ohjeita, jotka koskevat solunulkoisesta matriisista johdettuja injektoitavia hydrogeelejä. Opinnäytetyö on kirjoitettu kirjallisuuskatsauksena, jossa pääasiallisina lähteinä on käytetty Euroopan Unionin asetuksia ja Euroopan lääkeviraston ohjeita. Vastaavanlaista tuotetta ei ole vielä markkinoilla, joten näiden soveltaminen oikein on tärkeää. Työn alussa selvitetään tarvittavat tiedot tuotteesta eli millainen tuote on, mikä on sen käyttötarkoitus ja miten sitä käytetään. Näiden tietojen avulla voidaan selvittää tuotteen tyyppi, mikä määrittelee, mitä asetusta tulee soveltaa. Työn tarkoituksena on selvittää, miten kyseinen tuote voidaan tuoda markkinoille ja mitä markkinoilla tulee ottaa huomioon.

Hydrogeelit ovat herättäneet kiinnostusta uudenlaisina regeneratiivisina hoitomuotoina, sillä niillä on samanlaisia ominaisuuksia ihmisten pehmytkudosten kanssa. Luonnollisesta solunulkoisesta matriisista valmistettua injektoitavaa hydrogeeliä voidaan mahdollisesti käyttää sydänvaurioiden hoitoon kuljettamalla se sydänkudokseen katetrilla. Vaurioituneessa sydänkudoksessa se luo terveen kasvuympäristön soluille auttaen sydänkudosta uusiutumaan. Tuote on tarkoitus valmistaa sian sydänkudoksen solunulkoisesta matriisista poistaen siitä solut, jolloin jäljelle jää vain luonnollinen tukikudos. Näin voidaan hyödyntää matriisin omia ominaisuuksia.

Tämän työn perusteella solun ulkoisesta matriisista johdettu injektoitava hydrogeeli on luokan III lääkinnällinen laite, johon voidaan soveltaa lääkinnällisten laitteiden asetusta. Lääkinnällisten laitteiden sääntelykehys sisältää ohjeita ja vaatimuksia, jotka määrittelevät vaadittavat toimenpiteet, tutkimukset ja järjestelmät. Lääkinnällinen laite tarvitsee CE-merkinnän Euroopan Unionin markkinoilla ja sen saaminen edellyttää tiettyjä toimia.

Markkinoille päästäkseen tuotteelle tulee suorittaa vaatimuksenmukaisuuden arviointi, joka osoittaa, että tuote täyttää sille asetetut vaatimukset. Vaatimuksenmukaisuuden arviointi käsittelee tuotteen tyyppitarkastusta, vaatimusten toteutumista, tuotannon laadunvarmistusta ja tuotekohtaista arviointia. Valmistajan tulee luoda lisäksi laadunhallintajärjestelmä ja suunnitteluasiakirja sekä ylläpidettävä niitä. Valmis tuote tulee rekisteröidä Euroopan komission ylläpitämään Eudamed-rekisteriin. Lisäksi tuotteelle tulee suorittaa markkinoille saattamisen jälkeistä valvontaa, joka varmistaa tuotteen turvallisuuden jatkossakin.

Avainsanat: Injektoitava hydrogeeli, solunulkoinen matriisi, asetus, lääkinnällinen laite, EU

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

ATMP	Advanced therapy medicinal product
CE	Conformite Europeenne
CER	Clinical Evaluation Report
DoC	Declaration of Conformity
ECM	Extracellular matrix
EMA	European medicines agency
EU	European Union
ISO	International Organization for Standardization
MDCG	Medical Device Coordination Group
MDR	Medical device regulation
MI	Myocardial infarction
PMS	Post-market surveillance
UDI	Unique device identifiers

1. INTRODUCTION

The impact of cardiovascular diseases on human health is important, as heart diseases are the most common cause of death and disability worldwide (*World Health Organisation* 2021). Mature cardiac tissue has limited regenerative capacity and the lost cardiac muscle is replaced by scar tissue. The cardiac muscle is exposed to a greater hemodynamic load and as a result, it eventually fails. (*Max Planck Institute for Heart and Lung Research* 2021)

Due to the similar properties of hydrogels and soft tissues, hydrogels offer unique new regenerative treatments and open up many possibilities in biomedical applications. Interest in hydrogels is constantly growing and new applications are being developed. Hydrogels have the right properties to be injected into the body, so they arouse a lot of interest. However, they still have limitations such as biocompatibility, cytotoxicity and interaction with cells, that make their use and research difficult. Injectable hydrogels are hydrogels that can be injected as a liquid into the human body, forming a solid hydrogel (Alonso et al. 2021).

Injectable hydrogels derived from natural extracellular matrix (ECM) have been studied to have promising functions in helping damaged heart tissue to repair and regenerate. It's intented use is to support the cardiac tissues own regeneration by creating as normal a growth environment as possible for the cells. (Traverse et al. 2019) However, all medical applications are very strictly regulated in the European Union (EU), thus it is important to take these into account early in the beginning of development if the product is to be brought to market. EU regulations pose challenges in these developments due to their scope and complexity. Every detail must be taken into account so that the possible product meets the requirements. (Alonso et al. 2021) At the moment there is no product based on the technology in question on the market, so finding the right regulations can be difficult.

In this work, the regulations and requirements of injectable hydrogels derived from natural ECM, which are delivered to damaged cardiac scar tissue with a catheter, are in-

vestigated. There is one product under development based on that technology called Ventrigel, for which the regulations for a similar product are being studied. Ventrigel is the first biomaterial scaffold in development specifically designed to repair damaged cardiac tissue. It is an injectable hydrogel derived from porcine cardiac connective tissue. (Traverse et al. 2019)

The work focuses on the regulations that define the requirements for placing on the market and what happens after placing on the market. The aim of the work is therefore to find out what the product must be like in order to enter the EU market and what is still required on the market. The work answers the question "How can natural ECM derived injectable hydrogels be brought to the market, and what requirements apply to the product once in the market?".

This type of product is not yet available on the market, so there's not much literature regarding it available. The work applies products of the same style and instructions for them, as well as other examples that are helpful. The main challenge is finding the right information and applying it correctly. A key part of the work is also to specify the product type in order to know how to answer the research questions. Once the type is known, the correct EU regulation can be applied.

2. EXTRACELLULAR MATRIX CONTAINING INJECTABLE HYDROGEL

2.1 Injectable hydrogels

Three-dimensional polymer networks that are physically or chemically crosslinked are known as hydrogels. They can be of either synthetic, that is having an inherent hydrophilic nature due to their functional groups, or of natural origin. Hydrogels have many properties that make them unique, such as high water content, softness, flexibility, porosity, permeability and biocompatibility. Biocompatibility refers to the appropriate biological requirements of a biomaterial. They also have a strong affinity for bodily fluids like water. Since these properties are similar the properties of many living soft tissues, hydrogels open up many possibilities in biomedical applications. (Alonso et al. 2021)

An injectable hydrogel can be defined by the fact that when some biomaterials are injected as a liquid into the human body, a solid hydrogel is formed in situ (Alonso et al. 2021). In situ means an action that takes place in its final destination, so in this case it describes the formation of a hydrogel in the heart and not, for example, outside the human body (*Duodecim* 2021). Although there are other applications that also gel when injected into the human body, injectable hydrogels are the ones that have attracted the most interest for biomedical applications. They have the necessary physicochemical characteristics to be injected into the body in situ (Alonso et al. 2021).

As hydrogels are becoming increasingly useful biomaterials in many therapeutic approaches, the challenges associated with them should be taken into account right from the start of development. Therefore, it is important that the impact of diverse factors on processes is widely considered in research labs and the biomedical sector. Most of the time, these are ignored, which is why many potential products and good ideas do not reach the market or patients. (Alonso et al. 2021)

In addition to researching use cases, it would be important to attempt to construct novel innovations out of accessible materials using straightforward processes and basic designs

while assessing the impact of the process on the characteristics of the hydrogel that is generated. A successful approach necessitates consideration of economic, legal, and regulatory concerns in addition to monitoring chemical, physical, and biological data. If the product is meant to be commercialized, all of these should be taken into account at the beginning of the research, so that one can prepare for them. (Alonso et al. 2021)

Proteins and ECM components are commonly used to form natural hydrogels. Because they support numerous biological activities, this renders them naturally biocompatible, bioactive, and possibly ideal for various biomedical uses. Although they have similarities with native soft tissues in terms of structure and properties, they do have several drawbacks, chief among which is the difficulty in controlling them because of their considerable batch-to-batch variance. (Catoira et al. 2019)

Hydrogels still have limitations that make their use and research difficult. The biggest concerns center on their biocompatibility, cytotoxicity and interaction with cells. In addition, the restrictions increase the more detailed the product goes. Gelation mechanisms cause challenges with injectable hydrogels. The injectable hydrogel must gel at just the right time and cannot begin to gel until it is in the right place. There are many other aspects to this, such as speed kinetics, degradation time and mechanical resistance after gelation. (Alonso et al. 2021)

2.2 Function of extracellular matrix

ECM consist of extracellular macromolecules and minerals and it supports neighboring cells structurally and biochemically (Zhang et al. 2021). The binding cells structural coherence and stability are maintained by the ECM. The ECM also plays an important role in the transmission of biochemical signals that are essential for normal tissue development. All tissues contain it, but it occurs in each organ as a dispersion of certain matrix elements. Type I and type III collagen make up the majority of the cardiac's ECM. (Nikolov and Popovski 2022)

ECM has been demonstrated to be important for heart regeneration and repairing following cardiac damage. There is confirmation that the ECM may be utilized directly as a medication to encourage the proliferation of cardiomyocytes, in other words cardiac cell, and heart regeneration. When the cardiac's natural ECM is lost and replaced by a thick collagen scar, an aberrant microenvironment is generated that hinders the organ from functioning normally. (Vu et al. 2022)

For this reason an injectable hydrogel derived ECM can be considered a promising form of treatment. When it's injected, it supports the cardiac tissue's own regeneration by creating as normal a growth environment as possible for the cells. (*PhysicsWorld* 2019)

2.3 Case: Ventrigel

One product based on this technology is currently under development. A company called Ventrix aims to replace the abnormal microenvironment of damage caused by a heart attack to facilitate the heart's self-repair. Ventrigel, an injectable hydrogel that can be delivered using a catheter and is produced from porcine decellularized cardiac ECM, has been created specifically for this use. (Traverse et al. 2019)

Ventrigel is the first biomaterial scaffold under development that is specifically designed to repair damaged cardiac muscle tissue. It is an injectable hydrogel containing cardiomyocytespecific components of the ECM. It mimics the intrinsic properties of the cardiac ECM because it is produced from it. (Traverse et al. 2019) When permanent cells are removed from the tissue while leaving the original ECM structure and its constituents (laminin, collagens, and GAGs), a decellularized natural hydrogel called Ventrigel can be created. (Catoira et al. 2019)

Ventrigel is created using porcine cardiac connective tissue. It is first prepared by removing cardiac muscle cells through a purification process, after which the matrix is lyophilized, i.e. freeze-dried. It is subsequently reduced to a fine powder and partially digested by enzymes to allow it to pass through the catheter. Finally, it is turned into a liquid so that it can be injected into the cardiac muscle without surgery through a minimally invasive technique, such as using a catheter. This fluid develops a novel microenvironment when it hits body temperature and transforms into a semi-solid, porous gel. (*PhysicsWorld* 2019)

The purpose of Ventrigel is to provide a supportive environment for cell-matrix interactions, facilitating cell penetration and heart healing. When injected into the injured cardiac muscle, Ventrigel creates a scaffold that serves as a healing environment for healthy cells to move to, resulting in an increase in cardiac muscle tissue volume, a decrease in scar tissue volume, and an improvement in heart function. (Traverse et al. 2019)

Previously, in-vitro experiments have already been done with rat and pig myocardial infarction (MI) models using hydrogel derived from the extracellular matrix. These analyses already showed promising results, such as cardiac stem cell differentiation. The hydro-

gel decreased fibrosis or scarring, decreased border zone cardiomyocyte apoptosis, and promoted a pro-remodeling rather than a pro-inflammatory environment in rat MI models. Apoptosis means the process of programmed cell death. Instead, the hydrogel increased cardiac muscle, decreased fibrosis, and improved functional outcomes following MI in porcine MI models. Although the results are slightly different, both preclinical studies showed the most important thing, i.e. presence of biocompatibility and hemocompatibility. (Traverse et al. 2019)

Based on these results, Ventrigel was to be tested more. Between 2015 and 2019, Ventrigel participated in a phase I open safety study that was approved by the Food and Drug Administration and the institutional review boards of all participating clinical centers. This study examined the effects of administering extracellular matrix hydrogel percutaneously after myocardial infarction. Percutaneous procedure refers to a method that uses a needle puncture of the skin instead of open surgery. The study included 15 individuals who had experienced early and late post-MI within the previous 60 days to three years. Twelve of the study's participants were men, and they ranged in age from 45 to 69. Due to constraints on the number of injections that may be given, two patients received fewer injections than the other 13 patients. (Traverse et al. 2019)

The results of the trial were promising and there were no interruptions in the study. A few side effects occurred, but these cannot be directly linked to Ventrigel. The walking test, which was tested at baseline, 3 and 6 months into the trial, was used as a measure of functional training ability. It was noted that Ventrigel significantly increased maximum walking distance after injection at 3 and 6 months. The wide differences between patients can make it difficult to demonstrate the levels of efficacy. The most notable changes were in the left ventricular end systolic volume (LVESV) and increases in cardiac muscle. (Traverse et al. 2019) The amount of blood in the ventricle at the conclusion of contraction and just before filling up is known as the end-systolic volume, and it is influenced by afterload and the contractions of the heart (*End-diastolic volume* 2019). These are weakened by myocardial infarction, as was already mentioned. At six months after therapy, there were trending increases in viable mass and decreases in LVESV in these later individuals (Traverse et al. 2019).

3. EUROPEAN UNION REGULATORY FRAMEWORK

3.1 Legislation hierarchy of the European Union

As the name suggests, primary law is at the top of the hierarchy and consists of the European Union (EU) treaties, its protocols, the Charter of Fundamental Rights and the general principles established by the Court of Justice of the European Union. Secondary acts are laws and other acts adopted by the EU institutions that enable the EU to exercise its powers. Regulations, directives and decisions are related to these. Between of them there is no hierarchical difference, but their differences are related to the purpose of use. (Oikeusministeriö n.d.)

EU regulations are binding regulations that bind all EU countries in all respects. The regulations are automatically and uniformly applied as such in all EU countries as soon as they come into force. A directive refers to a regulation that sets an objective. All EU countries have to achieve this target. (Oikeusministeriö n.d.)

In addition to the aforementioned, implementing acts that define the detailed rules that enable the uniform implementation of EU acts. In addition to binding regulations, directive instruments such as recommendations, statements, resolutions, declarations and action programs can be issued in the EU. (Oikeusministeriö n.d.)

3.2 Regulation of medical devices

The regulatory framework for medical devices covers every regulation, guideline and standard that applies to them. These ultimately cover all the requirements for medical devices and with the help of these the legislation is correctly followed. In addition to the main regulation, the regulatory framework consists of other supplementary parts. There is a hierarchy in the legal system, where some regulations are higher than others and each of which contains specific provisions (Oikeusministeriö n.d.).

EU medical device legislation can be said to consist of four different levels, which are

international standards, national laws, EU laws and national laws. These are shown in figure 3.1. The regulatory framework theoretically defines the basic laws of each member state, but leaves room for national regulation. Its regulatory scope includes everything, for example product development, clinical trials and obtaining marketing approvals. In addition to the main regulation, it contains other instructions that are referred to in the main regulation. (Pitkänen et al. 2020)

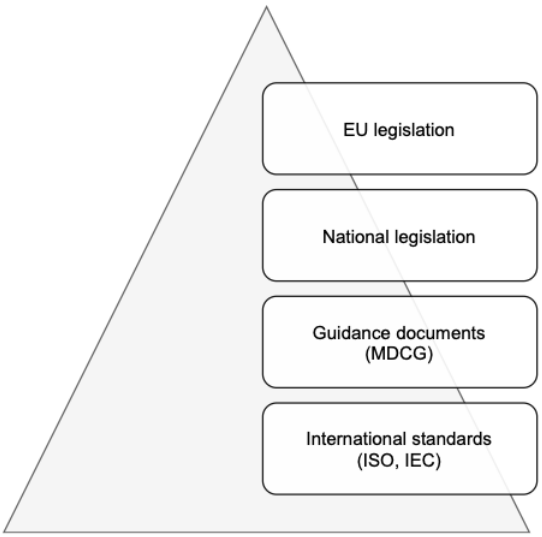

Figure 3.1. *The hierarchy of the EU regulatory framework (Toivakka 2021)*

The legislation set by the EU must be followed in the entire market area, but the implementations fall within the scope of national legislation. However, the regulatory guidelines support the definition of stakeholders, as they communicate the authorities expectations and thoughts. (Pitkänen et al. 2020)

Since EU regulations are challenging as a whole, guidance publications have been prepared that explain regulatory issues related to general safety, performance and standards. The Medical Device Coordination Group (MDCG) produces guidance and advice on key issues and compliance. (Pitkänen et al. 2020) It also supervises the operations of notified bodies.

Notified bodies are authorized by the European authorities to check companies and products that manufacture medical devices. Notified bodies are assessment organizations that perform conformity assessment tasks based on EU regulations. If a third-party assessment is required by law, this will be carried out by a notified body. On the website of the European Commission, can be found notified bodies for certain tasks. (*Finnish Accreditation Service* 2021)

However, following the relevant standards produced by the International Organization for Standardization (ISO) and using a standards-compliant development process is the most useful and practical way to understand the regulatory aspect. Manufacturers can say they comply with product legislation by using international standards set by regulatory authorities. (Pitkänen et al. 2020)

3.2.1 Medical device regulation

Medical devices have two main regulations, Medical device regulation (MDR) and In vitro diagnostic medical devices (IVDR), under which a possible medical device would fall. However, we will take a closer look only to MDR which is Regulation (EU) 2017/745 of the European Parliament and of the Council of April 5, 2017. The regulation entered into force definitively on May 26, 2021. (*Regulation (EU) 2017/745* 2017)

The MDR is designed to increase patient safety and make sure that new medical technology will benefit patients. Patient safety is improved by more precise and clear requirements and changes. These include, among other things, registration, quality system, responsibility of distributors and traceability of equipment. With the regulation, it is also necessary to ensure that the product is properly labeled. (*Regulation (EU) 2017/745* 2017)

The regulation improves medical device monitoring as well. Documented evidence must be obtained from device testing and safety monitoring. The supervision and responsibility of the authorities has also increased. (*Regulation (EU) 2017/745* 2017) Later in the text, the requirements of the regulation will be explored in more detail.

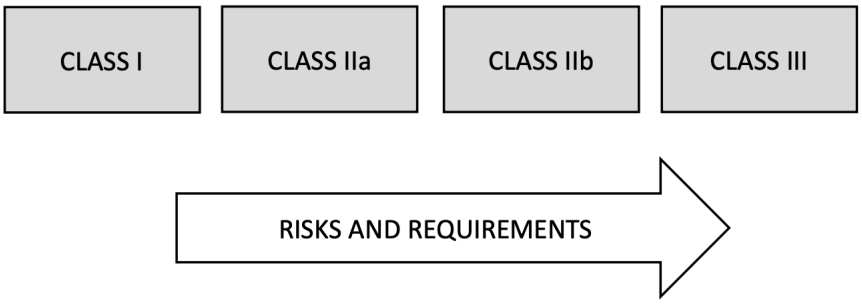

Figure 3.2. *Illustration of how risks and data requirements increase as classes go up.*

The MDR also introduces a classification of medical devices that reflects the potential harm that a medical device can cause. Product classification is determined by the intended use of the medical device, not its composition. In addition, the same product

classification exists regardless of the product type. (*Regulation (EU) 2017/745* 2017)

As figure 3.2 illustrates, the classes are divided into four different classes based on their purpose, characteristics and risks from lowest to highest risk: Class I, Class IIa, Class IIb and Class III. The class of medical devices can be determined according to Regulation (EU) 2017/745. The requirements related to the devices also follow the same classification, where the lowest category has the least requirements and the highest has the most. (*Regulation (EU) 2017/745* 2017)

3.3 European medicines agency

There are also other regulatory documents that are included in the EU Regulatory Framework and that are applicable to this study. European medicines agency (EMA) is agency relating to pharmaceutical development. The EMA is in charge of supervision and evaluating pharmaceutical products (EMA 2018a).

The EMA is an EU agency whose main responsibilities include protecting and promoting the evaluation and control of medicines and public and animal health. It provides scientific advice, develops technical guidelines and coordinates product evaluation and monitoring. Guidelines that reflect a coordinated strategy of the EU Member States and EMA are used to interpret and apply the standards for the demonstration of quality, safety, and efficacy outlined in the Community directives. (EMA 2018a)

EMA's scientific guidelines for medicinal products for human use are harmonized by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH works to guarantee that pharmaceuticals are created, registered, and maintained in the most resource-effective manner feasible. In addition, its goal is to enable compliance with high standards to increase global harmonization. (*ICH* n.d.)

3.4 European Union institutions

The European Union has several institutions whose task is to promote the interests of the EU and its citizens. Each institution has its own special tasks and often these are still specialized in certain fields such as medicine or environmental issues. The European Parliament, the European Council, the Council of the European Union and the European Commission participate in EU decision-making, and each of these has its own role in the legislative process. The areas of responsibility of all institutions are stipulated in the EU treaties. (EU Types of institutions n.d.)

The European Parliament represents the citizens and the Council of the EU represents the governments of the EU countries and together they make decisions regarding European laws. The European Council usually does not approve laws, but it defines the general political direction and priorities of the European Union. (EU Types of institutions n.d.)

The EU's primary executive body, the European Commission, represents the EU's common interests. It uses its "right of initiative" to propose new laws that can be approved by the European Parliament and the Council of the European Union. It also ensures that countries implement EU legislation properly. (EU Types of institutions n.d.)

4. DETERMINING THE TYPE OF THE PRODUCT

Before considering bringing a product to the market, the type of product should be determined by comparing the EU regulations intended for different products. There are different EU regulations for different products and their requirements vary, so it is necessary to find out what to use. Determining the type of product can be a simple process, but it can also require more research. The type of injectable hydrogel derived from ECM is not directly clear, so in this work figuring out the type is started by getting to know the possible options and one by one the options were excluded. There are several options, for example, advanced therapy medicinal product (ATMP), medical devices, medicinal product or tissue engineered products (TEP).

Determining the product type requires that the manufacturer knows the product. The manufacturer must know the intended use of the product and also what the product consists of. These can be used to determine the type of product, whether it would be, for example, an ATMP, a device or a medicine. When the right type is found out, it is also known which regulation should be applied.

There can also be different product categories within the regulations, which are determined by the regulation in question. Some requirements may also be different for products under the regulation. With the help of regulation, it can find out which requirements the product must meet.

4.1 As a medical device

An injectable hydrogel can be considered as a medical device according to MDR Article 2, paragraph 5, which says that an implantable device is a device that has been inserted into the human body by a clinical procedure and is intended to be left in place. Therefore, finding out the type of product starts with the fact that it could be a medical device. A medical device means an instrument, hardware, device, software, implant, reagent, material or other accessory that is intended to be used by a person for medical purposes, for

example to diagnose an illness or injury. Furthermore, the main effect of a medical device on the human body is not pharmacological, metabolic or immunological. The regulation does not separately define injectable devices, only implantable devices, so this article must be applied. According to Article 1, paragraph 5 of the regulation, a device that is designed to be fully inserted into the human body and remain there is referred to as an implantable device. (*Regulation (EU) 2017/745* 2017)

The classification of the category medical devices can be done with the help of Chapter 3 of Appendix 8 of the MDR. According to Rule 8, long-term invasive devices intended for use in direct contact with the heart are classified as Class III. In addition, according to rule 18, the product is classified as category III if animal tissues rendered non-viable or their derivatives are used in its manufacture. (*Regulation (EU) 2017/745* 2017) It is therefore stated that the product would be classified in class III. However, the product is derived from natural ECM and it's not synthetic, so other options have to be taken into account.

4.2 As a combined advanced therapy medicinal product

Regulation (EC) No. 1394/2007 defines the special rules regarding authorizations, supervision and pharmacovigilance of drugs used in advanced therapy, and the product could fall into this category. According to the definition, a product can be defined as such if it meets the following conditions: the product contains as an essential part at least one implantable medical device referred to in Article 1, paragraph 2, letter c of Directive 90/385/EEC, and its cellular or tissue part must contain living cells or tissues. (Regulation (EC) No 1394/2007 2007) According to the article 1, paragraph 2, letter c of Directive 90/385/EEC implantable medical device means any medical device which is meant to be fully or partially inserted into the human body and intended to stay there after the procedure, which in this case is true of the product (EUR-Lex 90/385/EEC 1990).

In order to either specify the product type here or exclude the option„ we need to consider the presence of ECM. First of all, it is necessary to find out how ECM is treated in the EU and by the EMA, i.e. whether it is counted as cell-based, for example. Since the ECM definition is not found separately, the type determination is done with the help of examples. According to the first comparative example, a cellular tubule graft consisting of human collagen types I and III and other extracellular matrix proteins such as fibronectin and vitronectin does not meet the criteria of a medicinal product used in advanced therapy according to the EMA, because it does not contain genes, cells or tissues. (EMA 2018b)

According to another example, the type determination of this product as a medical device can be more reliably justified. The example deals with a product that contains e.g. resorbable matrix and animal growth factors. According to the EMA, based on Regulation (EU) No 722/2012 and Regulation (EU) 2017/745, the product can be a medical device in class III, if it cannot be clearly demonstrated that the osteoinductive nature of the animal growth factors is only an additional part of the osteoconductive matrix, product belongs to the definition of the drug. (Directorate-General for Health and Food Safety 2022) In short, osteoinduction refers to the stimulation of cells to develop into preosteoblasts, i.e. early bone cells, and osteoconduction means that bone grows on the surface of the biomaterial. However, the product examined in this work does not contain growth factors, so based on this, the product can be a medical device in class III. Furthermore, Regulation (EU) No 722/2012 used in the example product cannot be applied to this product, as it only applies to tissues originating from TSE-susceptible species, which are not present in the product of this work (Regulation (EU) 722/2012 2012).

It was interesting to note that the directives referred to in the regulation are no longer valid even though the regulation is valid. Directive 90/385/EEC regulated active implantable medical devices and directive 93/42/EEC medical devices in general. However, both of these directives were replaced by the regulation on medical devices issued on April 5, 2017, and the new regulation entered into force on May 26, 2021. (*European Comission, New Regulations* 2023)

4.3 As a medicinal product for human use

In the end, the type determination between medical device and combined advanced therapy medicinal product was straightforward. However, ECM derived injectable hydrogels can be either a device or a drug depending on its main mode of action. If the product were to be a medicine, it would need a more thorough study of biocompatibility and therapeutic effect before approval for clinical use. (Almawash et al. 2022) Additionally, due to new regulations, it may be easier today to design product directly to meet medical device regulations.

An injectable hydrogel can be a medicinal product if its main mechanism of action is pharmacological, metabolic or immunological. Today, it has been observed that more and more products, which are usually devices, are beginning to approach the mechanism of action of drugs. This will probably change the regulatory framework, and in the future more effort will be put into the type of product according to the regulations already in

the design phase. Also, if the product is medicine instead of a device it slows down the process from three years to up to 12 years. (Almawash et al. 2022)

In this work, the product is exactly between devices and drugs. The purpose of this product is to form a support structure in the scar of damaged heart muscle and act as an environment for healthy cells to repair the damage (Almawash et al. 2022). Although the purpose of the product is to influence cell movements, it does not itself stimulate or affect the cells (Almawash et al. 2022). Based on this, it can be concluded that the main effect of this product is physical.

4.4 Reasoning for the chosen type

When the product's main mode of action is physical and not chemical in the body, the product is a device instead of a medicine. Physical interaction achieves the gel state by changing the forces between molecules. This can happen either from the internal arrangements of the polymer or from external stimuli such as pressure. Such hydrogels have a certain gelation time, mechanical strength and biodegradation mechanism. (Almawash et al. 2022)

Taking all options into account, we finally come to the conclusion that the ECM derived injectable hydrogel is a medical device in class III in the EU. Since the product type is now known, the relevant regulation can be applied in the following paragraphs. By applying it, answers to research questions are found. The regulation for medical devices is the MDR.

5. MARKETING APPROVAL

5.1 Marketing and implementation

Putting medical devices on the market is mainly based on the CE marking approval process (*Regulation (EU) 2017/745* 2017). This paragraph deals more generally with the requirements regarding ECM derived injectable hydrogel, and the following paragraphs focus on these requirements in more detail. The process can be thought of as certain steps shown in figure 5.1, each of which requires the success of the previous one.

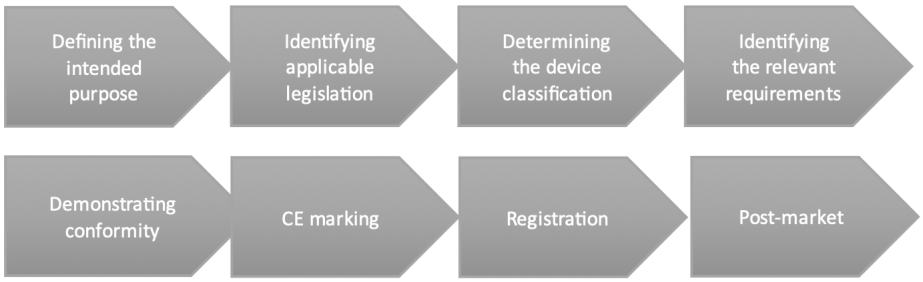

Figure 5.1. *Simplified steps to bring device to market*

The first step is to find out the intended use and the type of the product and determine the applicable legislation. After the classification and clarification of the requirements, it must be shown that these requirements for this risk category are met. A CE marking can be granted to the product when the above mentioned has been completed with approval. (*Regulation (EU) 2017/745* 2017) Product registration and post-market are discussed in the last paragraph.

Article 5 of the MDR defines the devices placing on the market and putting it into use. The medical device can only be placed on the market if it has been properly delivered and installed. The device must meet the applicable general safety and performance requirements, taking into account its intended use. These can be found in Annex I of the MDR. (*Regulation (EU) 2017/745* 2017)

Since we are looking at ECM based injectable hydrogels, appropriate requirements are

applied to them according to Annex I regarding desing and manufacture. Special attention must be paid to the compatibility of the materials used with the body. In addition, the risk of contamination must be minimized in planning, manufacturing and packaging. Furthermore, since the device contains a substance derived from an animal, according to point 13.2 of Annex I, the porcine from which the ECM was obtained must have undergone veterinary checks and, in particular, special attention must be paid to viruses and other pathogens. A clinical evaluation in accordance with Article 61 should be included in the demonstration of compliance with these requirements. (*Regulation (EU) 2017/745* 2017)

5.2 Conformity assessment

In order to get the product onto the European market, the manufacturer must carry out a conformity assessment for the product. For medical devices, the conformity assessment shows that the product meets all the requirements of the MDR and thus guarantees its reliability. A conformity assessment based on the quality management system and the evaluation of the technical documentation, which can be found in Annex IX of the MDR, is applied to a class III product. When the conformity assessment has been carried out with approval, the manufacturer may attach the Conformite Europeenne (CE) mark to the product. Without this mark, the product must not be on the European market but with it the product may move freely in any EU member state. (*Regulation (EU) 2017/745* 2017).

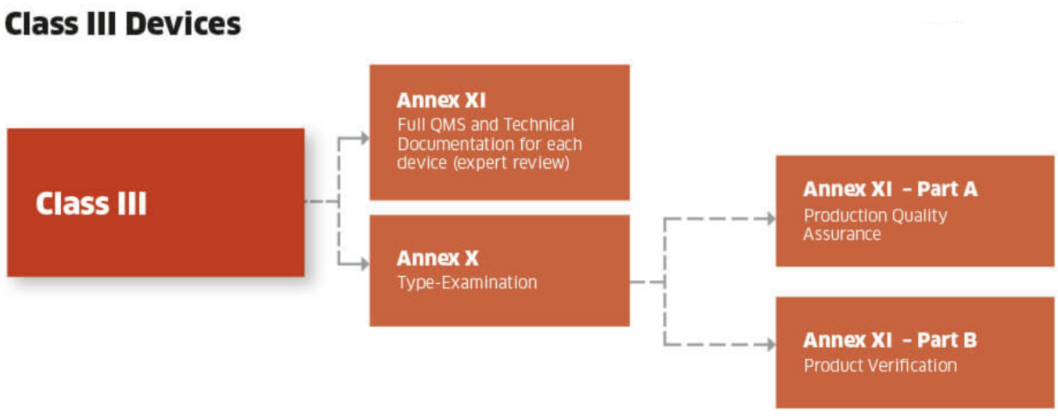

Figure 5.2. *Conformity assessment route (Mdi Europa n.d.)*

Figure 5.2 shows a diagram of class III medical devices of the conformity assessment route which includes Annexes X and XI of the MDR. Annex X concerns the conformity assessment based on the type inspection, with which the notified body ensures and proves

that the device meets the relevant regulations. Annex XI concerns the conformity assessment based on the inspection of the product's conformity to requirements. Part A of this deals with production quality assurance, i.e. the implementation of the quality management system and the final inspection and conformity assessment methods to be performed. Part B deals with the product-specific review, which requires a commitment to prepare and keep up to date a post-market surveillance plan.

5.3 Clinical trials and evaluations

Humans are involved in clinical trials of medical devices, so before that the company must perform preclinical testing. These initially determine whether the device is safe and effective enough to be used and tested in humans. Preclinical phases may include, for example, bench testing and animal experiments. When it is time to move on to clinical trials after preclinical testing, approval is needed for the proposed study. This approval is obtained by application. Before starting clinical trials, the sponsor must send an application to the Member State. A clinical trial can be started when the Member State notifies that it has granted permission to the sponsor. (*Regulation (EU) 2017/745* 2017)

Clinical trials are studies whose purpose is to discover or verify the effects of one or more research products. The regulation of clinical trials aims to ensure the credibility of the results of clinical trials and to protect the rights and well-being of those who participated in these trials. (*Regulation EU No 536/2014* 2023) Articles 62-82 of the MDR deal with clinical trials of medical devices and precisely define the details of their procedure from different perspectives. In this work, the focus is more on obtaining a marketing authorization, so the discuss about clinical trials is more brief. These are an essential part of bringing the device to market.

Clinical trials still require a clinical evaluation, which the manufacturer must plan, perform and document in accordance with Article 61. This is to demonstrate clinical trial level compliance with general safety and performance requirements. This includes, among other things, a critical review of the relevant scientific literature on the device's safety, performance, design features and intended use. In addition, the clinical evaluation includes a critical evaluation of the results of all available clinical trials in accordance with Articles 62-80 of the MDR, as well as a review of possible alternative forms of treatment. The findings of a clinical examination of your medical device are documented in a Clinical Evaluation Report (CER). A CER is made up of clinical data that has been examined and acquired from either a clinical investigation of one's own device or the findings of studies

on similar devices. (*Regulation (EU) 2017/745* 2017)

A clinical trial must comply with the legislation and its anticipated benefits must be greater than the anticipated risks. In addition, the subject must participate voluntarily and the subject must be subjected to as little stress as possible. Vulnerable population groups and subjects, for example disabled or pregnant, must be properly protected. The clinical study must be conducted in a space corresponding to the future purpose of use. (*Regulation (EU) 2017/745* 2017)

5.4 Quality Management System

For medical devices in class III, a quality management system must be implemented. The quality management system is defined in Article 10 of the MDR and its Annex IX. Product manufacturers must prepare, document and implement a quality management system that is constantly maintained and updated. An organization needs a quality management system to create high-quality products for customers and increase customer satisfaction.

Most commonly, companies use the ISO 13485 standard to achieve compatibility with their quality management system. The ability to produce these devices and services related to them that consistently meet customer approval and applicable regulatory requirements must demonstrated. In addition to this, other ISO standards can also be used, for example ISO 1415:2020, which deals with the clinical investigation of medical devices intended for humans. (*ISO 13485* 2021) An auditor from a Notified Body must audit the quality management system (*Regulation (EU) 2017/745* 2017).

5.5 Design dossiers

After the medical device has undergone sufficient testing, the manufacturer is required to put together a design dossier that shows the product complies with the design guidelines set during the product development process. Design dossier is a technical document intended for class III medical devices and it's comprehensive description of the device, the purpose of which is to show that the device complies with European regulations. This should include detailed information regarding function, design, use, composition, claims and clinical evaluation of the medical device. A design dossier is necessary to enable a design examination by a Notified Body, whose objective is to confirm that medical device complies with its technical requirements. A Notified Body must receive the design dossier for approval. (*Regulation (EU) 2017/745* 2017)

5.6 CE marking

When the product has been classified and tested and the quality management system and design dossier made and approved, the product can have the CE mark which every medical device on the EU market needs. The CE marking is applied in Article 30 of Regulation (EC) No. 765/2008. It's a mark with which the product manufacturer or an authorized representative assures that the product meets the performance, quality, safety and power requirements of the EU directives and regulations applicable to the product. A product with a CE mark can move freely within the EU. (*Regulation (EU) 2017/745* 2017) The CE marking can be found in the figure 5.3.

The CE marking must be affixed to the device before placing it on the market in a manner in which it is visible and understandable. The CE mark can only be affixed by the manufacturer of the product or an authorized representative and therefore it's not issued by any authority. If other markings are affixed to the CE marking, they must not mislead third parties in terms of purpose or graphic presentation. EU member states must ensure proper implementation of the CE marking system. (*Regulation (EU) 2017/745* 2017)

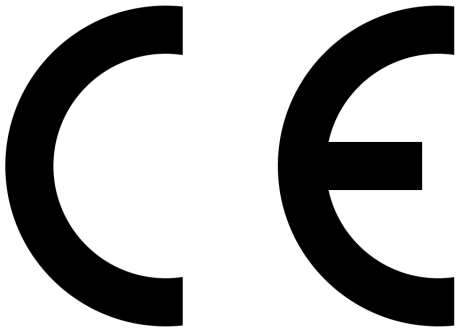

Figure 5.3. *CE mark (*CE marking *n.d.)*

CE mark issued by notified bodies are usually valid for three years. However, the status of your CE certification depends on maintaining the quality system certificate, so some risky devices may be valid for one year only. (*European CE Marking* n.d.) However, if the CE marking is misused, EU member states must take possible sanctions in accordance with Article 41 of Regulation (EC) No. 765/2008. EU member states must determine the applicable sanctions related to the violation of the rules and monitor their implementation. (*Regulation (EC) No 765/2008* 2008)

6. POST-MARKET

After the device has been placed on the market, manufacturers must design, establish, document, implement and maintain a control system. This should collect information about the quality, performance and safety of the device and analyze it. Manufacturers must comply with the The unique device identification (UDI) system obligations set out in Article 27 and the registration obligations set out in Articles 29 and 31. In the case of an implantable device, manufacturers must keep the technical documentation, the EU declaration of conformity and a copy of the relevant certificate issued in accordance with Article 56 (and its amendments and additions if necessary), accessible to authorities for a minimum of 15 years. In addition, manufacturers must ensure maintaining a quality management system and responsibility for the device while it is on the market. (*Regulation (EU) 2017/745* 2017)

6.1 Eudamed register and UDI

The device in question can be registered in Eudamed register maintained by the European Commission. Eudamed facilitates citizens and healthcare professionals access to information and enhances the coordination of actions between EU countries. It includes registration of operators, database of unique device identifiers (UDI) and registration of devices, notified bodies and certificates, clinical trials and performance studies, incident system and market surveillance. The use of Eudamed is not yet mandatory, but some parts can already be used freely. The European Commission cannot require the use of Eudamed until it's fully operational in accordance with the regulations, but the authorities have the authority to decide on registrations per member state even before this. Currently, the modules UDI/device registration, Actor registration and Notified Bodies and Certificates are in use. When the entire Eudamed system is found to be functional and put into use, the mandatory use of the medical device regulations system will start after six months. (*Regulation (EU) 2017/745* 2017)

The Unique Device Identification (UDI) is a unique numeric code which gives every device

on the market a unique identification number. This helps with, for example, their traceability. It contains a device identifier (UDI-DI) which can be used to retrieve information and documents from the database and a production identifier (UDI-PI) which is used to identify the production unit of the device. (*Regulation (EU) 2017/745* 2017)

6.2 EU declaration of comformity

The EU Declaration of Conformity (DoC) is a mandatory document that must be signed by the manufacturer or an authorized representative. It guarantees that the item conforms with EU regulations. The manufacturer fully accepts responsibility that the product complies with all relevant EU regulations by signing the certificate of conformity. (*Regulation (EU) 2017/745* 2017)

What must be in the DoC is explained in detail in Annex IV of the MDR. It must at least include the manufacturer's name, the product's name, its intended use, the risk category and its UDI-DI identification. Additionally, a declaration that the manufacturer accepts full responsibility for the product and that it's indeed compliant must be included in the DoC. The specifications upon which the declaration of compliance has been issued should also be mentioned. (*Regulation (EU) 2017/745* 2017)

6.3 Post-market surveillance activities

Post-market surveillance (PMS) is necessary for all manufacturers and others working within the medical device industry. PMS system needs to be an integrated part of the manufacturer's QMS and it shall include instructions on how to plan, document, maintain and update the system. The PMS system should be proactive, in which case the necessary wishes are more likely to be preventive and not curative. (*Regulation (EU) 2017/745* 2017)

When the product is on the market, in order to maintain the certificate, the company is monitored with planned annual audits. In addition, tests must be performed on the equipment to assess compliance with the requirements if possible. The auditing team checks that the product complies with the technical documents and the set statutory requirements. In addition, the company is subject to additional inspections at least once every three years, and their target is both the certified quality management system and the manufactured medical device. (*Regulation (EU) 2017/745* 2017)

Before receiving the CE mark, the manufacturer has had to make a CER for medical

devices. CER should be updated regularly. It should be updated at least every year, but more often if, after being placed on the market, new information emerges as a result of surveillance that may change the current assessment. (*Regulation (EU) 2017/745* 2017)

The manufacturer of the device is equally responsible for the device once the device enters the market. If the manufacturer notices or believes that the device no longer complies with the MDR, the manufacturer must immediately take the necessary corrective measures or remove the product from the market. However, if the manufacturer neglects his duties or the documents provided are incomplete or incorrect, appropriate measures may be taken by a competent authority to prohibit or make restrictions for the device's market status or remove it from the market altogether. (*Regulation (EU) 2017/745* 2017)

If dangerous side effects occur during use, with the exception of already reported side effects, these must be reported to the competent authorities under normal circumstances within 15 days of the dangerous situation at the latest and when the cause-and-effect relationship has been clarified. Notification is accelerated, for example, by a possible threat to public health or an unexpected serious deterioration of a person's health, such as death. If necessary, the member state also has the obligation to, for example, organize targeted information campaigns. The competent authority must also inform the manufacturer of the device about dangerous situations it learns about. (*Regulation (EU) 2017/745* 2017)

7. CONCLUSION

In this work, EU regulations regarding ECM based injectable hydrogel were investigated and applied. The primary goal of the work was to find out how to bring this type of product to the EU market and what should be taken into account in the market. The work started by finding out what type of product it is by comparing possible alternatives. When the type of product was clarified, it was known which regulation applies to it. By applying the regulation, the marketing process and the implementation after the launch were finally clarified.

According to this work, an ECM-based injectable hydrogel used for direct contact with the heart is a medical device of the highest risk class III. The requirements of the product are regulated by Regulation (EU) 2017/745 on medical devices. It defines that placing a product on the market requires CE marking. The CE mark is the only mark valid in the EU that shows that it meets the requirements set for it. Obtaining the CE mark requires clinical trials and clinical evaluation, conformity assessment, quality system and design dossier.

After entering the market, the quality of the product must continue to be monitored with post-market surveillance. It includes everything from systems design, documentation, maintenance and updating. In addition, it takes into account possible risks and sets the necessary measures when they occur. The medical device must also be registered in Eudamed, where it has its own UDI code, which is a unique identification number for every device on the market.

Figuring out the type of product turned out to be the most difficult phase of the work. The product in question could potentially fit under a few different types, so the details were an important part. Especially when comparing a device and a drug, the final type of product could have been different with a small change. While looking for information, I came across many different sources where the fact that many people find it difficult to classify such products was stated.

For simple products, finding the type is easier, but in complex cases it takes time. Perhaps

in the future, the guidelines for regenerative medicine will be developed even clearer, so that more products can be manufactured. Development would certainly be helped by clear instructions, according to which products could be developed and steered in the right direction.

The selection of the type was further complicated by the fact that the work sample product was developed in the USA and all literature is based on US and US Food and Drug Administration (FDA) definitions. Many of the FDA's regulations and guidelines have been confusing in this study, as some information and product claims may refer more to a different category. However, in the EU products are defined according to their own regulations.

Obtaining the CE mark in class III requires stricter regulations and quality requirements compared to other classes. However, since it is supposed to work in contact with the heart, I think this is also necessary. Hopefully the laws will evolve in the future and keep up with the development of medical applications so they don't slow down their use. We certainly also need more experts to work between laws and companies, so that new forms of treatment can be introduced effectively.

If the research were to be continued, the question could be to consider the path of ECM based injectable hydrogels to the market in other countries or how the path changes when the product type changes. In addition, it would be interesting to think about what, for example, adding cells to the product would do to market access.

REFERENCES

Almawash, S., S. K. Osman, G. Mustafa, and M. A. El Hamd (Mar. 18, 2022). “Current and Future Prospective of Injectable Hydrogels—Design Challenges and Limitations”. In: *Pharmaceuticals* 15.3, p. 371. DOI: 10.3390/ph15030371. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8948902/ (visited on 04/25/2023).

Alonso, J. M., J. Andrade del Olmo, R. Perez Gonzalez, and V. Saez-Martinez (Feb. 22, 2021). “Injectable Hydrogels: From Laboratory to Industrialization”. In: *Polymers* 13.4, p. 650. DOI: 10.3390/polym13040650. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7926321/ (visited on 04/25/2023).

Catoira, M. C., L. Fusaro, D. Di Francesco, M. Ramella, and F. Boccafoschi (Oct. 10, 2019). “Overview of natural hydrogels for regenerative medicine applications”. In: *Journal of Materials Science: Materials in Medicine* 30.10, p. 115. DOI: 10.1007/s10856-019-6318-7. URL: https://doi.org/10.1007/s10856-019-6318-7 (visited on 04/25/2023).

CE marking (n.d.). URL: https://single-market-economy.ec.europa.eu/single-market/ce-marking_en (visited on 05/21/2023).

Directorate-General for Health and Food Safety (Sept. 7, 2022). *Manual on Borderline and Classification in the Community Regulatory Framework for Medical Devices (September 2022)*. URL: https://health.ec.europa.eu/latest-updates/manual-borderline-and-classification-community-regulatory-framework-medical-devices-september-2022-2022-09-07_en (visited on 04/25/2023).

Duodecim (2021). *Duodecim in situ*. Duodecim Terveyskirjasto. URL: https://www.terveyskirjasto.fi/ltt04179 (visited on 04/25/2023).

EMA (Sept. 17, 2018a). *European Medicines Agency (EMA), What we do*. European Medicines Agency. URL: https://www.ema.europa.eu/en/about-us/what-we-do (visited on 04/25/2023).

EMA (Sept. 17, 2018b). *Scientific recommendations on classification of advanced therapy medicinal products*. European Medicines Agency. URL: https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/advanced-therapy-classification/scientific-recommendations-classification-advanced-therapy-medicinal-products (visited on 04/25/2023).

End-diastolic volume (June 18, 2019). *End-diastolic volume: What is it, and how do doctors use it?* URL: https://www.medicalnewstoday.com/articles/325498 (visited on 04/26/2023).

EU Types of institutions (n.d.). *Types of institutions, bodies and agencies | European Union*. URL: https://european-union.europa.eu/institutions-law-budget/institutions-and-bodies/types-institutions-and-bodies_en (visited on 05/21/2023).

EUR-Lex 90/385/EEC (June 20, 1990). *Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices*. URL: http://data.europa.eu/eli/dir/1990/385/oj/eng (visited on 04/25/2023).

European CE Marking (n.d.). *European CE Marking Strategy for Medical Devices*. Emergo by UL. URL: https://www.emergobyul.com/services/european-ce-marking-strategy-medical-devices (visited on 04/26/2023).

European Comission, New Regulations (Apr. 21, 2023). URL: https://health.ec.europa.eu/medical-devices-sector/new-regulations_en (visited on 04/25/2023).

Finnish Accreditation Service (June 10, 2021). *Finnish Accreditation Service*. URL: https://www.finas.fi/sites/en/accreditation/activity/Pages/Operation-of-Notified-Bodies-.aspx (visited on 04/25/2023).

ICH (n.d.). *International Council for Harmonisation (ICH), Official web site*. URL: https://www.ich.org/ (visited on 04/25/2023).

ISO 13485 (June 2, 2021). *ISO 13485:2016*. ISO. URL: https://www.iso.org/standard/59752.html (visited on 04/25/2023).

Max Planck Institute for Heart and Lung Research (Sept. 24, 2021). *Max Planck Institute for Heart and Lung Research, Heart regeneration after reprogramming of cardiac muscle cells*. URL: https://www.mpg.de/17679417/heart-regeneration-after-reprogramming-of-cardiac-muscle-cells (visited on 04/25/2023).

Mdi Europa (n.d.). *Full collection of charts about the Conformity Assessment Routes under the MDR*. mdi Europa. URL: https://mdi-europa.com/the-mdi-europa-online-library/full-collection-of-charts-about-the-conformity-assessment-routes-under-the-mdr/ (visited on 05/29/2023).

Nikolov, A. and N. Popovski (Mar. 28, 2022). “Extracellular Matrix in Heart Disease: Focus on Circulating Collagen Type I and III Derived Peptides as Biomarkers of Myocardial Fibrosis and Their Potential in the Prognosis of Heart Failure: A Concise Review”. In: *Metabolites* 12.4, p. 297. DOI: 10.3390/metabo12040297. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9025448/ (visited on 04/25/2023).

Oikeusministeriö (n.d.). *6.3 EU-säädökset*. Lainkirjoittajan opas. URL: http://lainkirjoittaja.finlex.fi/6-euroopan-unionin-oikeus-osana-suomen-oikeusjarjestysta/6-3/ (visited on 05/21/2023).

PhysicsWorld (Sept. 12, 2019). *PhysicsWorld, Hydrogel to repair heart proves safe to inject in humans*. Physics World. URL: https://physicsworld.com/a/hydrogel-to-repair-heart-proves-safe-to-inject-in-humans/ (visited on 04/25/2023).

Pitkänen, H., L. Raunio, I. Santavaara, and T. Ståhlberg (2020). “European Medical Device Regulations MDR & IVDR - A Guide to Market”. In: URL: https://www.leanentries.

com/wp-content/uploads/european-medical-device-regulations-mdr-ivdr-a-guide-to-market.pdf.

Regulation (EC) No 1394/2007 (Nov. 13, 2007). *EUR-Lex, Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (Text with EEA relevance)*. Legislative Body: CONSIL, EP. URL: http://data.europa.eu/eli/reg/2007/1394/oj/eng (visited on 04/25/2023).

Regulation (EC) No 765/2008 (July 9, 2008). *Regulation (EC) No 765/2008 of the European Parliament and of the Council of 9 July 2008 setting out the requirements for accreditation and market surveillance relating to the marketing of products and repealing Regulation (EEC) No 339/93 (Text with EEA relevance)*. Legislative Body: EP, CONSIL. URL: http://data.europa.eu/eli/reg/2008/765/oj/eng (visited on 04/25/2023).

Regulation (EU) 2017/745 (Apr. 5, 2017). Legislative Body: CONSIL, EP. URL: http://data.europa.eu/eli/reg/2017/745/oj/eng (visited on 04/25/2023).

Regulation (EU) 722/2012 (Aug. 8, 2012). *Commission Regulation (EU) No 722/2012*. Legislative Body: COM. URL: http://data.europa.eu/eli/reg/2012/722/oj/eng (visited on 04/25/2023).

Regulation EU No 536/2014 (Jan. 30, 2023). URL: https://health.ec.europa.eu/medicinal-products/clinical-trials/clinical-trials-regulation-eu-no-5362014_en (visited on 04/25/2023).

Toivakka, H. (Sept. 1, 2021). "Integration of EU medical device regulatory requirements into a CI/CD pipeline". PhD thesis. Tampere University. URL: https://trepo.tuni.fi/bitstream/handle/10024/134314/ToivakkaHenrik.pdf?sequence=2&isAllowed=y.

Traverse, J. H. et al. (Oct. 1, 2019). "First-in-Man Study of a Cardiac Extracellular Matrix Hydrogel in Early and Late Myocardial Infarction Patients". In: *JACC: Basic to Translational Science* 4.6, pp. 659–669. DOI: 10.1016/j.jacbts.2019.07.012. URL: https://www.sciencedirect.com/science/article/pii/S2452302X1930227X (visited on 04/25/2023).

Vu, T. V. A., D. Lorizio, R. Vuerich, M. Lippi, D. S. Nascimento, and S. Zacchigna (Dec. 2022). "Extracellular Matrix-Based Approaches in Cardiac Regeneration: Challenges and Opportunities". In: *International Journal of Molecular Sciences* 23.24, p. 15783. DOI: 10.3390/ijms232415783. (Visited on 04/26/2023).

World Health Organisation (June 11, 2021). URL: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (visited on 04/25/2023).

Zhang, W., Y. Liu, and H. Zhang (Mar. 31, 2021). "Extracellular matrix: an important regulator of cell functions and skeletal muscle development". In: *Cell & Bioscience* 11.1, p. 65. DOI: 10.1186/s13578-021-00579-4. URL: https://doi.org/10.1186/s13578-021-00579-4 (visited on 04/25/2023).