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BIOMATERIALS IN THE MANUFACTURING OF ADVANCED THERAPY MEDICINAL PRODUCTS USED IN BONE REPLACEMENT

Regulatory requirements in the EU

Faculty of Medicine and Health Technology
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ABSTRACT

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Advanced therapy medicinal products (ATMPs) for bone replacement are relatively new regenerative medicinal products. These products are normally composed of stem cells and a biomaterial and are therefore defined to be combined ATMPs. There are many regulations concerning the biomaterials in these products. These regulations are often very detailed and complex. This literature review studies the biomaterials that are used in ATMPs for bone replacement as well as European Union's (EU) regulations and directives concerning these materials. The purpose of this literature review is to gain a good overview of the topic and to apply the information to Tampere University's RegeOS bone replacement product.

The review is divided into two main parts. The first part describes the most common biomaterials and the manufacturing process of combined ATMPs used in bone replacement. When investigating the biomaterials, it was found that there are only few biomaterials that have been clinically used for this purpose. These biomaterials are β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA), which are both calcium phosphates (CaPs). This is understandable considering that calcium phosphates mimic well the natural composition of bone and have been used as such for bone replacement already for several years.

From the manufacturing of the ATMPs used in bone replacement, it was noticed that only few manufacturers have developed these kinds of products and the manufacturing methods were very similar between different manufacturers. ATMPs studied in this literature review were manufactured combining patient's own stem cells and bioceramic granules. Only differences that were found were the type of cells used in the product and the stage in which cells were mixed with the biomaterial.

The second part of the review focuses on regulations concerning these biomaterials and their use in ATMPs. Regulations applied to the product depend on its classification. Regulations on gene therapy medicinal products, somatic-cell therapy medicinal products and tissue engineered products differ somewhat from regulations on combined ATMPs. This is due to the fact that in combined ATMPs there is the biomaterial as part of the product. The biomaterial is defined as a medical device in combined ATMPs. Medical devices have their own additional regulatory requirements, and it complicates a bit the regulations on combined ATMPs.

The review shows that the most important regulatory requirements for biomaterials are biocompatibility, non-toxicity and sterility. These topics arise in many parts of regulations and directives. If these properties are shown to be suitable, the biomaterial should be safe to use as a medical device. In addition to biomaterials, the manufacture of these products is guided by many regulations. Most of the regulations are related to manufacturing facilities, personnel, documentation and quality control. The aim of these regulations is to ensure that medicinal products, that are used to treat patients, are safe and have the intended efficacy.

Keywords: Advanced therapy medicinal product, bone replacement, biomaterial, bioceramic, combined ATMP, manufacturing.

TIIVISTELMÄ

Katri Ala-Mononen: Biomateriaalit pitkälle kehitetyssä terapiassa käytettävissä lääkkeissä, joita käytetään luukudoksen korvaamiseen: lainsäädännön asettamat vaatimukset EU:ssa
kandidaatintyö
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Pitkälle kehitetyssä terapiassa käytettävät lääkkeet (engl. Advanced therapy medicinal products, ATMP) ovat melko uusia regeneratiivisen lääketieteen tuotteita, joita käytetään myös luukudoksen korvaamisessa. Useimmat luukudoksen korvaamisessa käytettävät ATMP-lääkkeet on valmistettu ihmisen kantasoluista sekä jostakin biomateriaalista ja niitä kutsutaan yhdistelmä-ATMP-valmisteiksi. Yhdistelmä-ATMP-valmisteissa käytettäviä biomateriaaleja koskevat useat määräykset, jotka ovat usein todella pikkutarkkoja ja monimutkaisia. Tämä kirjallisuuskatsaus keskittyy biomateriaaleihin, joita käytetään luukudoksen korvauksessa käytettävissä ATMP-valmisteissa. Työ käsittelee myös Euroopan Unionin lainsäädäntöä, joka koskee ATMP-lääkkeitä ja erityisesti niissä käytettäviä biomateriaaleja. Kirjallisuuskatsauksen tavoitteena on saada hyvä yleiskuva aiheesta ja tämän jälkeen soveltaa hankittua tietoa Tampereen yliopiston RegeOS tuotteeseen, jota käytetään luukudoksen korvaamiseen.

Kirjallisuuskatsaus on jaettu kahteen osaan, joista ensimmäisessä käsitellään yleisimpiä luukudoksen korvaamisessa käytettäviä biomateriaaleja sekä samassa tarkoituksessa käytettävien yhdistelmä-ATMP-valmisteiden valmistusta. Luukudoksen korjauksessa käytettäviä biomateriaaleja tutkittaessa tieteellisen kirjallisuuden avulla huomattiin, että vain muutamaa biomateriaalia on käytetty tähän tarkoitukseen. Nämä biomateriaalit ovat β-trikalsiumfosfaatti sekä hydroksiapatiitti, jotka ovat molemmat kalsiumfosfaatteja. Kyseisten biokeraamien käyttö perustuu siihen, että ne matkivat hyvin luontaisen luun rakennetta ja että niitä on käytetty onnistuneesti jo useita vuosia luukudoksen korvaamisessa.

Luukudoksen korvaamisessa käytettävien yhdistelmä-ATMP-valmisteiden osalta huomattiin, että vain muutamat tahot ovat tutkineet ja kehittäneet kyseisiä tuotteita. Valmistustavat ovat myös hyvin samankaltaisia eri valmistajien välillä. Tässä kirjallisuuskatsauksessa esiteltävät yhdistelmä-ATMP-valmisteet on valmistettu yhdistämällä potilaan omia, laboratoriossa viljeltyjä kantasoluja biokeraamijyväsiin. Suurimmat erot tuotteiden valmistuksessa eri valmistajien välillä ovat tuotteessa käytettyjen kantasolujen alkuperä sekä vaihe, jossa solut ja biomateriaali yhdistetään.

Kirjallisuuskatsauksen toinen osa käsittelee ATMP-lääkkeitä koskevaa lainsäädäntöä yleisesti sekä erityisesti yhdistelmä-ATMP-valmisteissa käytettäviä biomateriaaleja koskevia säädöksiä. ATMP-lääkkeitä koskevat määräykset riippuvat isolta osalta ATMP-lääkkeiden määrittelystä. Yhdistelmä-ATMP-valmisteita koskevat säädökset eroavat jonkin verran geeniterapiatuotteita, somaattisia soluterapiatuotteita sekä kudosmuokkaustuotteita koskevista säädöksistä. Tämä johtuu siitä, että yhdistelmä-ATMP-valmisteissa on mukana biomateriaali, joka on yhdistelmä-ATMP-valmisteiden määritelmän mukaan lääkinnällinen laite. Lääkinnällisiä laitteita koskevat omat erityiset määräyksensä ja tämän vuoksi yhdistelmä-ATMP-valmisteita koskevat määräykset ovat hieman monimutkaisempia.

Kirjallisuuskatsaus osoittaa, että tärkeimmät ATMP-lääkkeitä koskevat laadulliset vaatimukset liittyvät bioyhteensopivuuteen, myrkyttömyyteen sekä steriiliyteen, minkä vuoksi nämä ominaisuudet nousevat esille useissa lainsäädännön kohdissa. Kun nämä vaatimukset täyttyvät, voidaan ajatella, että biomateriaali on turvallinen käytettäväksi lääkinnällisenä laitteena osana ATMP-lääkettä. Myös useat määräykset koskevat ATMP-lääkkeiden valmistusta. Pääasiassa valmistusta koskevat määräykset liittyvät tuotantotiloihin, henkilöstöön sekä dokumentointiin ja laadunvalvontaan. ATMP-lääkkeitä koskevan lainsäädännön tarkoituksena on varmistaa lääkinnällisissä tarkoituksissa käytettävien tuotteiden turvallisuus sekä tarkoitetun vaikutuksen toteutuminen.

Avainsanat: Pitkälle kehitetyssä terapiassa käytettävä lääke, luukudoksen korvaaminen, biomateriaali, biokeraami, yhdistelmä-ATMP-valmiste, valmistus.

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

ASC	Adipose stem cell
ATMP	Advanced therapy medicinal product
BM-hMSC	Bone marrow-derived mesenchymal stromal cell
CaP	Calcium phosphate
CAT	Committee for Advanced Therapies
CHMP	Committee for Medicinal Products for Human Use
EC	European Commission
ECM	Extracellular matrix
EMA	European Medicines Agency
EU	European Union
FACS	Fluorescence-activated cell sorting
GMP	Good Manufacturing Practice
GTMP	Gene therapy medicinal product
HA	Hydroxyapatite
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HE	Hospital exemption
HIV	Human immunodeficiency virus
MNC	Multinucleated cell
MSC	Mesenchymal stem cell
rDNA	Recombinant DNA
RT-PCR	Real-time polymerase chain reaction
sCTMP	β-tricalcium phosphate
β-TCP	Somatic cell therapy medicinal product
TEP	Tissue engineered product
UDI	Unique device identification

1. INTRODUCTION

Products for fixing bone deficiencies are currently widely researched all around the world. Many of these products have the ability to use repair mechanisms of human body to restore normal function of the bone tissue. These products belong to the field of regenerative medicine, which usually uses engineered human stem cells. Some product developers are currently examining bone replacement products that combine stem cells and supporting biomaterial. These products can promote bone tissue growth into the defect site. Some product developers have already treated patients with these products with promising results. [1-7] Bone deficiencies can be caused by diseases or injuries and advanced therapy medicinal products (ATMPs) can provide regenerative treatment for various cases and patients. Such products will most likely become more common in the future, because these regenerative products can induce natural growth of the tissue and therefore help the human body to repair itself. They also offer the possibility of personalised treatments.

ATMPs are medicinal products, that have their own regulatory requirements. Regulation concerning ATMPs is set out by European Commission in cooperation with European Medicines Agency (EMA). Regulations and directives define, how these products should be designed and manufactured. Legislation concerning ATMPs is very complex and even incomplete on some parts, which complicates the work of product developers. In order to manufacture these kinds of medicinal products, the regulatory system regarding these products must be well known by the product developer. In addition, the manufacturer must be able to demonstrate the safety and intended efficacy of the product in order to obtain a marketing authorisation [8,9].

The first chapter of this literature review discusses ATMPs used in bone replacement starting from the classification of ATMPs in general. After general information, review focuses on products that are used for replacing bone deficiencies and especially biomaterials in them. ATMPs that have cells or tissues combined with biomaterials are defined to be combined ATMPs [10]. ATMPs used for replacing bone tissue are usually combined ATMPs that are made of human stem cells combined with bioceramic. [1-7] These bioceramics are most often calcium phosphates because they are similar in structure and mechanical properties to native bone [16]. In addition to these topics, the regulation concerning biomaterials and the manufacturing of these products is discussed. One aim of this review is to get acquainted with the regulations concerning ATMPs and to apply gained knowledge to Tampere University's RegeOS product.

In the third chapter the review focuses on regulation requirements concerning the themes of the second chapter. First, the regulation of ATMPs is discussed in general and then

the regulation concerning biomaterials in them and regulations concerning their manufacture are discussed. There is extensive and precise legislation on biomaterials used in ATMPs. The biomaterials must be such that they do not endanger the patient in which they are implanted. The biomaterials must therefore be biocompatible, non-toxic and sterile. The suitability of materials for their intended use must also be able to be scientifically proven. [8] At the end of the chapter, regulation concerning Tampere University's RegeOS product is dealt with. RegeOS product is an investigational combined ATMP, that is designed to replace small bone deficiencies. The product is made of patient's own adipose stem cells and porous β-tricalcium phosphate granules. The product has so far been used in several patients to replace bone tissue in maxillary, mandibular and cranial bones. [1-5] At the end of review, all the things presented in the previous paragraphs are brought together and summarised.

2. ADVANCED THERAPY MEDICINAL PRODUCTS USED IN BONE REPLACEMENT

Advanced therapy medicinal products (ATMPs) are biological medicinal products, which are used for treating or preventing diseases in humans [10]. Their therapeutic effect is based on genes, tissues, or cells. The basic idea of ATMPs is that the genes, cells and tissues are significantly modified or manipulated for the intended therapeutic purpose. Modifications and manipulations are made so that the biological function of cells or tissues change. Cells used in ATMPs can also be non-manipulated. In this case, cells are intended to be used in different function than naturally. Most ATMPs are still at research level and only some of them have been used in clinical trials and even fewer have received marketing authorisation. This chapter presents ATMPs in more detail, and then focuses on biomaterials and ATMPs used for bone replacement therapies.

2.1 Classification

ATMPs are classified into gene therapy medicinal products (GTMPs), somatic cell therapy medicinal products (sCTMPs) and tissue engineered products (TEPs). In addition to these, there are combined ATMPs, which are very similar to TEPs with the difference that combined ATMPs include one or more medical devices as part of the product.

Gene therapy medicinal products contain recombinant DNA (rDNA), which have therapeutic, prophylactic, or diagnostic effect in human body. Recombinant gene is artificially made in laboratory, and it includes DNA from different sources. Recombinant genes can even contain DNA from different species. The function of the recombinant gene is regulating, repairing, replacing, adding, or deleting a genetic sequence in human body. European Parliament also defines, that GTMPs' effect in human body must be in direct relation to its recombinant gene or to the product of the gene, which is usually some kind of protein. [11]

Somatic-cell therapy medicinal products are made of cells or tissues. Their biological characteristics, physiological functions or structural properties must be altered, or they must be used for a different function than their natural function. Manipulation of cells or tissues must be significant, and modifications must be related to the intended clinical use. Properties of the product must also be used for treating, preventing, or diagnosing a disease. [11] These products contain only cells or tissues and do not include any additional structural components. This feature distinguishes sCTMPs from tissue engineered medicinal products.

Tissue engineered medicinal products i.e. tissue engineered products consist of engineered cells or tissues with a view to repair, regenerate or replace human tissues. Definition of engineered tissues or cells is that they have been subjected to significant modification or they are used for a different function than their natural function. European

Parliament defines, that cells or tissues in TEPs can be human or animal derived, or both. In addition to cells and tissues, TEPs can contain cellular products, biomolecules, biomaterials, chemical substances, scaffolds, or matrices. Cells and tissues in TEP can be viable or non-viable, but if they are only non-viable, they must act mainly by pharmacological, immunological, or metabolic action. [10]

Combined advanced therapy medicinal products are defined to be ATMPs, that include a medical device or an active implantable medical device as an integral part of the product. There may also be one or more devices in the product. [10] Medical device is defined in directive 93/42/EEC to be any instrument, apparatus, appliance, material, or other article used for human beings for diagnosing, preventing, monitoring, treating or alleviating diseases, injuries, or handicaps. Medical device can also be used for the purpose of investigation, replacement, or modification of the anatomy or of a physiological process. In addition to these, the purpose of medical device can be a control of conception. Medical device must not gain its primary action by pharmacological, immunological, or metabolic means, but its function may be assisted by these means. [12] Active implantable medical device is defined in directive 90/385/EEC to be any active medical device, which is surgically or medically put in the human body and is intended to remain in the human body after the implantation. An active medical device is defined to be a medical device, which uses for its functioning electrical energy or any other source of energy other than the energy that is directly generated by the human body or by the gravity.[13] Cellular or tissue part of the combined ATMP must be viable or if they are non-viable, those parts must primarily support the functions of the medical device in the human body.[10] Devices in combined ATMPs used in bone replacement are mainly medical devices, that are defined in directive 93/42/EEC and those medical devices are usually biomaterials. Regulation 2017/745 has replaced Directives 90/385/EEC and 93/42/EEC, but definitions of medical devices have remained the same. [8]

2.2 Biomaterials

Chengdu Definitions in Biomaterials Conference 2019 defined a biomaterial as follows: “A material designed to take a form that can direct, through interactions with living systems, the course of any therapeutic or diagnostic procedure”. They also defined biomedical materials to be synonymous with biomaterial. [14] Biomaterials can be divided into natural biomaterials and synthetic biomaterials. Natural biomaterials are made in living organisms and synthetic biomaterials are made synthetically in laboratory. Biomaterials can also be classified to bioactive materials, bioresorbable materials and bioinert materials. These classes describe the behaviour of the biomaterial in the body. Bioactive material interacts in the body by forming bonds between the material and tissue, and therefore has an active role in it. Bioresorbable materials degrade in the body and their degradation products are eliminated by biological processes. Bioinert materials do not have any interaction with tissues in the body and they are usually surrounded by a fibrous capsule. [15] Biomedical materials can be different polymers, metals, ceramics, or composites. ATMPs containing biomaterials are mostly TEPs and combined ATMPs. Biomaterials in ATMPs used in bone replacement are mainly bioceramics due to their prop-

erties that are suitable for their intended use. Biomaterials in ATMPs used in bone replacement are usually bioactive materials or bioresorbable materials. This is because the biomaterial must interact with the surrounding tissues to allow new bone tissue to form.

2.2.1 Biomaterials in the Natural Bone Tissue

Natural bone tissue consists of extracellular matrix (ECM) and bone cells, such as osteoblasts, osteocytes, and bone-lining cells. The ECM is composed of inorganic hydroxyapatite (HA) and organic collagens. In natural bone tissue, HA is not in its pure form, which means it has some impurities in its crystalline structure. These impurities can be potassium, magnesium, sodium, strontium, carbonate, chloride and fluoride. The chemical structure of HA is discussed in more detail in the chapter 2.2.4. The organic collagen is 90% type I collagen, and the rest is other collagen types. Calcium phosphate strengthens and hardens the structure and collagen gives it flexibility. These properties together enable bone to withstand stress. Other non-collagenous proteins that are present in bone tissue are proteoglycans, glycoproteins, carboxylated proteins, growth factors and other proteins that are presented in small amounts. These proteins have an essential role in regulation of bone mineral deposition and bone cell activity. [16]

Bone remodelling is a phenomenon, in which bone tissue is resorbed and replaced with new bone tissue. In natural bone remodelling, the resorption and bone formation are in balance. This ensures that there are no significant changes in bone mass or in its mechanical strength. The remodelling consists of four phases. First phase is an activation of bone remodelling. In this phase mononuclear monocyte-macrophages are recruited from the blood circulation and they interact with bone lining cells, which form a continuous layer on top of the bone tissue. This interaction leads to retraction of bone lining cells. After this osteoclast precursors attach to the retraction site and form osteoclasts. Second phase is a bone resorption and recruitment of mesenchymal stem cells (MSCs) and osteoprogenitors. In this phase osteoclasts resorb the bone. Phase three is an osteoblast differentiation and function, which begins when the function of osteoblasts overtakes the bone resorption. Phase four is a mineralisation of osteoid and completion of bone remodelling. In this phase the bone is mineralised by enzymes secreted by osteoblasts. These enzymes provide inorganic phosphate for HA crystallisation. [16]

2.2.2 Ceramics

Ceramic biomaterials or bioceramics are polycrystalline and inorganic materials. They are very hard, but brittle biomaterials. Their surfaces can be highly polished, but they are difficult to fabricate and reshape. [15] Bioceramics can be categorised as bioinert ceramics and bioactive ceramics. [17] Bioinert ceramics are nontoxic and biologically inactive materials that does not interact with human body. Bioactive ceramics are biologically active, which means that the material interacts and forms bonds with human tissue. [15]

Bioactive ceramics can be further categorised into resorbable and non-resorbable ceramics [17]. Resorbable bioceramics are mainly used in bone replacement products because dissolution of the biomaterial enables bone tissue to grow into defect site. [18]

Bioresorbable bioceramics can degrade in human body by different mechanisms: physico-chemical dissolution, cellular degradation, or mechanical fragmentation. Physico-chemical degradation is caused by ion exchange between material and surrounding liquid. Acidity of environment and low Ca/P ratio of ceramic accelerates degradation. There are other factors too affecting physico-chemical dissolution. The crystalline structure of the material has an effect on the degradation, because the higher the crystallinity is, the lower the degradation rate is. This is because high crystallinity means that the material has highly organised structure which means strong bonds between molecules in the structure. Strong bonds reduce the degradation of the material in the liquid environment. Also, impurities in crystalline structure affect the crystalline structure and therefore affect degradation. Impurities make crystalline structure less organised and affect degradation that way. For example, hydroxyapatite can have substitutes in its crystalline structure. In addition to these features, the physical structure of bioceramic has an effect on its degradation rate. The shape and structure of the material determines the surface area that interacts with the environment. Cellular degradation is done by multinucleated cells (MNC). MNCs resorb the material from its surface and phagocytose it. In bone tissue, these MNCs are osteoclasts, which have significant role in natural bone resorption and replacement. Osteoclasts form microenvironment between themselves and the material and use acid dissolution to degrade it. Osteoclasts can degrade calcium phosphate bioceramics same the way as they degrade natural bone. [18]

Most used ceramics in ATMPs are calcium phosphates (CaPs) which are bioceramics containing calcium and phosphate anions [15]. Most common CaPs in ATMPs are β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) and those combinations in different compositional percentages [18]. HA and β-TCP are very biocompatible materials because they occur naturally in bone tissue and therefore do not cause rejection reaction [15]. They are also osteoinductive and biodegradable materials. Osteoinductivity means that they stimulate cell differentiation toward osteoblast lineage. These properties promote bone tissue formation and enable it to grow into defect site. HA and β-TCP have both been used as bone replacement products for a long time, because of their biocompatibility, osteoinductivity and biodegradability. [16]

One of the most important properties of CaP bioceramics used in bone replacement products is porosity. Pore size and interconnectivity are important features when thinking about the porosity of the bioceramic. Pore size determines materials' surface area and surface for cell adhesion. It determines how well bone tissue grows inside the bone replacement product. Interconnectivity enables cell migration and distribution inside the implant. It also allows vascularisation inside the implant which is crucial for the healing of the defect site. [18] Porosity is often gained with foam replica technique, gas foaming technique or surface-active foaming agents. Toxicity, biocompatibility, and mechanical properties are also important features that need to be taken into account when designing a scaffold for bone replacement product [16].

2.2.3 β-tricalcium phosphate

Most used tricalcium phosphate in bone replacement products is β-tricalcium phosphate (β-TCP). Its chemical formula is $Ca_3(PO_4)_2$ and its ortho type calcium phosphate. β-TCP's crystalline structure is composed of two types of columns arranged in parallel to vertical axis of the structure. The columns A and B both contain phosphate (PO_4^{3-}) anions and Ca^{2+} cations organised different ways. These columns are shown in Figure 1.

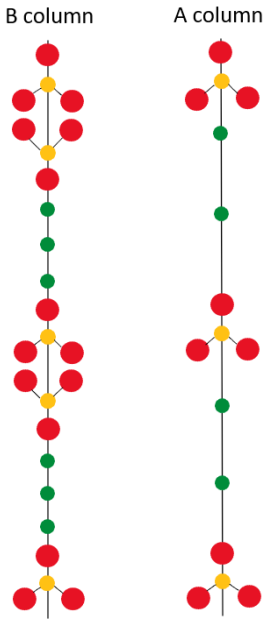

Figure 1. *Two columns from which β-TCP is composed. Oxygen is presented as red, phosphorus as yellow and calcium as green. [16]*

The crystalline structure is composed of A columns, which are surrounded by B columns in a hexagonal pattern. β-TCP's Ca/P ratio is 1.5 and the ratio is closely correlated to its solubility like mentioned above. Therefore β-TCP is quite soluble in human body. [16] β-TCP is a bioactive and bioresorbable material, which means it dissolves after implantation and enables new bone tissue to grow into its surface irregularities without being in direct contact with the tissue. This replacement process is very similar to natural bone repair reaction in human body. Bone replacement is successful only when the structure remains stable at the tissue material interface during the degradation and when the resorption rate is correct with respect to the bone formation rate. Porosity of β-TCP scaffold increases osteoconductivity and solubility of the material and therefore β-TCP is mostly used as porous blocks or granules in medical applications. β-TCP is mainly used for replacing small bone defects, because it is brittle, rigid and has low wear resistance. [16]

2.2.4 Hydroxyapatite

Hydroxyapatite's (HA) chemical formula is $Ca_{10}(PO_4)_6(OH)_2$ and it is ortho type calcium phosphate like β-TCP. HA's crystalline structure consists of phosphate (PO_4^{3-}) anions, calcium (Ca^{2+}) cations and hydroxide (OH^-) anions. The structure is presented in Figure 2. It is also biocompatible, bioactive and bioresorbable, because its structure is similar

to the mineral structure of bone and teeth in human body. Although HA in the human body is not identical to the stoichiometric HA, because in the human body HA is calcium-deficient, carbonated and ionically substituted. Stoichiometric HA structure can be substituted to improve its bioactivity.[16] Un-stoichiometric HA is also used in medical applications, because natural HA in bone tissue is non-stoichiometric and impurities in the HA structure make it more soluble in human body.

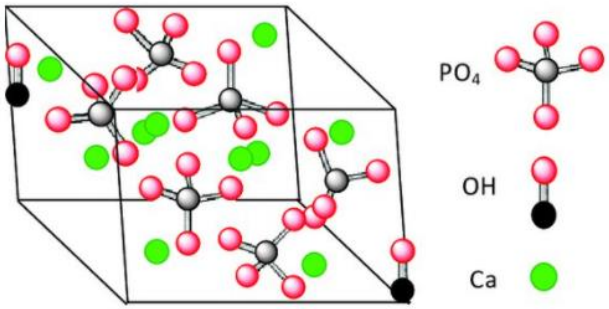

Figure 2. *The structure of stoichiometric hydroxyapatite. [19]*

HA's Ca/P ratio is 1.67, which is little bit higher than the ratio of β-TCP. Higher Ca/P ratio means, that HA is less soluble than β-TCP. Another aspect which affects HA's properties is its synthesis route. Aqueous precipitation methods are most used, but if dense HAs are wanted, solid-state processing is used. Stoichiometric HA needs to be sintered in order to be usable for clinical applications. In sintering process HA is kept over 900°C for several hours. This process removes moisture, carbonates and residuals from the material and increases the crystal size and decreases surface area. This improves HA's mechanical properties such as toughness and mechanical strength. Despite the sintering process, HA is a brittle material, and it can not be used for load bearing applications. HA is most often used as porous blocks or granules in medical applications. [16]

2.3 Manufacturing Process of ATMPs Used in Bone Replacement

ATMPs used for bone replacement are mostly combined ATMPs and they are composed of biomaterial and stem cells. At the first stage of manufacturing, cells are harvested from the patient or donor and cell product is manufactured following good manufacturing practice (GMP). After this the cell product is combined with biomaterial in order to make cells attach to the material. Biomaterial and cell product can be mixed in the laboratory before implantation or just before surgery. Biomaterials are often acquired from a commercial manufacturer. [1-7]

Like mentioned in paragraph 2.2, biomaterials in ATMPs used for bone replacement are mainly bioceramics and usually they are calcium phosphates (CaPs). Those ceramics are often in a form that mimics human cancellous bone, which has high porosity and pore interconnectivity. These materials must be suitable for the implantation site, and they must promote tissue ingrowth. They also must be non-toxic and stimulate osteogenesis. [16] Because of these conditions, biomaterials in bone replacement products

are often porous granules, because cells can be easily mixed to the material and the material can be easily fitted to the bone defect site.

The cell source for combined ATMP can be autologous or allogeneic. Autologous cells are harvested from the same person to which they are transferred to. Allogeneic cells are from another individual as the person they are transferred. Before harvesting cells or tissues, donor must be tested negative for hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV) and syphilis [6] in order to avoid transmitting these diseases. In ATMPs used in bone replacement, manufacturers use mostly bone marrow-derived mesenchymal stromal cells (BM-hMSCs) or adipose stem cells (ASCs) [1-7]. Mesenchymal stromal cells are multipotent cells, which means they can differentiate into specialised cell types of specific tissue or organ. MSCs can differentiate into several mesodermal cell lineages, for example osteoblasts, chondrocytes, adipocytes, tenocytes, and myoblasts. [20] ASCs are harvested from subcutaneous fat [1-5]. The harvesting procedure is easier for the patient and the cell source is bigger than with BM-hMCSs. ASCs can also be differentiated into several cell types, for example chondrocytes, myocytes, osteoblasts, and adipocytes [21].

After harvesting stem cells, they are expanded [1-7]. Before expansion, cells can be isolated in mechanical and enzymatic way. The purpose of isolating is to separate cells from ECM. [1-5,7] Cells are cultured in cell culture media with cell-specific additive substances. Those additives can be for example patient's autologous serum [1-5] or platelet lysate and heparin [6,7]. Cells are typically cultured without antibiotics, because antibiotics expose cells to non-human origin substances, and they can make false-negative in bacterial testing. [1-7] Cells are expanded for a few weeks and after that, the expanded culture is mixed with biomaterial or other substances. In the clinical cases using BM-hMSCs cultured cells are suspended in 5% human albumin and the cell product was added to the HA/β-TCP biomaterial before implantation. [6] In the clinical cases using ASCs, cell product is combined with porous β-TCP before implantation and β-TCP is incubated for two days before adding the cell product. Cell product is mixed with biomaterial two days before implantation in order to allow cells to attach to material. [1-5,22]

In all cases, the sterility, endotoxins, and negativity for mycoplasma were tested before combining the cell product to the biomaterial. These tests are usually made in laboratories, that are specialised and licensed for these analyses. Sterility, endotoxin, and mycoplasma tests are done according to methods described in European Pharmacopoeia. [1-7,22] Properties of the cells are also tested throughout the manufacturing process. Differentiation capacity, clonogenicity, cell attachment and cell viability are properties, that can be tested with control samples in the manufacturing process. There may be multiple control samples, that are processed the same way than the main culture. Differentiation of cells can be tested with differentiation mediums and real-time reverse transcriptase polymerase chain reaction (RT-PCR) and semi-quantitative PCR. In the staining method, cells are differentiated with specific differentiation mediums, and they are stained to detect some cell type specific substance activity. Substances in differentiation medium induce cellular differentiation in certain direction and staining reveals specific substances from the culture. Differentiation can be tested from the pure cell culture or

cell culture with biomaterial depending on manufacturing process. For example, in osteogenic differentiation the detected substance is alkaline phosphatase. [1-5,22] In addition to staining method, cell specific genes can be detected from the differentiated cell culture. In this method, gene specific RNAs are transcribed into cDNA and the cDNA is amplified with RT-PCR. After PCR, results are compared to the results from control culture at day 0. [5] Karyotyping of cells can also be performed with flow cytometry method. In these specific cases fluorescence-activated cell sorting (FACS) was used. [1-5,22] This method sorts cells based on their specific light scattering and fluorescence characteristics. Measurement of fluorescence and light scattering is based on the molecules on cell surface. For each cell type, there are surface molecules specific to them. Due to this fact, FACS allows the cells to be identified as cells of a particular cell line. [23] Clonogenicity means the ability of a cell to form colonies by cloning itself. This can be tested from control samples, that have same cell density as the main culture. In this method cells are stained with trypan blue to count viable cells. The size of colonies is counted and compared to earlier counting. [22] Viability of cell culture and attachment to the biomaterial can be studied with live/dead staining. Staining is based on substances, that mark viable cells with green fluorescence and dead cells with red fluorescence. Viability is detected with a fluorescence microscope. [4]

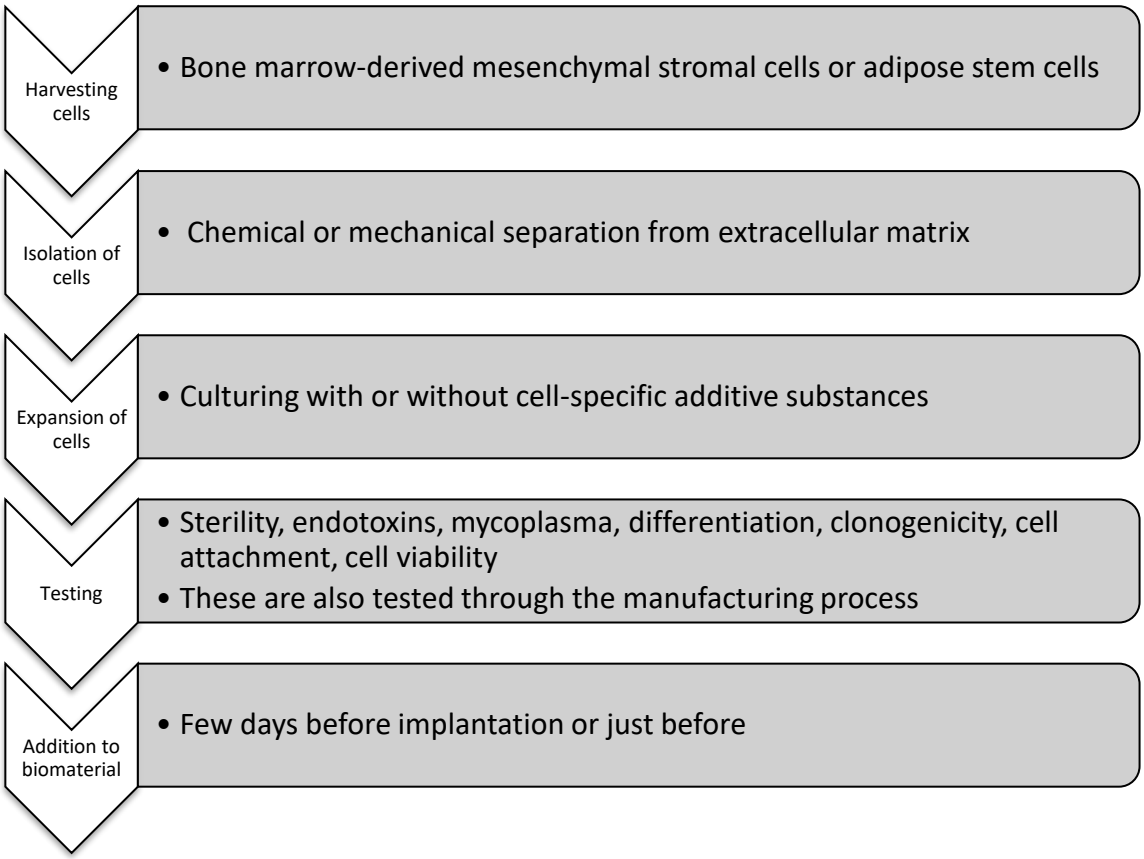

Figure 3. *Flowchart describing the steps in the manufacture of a combined ATMP for bone repair.*

3. REGULATION ON ADVANCED THERAPY MEDICINAL PRODUCTS USED IN BONE REPLACEMENT

European Union's (EU) regulation on advanced therapy medicinal products includes all requirements for all the stages of ATMP's life cycle. Regulations and directives include all requirements for the development, manufacturing, and marketing authorisation of ATMPs. These requirements can be found in Directive 2001/83/EC and Regulation 1394/2007 which is amending Directive 2001/83/EC. In addition to these documents, regulatory requirements for medical devices are found in Regulation 2017/745. This regulation is relevant for combined ATMPs which include one or more medical devices as an integral part of the product. This chapter will focus on the regulation is relevant biomaterials used in ATMPs and the manufacturing of ATMPs. The marketing authorisation process of ATMPs and the hospital exemption are also presented. Finally, these regulatory requirements are applied to Tampere University's RegeOS product which is a combined ATMP used for replacing bone deficiencies.

3.1 Marketing Authorisation in European Union

European Medicines Agency (EMA) authorises all ATMPs within the EU and is responsible for drawing up instructions for monitoring these products after they are approved. EMA has a Committee for Advanced Therapies (CAT), which evaluates the quality, safety, and efficacy of ATMPs. CAT is responsible for making a draft opinion on every ATMP application, which is submitted to EMA. After this, the Committee for Medicinal Products for Human Use (CHMP) can make the final opinion on the marketing authorisation of the product. CAT also draws up an opinion regarding any scientific matter on ATMPs if the Executive director of EMA or European Commission (EC) requests it. [24] Directives and regulations concerning medicinal products are drafted by the European Commission, which receives advice for legislation from the Pharmaceutical Committee and expert groups. [25] Final directives and regulations are adopted by the European Commission or the European Parliament together with the Council of the European Union. [26] Compliance with these directives and regulations is a prerequisite for the marketing authorisation of the product.

Before getting the marketing authorisation, the product must go through clinical trials that demonstrate the safety and efficacy of the product. Clinical trials must be done in accordance with Regulation (EU) No 536/2014. [9] Clinical trials are authorised by the EU country in which the trials are conducted. The sponsor of clinical trials must do application for clinical trials through the EU portal. The sponsor also proposes one Member State to be a reporting Member State that will assess the clinical trial application. Clinical trial application and assessment are divided into two parts. The first part deals with benefits, risks, and regulatory aspects of the clinical trial and the second part deals with the

regulations on individuals participating the clinical trial. The reporting Member State will assess the clinical trial application part I and submit an assessment report through the EU portal. Assessment report concludes whether the clinical trial application part I is accepted or not. If there are more than one country concerned, one Member State must assess the application for clinical trials and make an assessment report about it. These reports are submitted through the EU portal. After this, all other Member States concerned should review the application and share any aspects that might be relevant to the application. Then the reporting Member State need to go through reviews and report how aspects that other Member States have brought up have been taken into account. Then the reporting Member State submits the final assessment report to the sponsor and other Member States. Part II of the clinical trial application is assessed by every Member State concerned. Each Member State should write an assessment report, which concludes whether the clinical trial is accepted or not. Member States may also request additional information from the sponsor if they need it for the assessment report. Finally, each Member State concerned will notify the sponsor via the EU portal whether the clinical trial is authorised fully or subject to certain conditions or not authorised. [27]

After authorisation, there are still several things that are strictly regulated. All patients participating in clinical trials must have informed consent. Informed consent means that the patient or the person representing him or her knows and understands all the risks and benefits associated with the trials and gives his or her informed consent to perform them. Informed consent must be given in writing and signed but may be withdrawn at any stage of the trials. The start and end of clinical trials must also be reported via the EU portal. In addition, the party conducting the trials should report any adverse reactions and adverse events to the party who sponsors the trials. Overall, the trials should be performed in such a way that the expected risks are balanced against the potential benefits of the product. All clinical trials should also be performed in accordance with good clinical practice. [27]

The ATMP manufacturer must also prove the safety and proper performance of the medical device in combined ATMP with clinical results. Undesirable side-effects and the benefit-risk ratio are studied in clinical trials. Clinical evaluation of the product is made based on clinical trials and non-clinical studies which are relevant for the product. Non-clinical data is gathered from a systematic scientific literature review. All the data is evaluated, and a clinical evaluation report is written based on it. The idea of the clinical evaluation report is to support the assessment of the conformity of the device. [8]

Marketing authorisation for combined ATMPs should also include the results of the assessment of the conformity of the medical device part by a notified body. The assessment concerns the medical device i.e., the biomaterial in the combined ATMP. [8,10] Notified body means an organisation in EU member state which has been designated to evaluate the uniformity of certain medical devices. The manufacturer must make an application for a notified body that is designated to make assessments related to medical device in combined ATMPs. If the device fulfils all requirements laid down by regulations, the manufacturer gets a certificate of conformity from the notified body. After getting the

certificate of conformity, the medical device may be CE marked. CE marking indicates that the device is in conformity with all requirements that are applicable. [8]

3.2 Regulation on Biomaterials

Regulatory requirements for the materials used in ATMPs are presented in Directive 2001/83/EC. Most of the regulation of materials used in medicinal products is related to active substances and starting materials used in them. Directive 2001/83/EC defines that active substance is any substance or mixture of substances, which is used in the manufacture of a medicinal product. In addition to that, the active substance must be an active ingredient of the final product and to be intended to drive a pharmacological, immunological, or metabolic action. Target of these actions must be restoring, correcting, or modifying physiological functions or making diagnosis. Directive also uses term starting materials, which means all the materials that are used for manufacturing the active substance. Starting materials in ATMPs mean substances that are derived from biological origin and are the materials from which the active substance is manufactured. Starting materials in ATMPs are usually cells and tissues. Raw materials are the materials that are used in the manufacturing of active substance but are not part of the active substance itself. Raw materials in ATMPs can be, for example, culture media, serums, additives or any other reagents used in the manufacturing of active substance. [11] Biomaterials in combined ATMPs are defined to be medical devices and therefore regulations concerning them are found from Regulation 2017/745, which handles regulations on medical devices. Regulations on biomaterials used in other ATMPs are found from Regulation 1394/2007, which is “a lex specialist, which introduces additional provisions to those laid down in Directive 2001/83/EC.” [10]

Directive 2001/83/EC states that additional substances, such as biomaterials, used in TEPs or sCTMPs are defined as starting materials, even if they are not from biological origin. It says that the information about safety, biocompatibility, and suitability of all structural components, which are part of the final product, should be provided. Also, the mechanical, chemical, and biological properties of the materials need to be taken account and the toxicity of the material should be tested. All the testing for materials must be described and justified. If the material used as starting material can be found from the monographs of European Pharmacopoeia, the manufacturer can apply for a certificate of suitability from the European Directorate for the Quality of Medicines without any testing. Same directive states, that the interaction and compatibility between genes, cells, tissues, and the structural components must be described. [11] More quality requirements are found from European Commission’s Good Manufacturing Practice: Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products -document.

The Good Manufacturing Practice (GMP) document states, that the most important aspects when choosing materials for ATMPs is to avoid contaminations and to minimise the variability of the starting materials. Document also states that it is acceptable to rely on the documentation from third parties, such as material supplier, when examining risks of the materials. Identity verification or testing should be done by manufacturer if it is

necessary. [9] GMP document gives more regulatory information about manufacturing facilities and methods, and it is discussed in more detail in chapter 3.3.

Regulations on biomaterials in combined ATMPs are found from Regulation 2017/745, which replaces Directives 90/385/EEC and 93/42/EEC. In combined ATMPs the biomaterial is defined to be medical device. In general, the biomaterial must fulfil its intended use, but be safe to the patient. There are three categories in the Regulation 2017/745, that are relevant to biomaterials in bone replacement products. Those categories are chemical, physical and biological properties, infection and microbial contamination and information supplied by the manufacturer. [8]

Chemical, physical and biological properties of the biomaterial should be such that the product is as safe as possible for the patient and other persons. Any risks regarding the material must be acceptable when weighted against its benefits to patient. When designing the biomaterial for combined ATMP, special attention should be paid to toxicity of the material and its biocompatibility [8]. This means that the biomaterial itself must not cause any intoxications and its degradation products must be non-toxic too. Absorption, distribution, metabolism, and excretion of the material should also be taken account when designing biomaterial for combined ATMPs. Toxic substances could cause significant harm to the patient and therefore toxic materials should be avoided when designing biomaterial for combined ATMP. Biocompatibility means that the material is compatible with tissues, cells, and body fluids in its intended implantation site. Biocompatible material does not cause toxic or immunological response when implanted in human body. In addition to these, the material should be safe to use with other parts of the combined ATMP. In ATMPs used for bone replacement this means that the biomaterial must be compatible with the cell product and all the substances it includes. Other properties that need to be taken account according to Regulation 2017/745 are the mechanical properties of the material. These properties include for example strength, ductility, fracture resistance, wear resistance and fatigue resistance. [8] In bone replacement products, these mechanical properties play a vital role, because the biomaterial should give mechanical support to the defect site. In addition to these properties, the surface properties of the material need to be taken into consideration [8]. Risks arising from the release of particles or substances from the device should be avoided too. These particles or substances can be degradation products, wear debris or processing residues. Materials that are in direct contact with human tissues, should have substances that are carcinogenic, mutagenic, toxic to reproduction or endocrine-disrupting in a concentration less than 0,1 % weight by weight. The concentration of these substances can be higher only, if there are not any other suitable materials for the intended use. These substances must be mentioned on the label of the product if the concentration limit is exceeded. [8] When complying with the requirements mentioned above, one must consider the intended purpose of the biomaterial in the ATMP.

There are also regulations concerning sterility of the biomaterial used as medical device in combined ATMP. The biomaterial in ATMP used for bone replacement must be sterile. Therefore, it should not cause any infection in its implantation site. The biomaterial must be manufactured in such way that the risk of infection to the patient or other persons is

minimised. This means that the manufacturing conditions of the material must be aseptic and manufacturing methods must be validated and appropriate for the final product. In addition, the design of the material must support the ease of use of the product. Design should also minimise the contamination of the product if it is possible. Ease of use minimises contaminations caused by the handling of the material. The biomaterial must also be sterilised by a method suitable for its use and the sterilisation method must be validated. Package of the material must be designed and manufactured in such way that the material remains sterile until the package is opened or damaged. It should also be clear to the final user if the package is somehow damaged. [8]

Manufacturer has a responsibility to provide the required information on the biomaterial for the customer. The product must have certain information written in its label. For the biomaterial used in medicinal product the following information is relevant. This information includes the name of the product, the name and the address of the manufacturer, a unique device identification (UDI) carrier, information of sterility and sterilisation method, a serial number or a LOT number and a use-by date and a manufacturing date. In addition to these, if it is necessary the label should have information of storage conditions and handling conditions of the material, operating instructions, mention of single-use product and warnings regarding the material and its use. If the product is intended to be used in clinical investigation, that should be mentioned in the label too. Same rule also applies to custom-made products. In addition to the label, the manufacturer must provide instructions for use. Instructions for the biomaterial used in ATMP should include some of the same information as in the label of the product: the name of the product, the name and address of the manufacturer, information about storing and handling of the product, information about the sterility of the product and used sterilisation method and if necessary, mention of the single-use product. Other information that needs to be provided in the instructions are intended use of the product, contra-indications, the patient target group, side-effects, information to avoid risks related to implantation, information about correct sterilisation of the product and instructions for the possible pretreatment of the product before use. Instructions should also include the performance characteristics of the material, the information needed to make sure that the material is properly installed and instructions for package breakage. In addition to all these things, the instructions should include all possible risks associated with the product and instructions on how to act in a problem situation. [8] The correct labelling and instructions are essential for the user of the product and the patient in which the material is implanted. Accurate instructions enable the correct and safe use of the product and therefore promote the safety of the patient.

3.3 Regulation on Manufacturing

The manufacturing of ATMPs plays a significant role in the safety and efficacy of these products. Manufacturer has the responsibility to use validated and appropriate methods through the manufacture of the product. Regulations on the manufacturing of ATMPs can be found from European Commission’s document Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products. The document includes

guidelines on good manufacturing practice (GMP) specific to ATMPs. The GMP document handles risk-based approach on ATMPs, manufacturing personnel, production facilities, equipment used in the manufacturing, documentation, materials used in the manufacturing, cell bank system, production from beginning to end, qualification and validation, qualified person and batch release, quality control, outsourced activities in the manufacturing, quality defects and recalls, ATMPs containing GMOs, reconstitution activities of released products and automated production of ATMPs. [9]

Risk-based approach guides the whole manufacturing process of ATMPs, and its purpose is to make sure that the risks specific to each ATMP are taken into account. ATMPs are usually complex and varying class of medicinal products. Therefore, it is important that there is some flexibility in rules governing the manufacture of ATMPs. On the other hand, the manufacturer is responsible to ensure quality, safety and efficacy of the ATMP by following the GMP when necessary for the product. Risk-based approach must be followed in investigational ATMPs and in authorised ATMPs. In investigational ATMPs the main point is to protect the clinical trials subjects and to ensure reliable results in all phases of the investigation. When following GMP, the consistency of the production and reliable results from the clinical trials can be obtained. For authorised ATMPs, the risk-based approach should be uniform with the terms of the marketing authorisation. [9]

Manufacturing of ATMPs should be made in appropriate facilities with appropriate equipment and methods. Manufacturing facilities must be designed to minimise the risk of contaminations, cross-contaminations, and errors. The main idea is that the facilities are suitable for high-quality manufacturing. There are some basic principles that need to be followed when manufacturing ATMPs. Manufacturing facilities need to be kept clean, which means that the facilities must be disinfected and cleaned appropriately. Facilities must also be free of technical poisons. All the repair and maintenance actions of the production facilities should be carried out without affecting the quality of the products. Circumstances must be appropriate for the manufacturing activities without affecting negatively to the equipment or the product itself and there must be appropriate measures to monitor essential environmental parameters. In addition to these, the facilities must be designed in such way that all animals, insects, and unauthorised people remain out of the facilities. Equipment in the manufacturing facilities should also be appropriate for the manufacturing process and not to cause any harm to the product. This means that equipment must not affect the quality of the product. All the parts that are in direct contact with cells or tissues must be sterile. Equipment should be identified clearly and located logically in order to prevent mix-ups and contaminations. In addition, maintenance of the equipment must be appropriate. All equipment must be calibrated and examined at certain intervals to ensure their correct operation. [9]

Manufacturing of the product should be done in such way that the quality of the product is ensured. These procedures include handling of the materials and products, prevention of cross-contaminations, aseptic manufacturing and sterilisation and packaging. All the procedures should be reviewed on regular basis, and they should be improved if it is necessary. Most cross-contaminations can be prevented with aseptic manufacturing methods. Aseptic manufacturing methods prevent microbial contamination of products

and are especially important in the manufacturing of ATMPs, because most of ATMPs cannot be sterilised. ATMPs should be manufactured in an isolator or a clean room, which isolates the product from the surrounding environment. [9]

Quality control is a major part of GMP. ATMP manufacturer must have a person responsible for quality control. The person responsible for quality control ensures that facilities and equipment used in quality control are appropriate and personnel performing quality control is adequately trained. The person responsible for quality control is also responsible for approving test methods and test conditions. In addition to these responsibilities, this person is responsible for the quality of raw materials, starting materials and medical devices in the manufacturing of the product. Documentation is a crucial part of the quality control. The main idea of the documentation is to monitor and record all the activities that can affect the quality of the product. Documentation also helps to trace errors in the manufacturing of the product. Therefore, all the documentation should be unambiguous and securely preserved. There are two main types of documentation: specifications and instructions, and record and reports. Specifications include the materials used in the product and the finished product itself. Instructions consists of manufacturing instructions which are necessary for the marketing authorisation or clinical trial authorisation, product consistency and required level of quality. Records and reports should be made at the time each production action is made. The idea of records is to make all the production steps traceable. [9]

3.4 The Hospital Exemption

In Regulation 1394/2007 there is Article 28 (2), which amends the Directive 2001/83/EC. This article states that ATMPs can be manufactured for specific purposes without a marketing authorisation. Authorisation is given by competent authority of the EU member state. This exemption of Directive 2001/83/EC is called a hospital exemption (HE) and its application takes place with certain specific requirements. In order to apply HE, the ATMP must be intended for the treatment of an individual patient on the prescription of a medical practitioner. The ATMP must be manufactured on non-routine basis, but according to specific quality standards. These custom-made ATMPs should only be used in a hospital in the same member state whose competent authority has authorised the use of the product. [10] The criteria set out in the article leave considerable room for interpretation to the Member States when deciding which products can be manufactured with HE. In particular, the terms non-routine basis and specific quality standards are understood differently in different Member States. For example, in some countries the non-routine basis is understood to mean non-commercial manufacturing and in others there is a maximum number of patients for which the product can be manufactured. Most Member States have required compliance with the European Union's GMP guide for ATMPs manufactured under HE. In some Member States, other criteria are also required for the application of the HE. As a result, HE is applied somewhat differently in different Member States. [28]

For example, in Finland, these HE authorisations are granted by Finnish medicines agency Fimea. Fimea requires that the quality and safety of ATMPs meet product-specific requirements and that they are manufactured in accordance with GMP. In addition, there should be procedures for monitoring and reporting adverse reactions and all materials used in the product should be traceable. In addition, Fimea requires a risk assessment attached to the application for authorisation, which states the known risk factors of the product. The risk assessment should include information on non-clinical studies and clinical use of the product to determine the safety of the product. ATMP must be manufactured in accordance with the European Union's GMP guide, and the labelling must be made according to the requirements of Regulation 1394/2007. [29]

3.5 Case: RegeOS-product

The RegeOS product is Tampere University's investigational product which has been used to repair bone defects in maxillary, mandibular or cranial bone. Maxillary bone means the upper jaw and mandibular bone means the lower jaw. Cranial bones form "a dome" that protects the brain. Therefore, all these bones are located in the human skull. RegeOS product consists of autologous adipose stem cells and chronOS -biomaterial from DePuy Synthes. The use of RegeOS product has been approved under the hospital exemption framework in Finland. RegeOS product has been used in several patients. [1-5] In one of them, the product has been used to replace bone tissue due to the removal of a large recurrent keratocyst on the orbital floor. [5] In some patients, the product has been used to replace bone tissue in the mandible due to removal of recurrent ameloblastoma. [1,4] In addition to these, the product has been used to repair bone defects in the skull and frontal sinus. Bone defects have been caused, for example, by infections or resorption and loosening of previous bone replacement products. The product was also used in patients who did not have previous bone replacement products in their skull. [2,3]

Autologous adipose stem cells are harvested from patient's own adipose tissue. The cells are then isolated and expanded in GMP-class clean rooms. Isolated cells are cultured for few weeks in basal media with autologous serum collected from the patient. The cells are then combined with β-TCP granules. Before implantation operation, the cells and β-TCP granules are incubated for two days to allow the cells to adhere to the surface of the granules. Prior to combining cells and biomaterial, β-TCP granules are incubated in basal media for two days. Prior to surgical implantation of the product, cell sterility and endotoxins are tested with methods that are described in the European Pharmacopoeia. The cells are also tested for mycoplasma contamination. In addition to these analyses, various in vitro analyses are also performed to examine cell viability, differentiation capacity and cell characteristics throughout the manufacture of the cell product. Cell viability and adhesion to β-TCP are tested by live/dead staining. The FACS method was also used to characterise the cells. [1-5] This method sorts the cells based on the molecules on their cell surface. For each cell type, there are surface molecules specific to them. Due to this fact, FACS allows the cells to be identified as cells of a particular cell line. [23] Some additional research has been made to study the differentiation of ASCs. In these studies, stem cells are cultured in three different culturing environments

to study osteogenic, adipogenic, and chondrogenic differentiation of the cells. Cell differentiation into the osteogenic direction was examined by alkaline phosphatase staining as well as RT-PCR. RT-PCR was used to examine whether cells that differentiate in the osteogenic direction express genes that are typical for cells in bone tissue. Adipogenic and chondrogenic differentiation were examined by histochemical staining. [1,5]

ChronOS is a β-TCP bioceramic which is in granule form. [1-5] β-TCP in chronOS is biocompatible and manufactured synthetically in a clean-room environment. Biocompatibility of the biomaterial has been tested and it has been proven to be free of any cytotoxic material. It has been clinically used more than 25 years as a bone substitute for void filling. It has high porosity (60%), and its mechanical properties are similar to human cancellous bone. It has interconnected macropores which supports the bone formation inside the biomaterial. Macropores also support vascularisation of the defect site. In addition to micropores, chronOS® granules have micropores that increase the surface area of the biomaterial in order to accelerate bone remodelling process. In human body, it is replaced with natural bone in 6 to 18 months. The manufacturer says that chronOS can be used together with autogenous blood and/or bone marrow. In RegeOS product, chronOS is used with autologous adipose stem cells which means its use in RegeOS differs from the instructions that the manufacturer has given. [30,31]

RegeOS bone replacement product is a combined ATMP and the biomaterial (chronOS) is defined to be a medical device. The Regulation 2017/745 on medical devices states that biomaterial in medical device must be biocompatible and non-toxic [8]. These requirements are met with the biomaterial in RegeOS product, because β-TCP is highly biocompatible biomaterial that mimics the mineral composition of natural bone. Its decomposition properties are well known, and it is replaced in the human body the same way as natural bone. [16] Requirements for mechanical properties are met as well because the biomaterial has similar compressive strength as cancellous bone [31]. These mechanical, chemical and physical properties are appropriate to RegeOS product's intended use as a bone replacement product and therefore acceptable according to the Regulation 2017/745 [8]. In addition to these properties, chronOS has been manufactured in a clean-room environment and undergone sterilisation process which means it meets the regulatory requirements relating to sterility [30,31].

Because the biomaterial is used in a combined ATMP, the manufacturer of RegeOS should apply for assessment of the conformity by a notified body for a marketing authorisation. This should be done even if the biomaterial has the CE mark already, because in combined ATMP the biomaterial is used for a different purpose than normally. In this specific case, chronOS is used for very similar purpose in RegeOS as it is intended to be used originally. [8] chronOS is normally used as bone void filler material, but in the RegeOS product it is used as a part of combined ATMP. The difference between these uses is that in RegeOS product there are stem cells as a part of the product and therefore the product is classified as combined ATMP. Because of these differences the assessment of the conformity must be done if the marketing authorisation is applied for the product. As the RegeOS product has only been manufactured in accordance with HE

legislation without a marketing authorisation, it has not been necessary to apply for this assessment.

The manufacturing of RegeOS product must be done as guided in European Commission's GMP guideline. RegeOS product is used under the hospital exemption legislation. Therefore, the most important aspects that should be taken into account in manufacturing are to ensure the safety of the product and the consistency of the production. Consistency of the biomaterial and the cell product is vital for getting good treatment results. Also, the manufacturing process must be consistent in order to make batches of uniform quality. Manufacturing requirements concern mainly the manufacturing of the cell product, because the biomaterial in RegeOS product is bought from a commercial manufacturer.

4. CONCLUSIONS

The products used in bone replacement have developed a lot in recent years. New products combining stem cells and bioceramics have made it possible to offer individualised treatments for repairing large bone defects. In such products, the bioceramic is intended to degrade over time in order to allow the formation of new bone tissue in its place. It is important that the bioceramic used degrades at just the right rate to be able to provide mechanical support. The rate of degradation of the bioceramic should therefore be as close as possible to the rate of bone formation. Another really important property for bioceramics in a bone replacement product is porosity. The porosity of the ceramic increases its surface area and thus provides a wider adhesion surface to the surrounding cells. The interconnectivity of the pores, in turn, allows for the migration of cells and the formation of blood vessels within the implant. In particular, the formation of blood vessels is important for the functioning of the implant and the formation of new bone tissue. The most common bioceramics used in bone replacement products are β-TCP and HA because they are very similar to the mineral structure of natural bone.

The stem cells in bone replacement products help the body form new bone tissue. The use of the patient's own stem cells also improves the biocompatibility of the product. These autologous stem cells are collected from the patient's adipose tissue or bone marrow. Thus, the stem cells used in bone replacement products are such that they have the ability to differentiate in the direction of the cells in bone tissue. Harvested autologous stem cells are isolated and expanded in the laboratory. Cultured cells are combined with the biomaterial and the product is surgically implanted into the bone defect site. Products like this can be used to correct bone defects in the head area, for example. Such products combining cells and biomaterials are combined advanced therapy medicinal products (ATMPs) and they are subject to specific regulatory requirements.

The biomaterial in combined ATMP is defined as a medical device. Therefore, combined ATMPs are subject to additional regulations. The regulations for biomaterials are largely related to the biocompatibility, toxicity, and sterility of the material. The material must therefore be such that it is compatible with the human body, that is, it must not cause adverse effects when in contact with human tissues. The material itself must be non-toxic, or its degradation products must not be toxic. The material must also be prepared under sterile conditions and must remain sterile until implantation. Other regulations for biomaterials are related to the content of the biomaterial documentation. The manufacturer of the biomaterial has an obligation to provide adequate information on the packaging of the material. This ensures that the right kind of materials are used for the right kind of purposes and that the buyer of the material knows all the essential properties of the material. The serial number on the packaging also makes it easier to find defective batches.

In addition to biomaterials, extensive regulations apply to the manufacture of ATMPs. The regulations largely apply to manufacturing facilities, personnel, and documentation. The manufacturing facilities must be such as to ensure the quality and safety of the product. Products should only be manufactured in clean rooms where adequate air cleanliness class and prevention of contaminations can be ensured. The manufacturing personnel must also be sufficiently trained to ensure that the products are of adequate quality. There must also be someone on the personnel who ensures that the regulatory requirements for the product are met. In order for such products to be manufactured for commercial use, the manufacturer must apply for a marketing authorisation. There are many steps involved in applying for a marketing authorisation and it can take a really long time because the safety and efficacy of products must be able to be demonstrated in a number of clinical and non-clinical trials. However, the products can be used for treating individual patients with a hospital exemption. This exception applies in situations where the product is used on a prescription in a hospital. The product must be manufactured on non-routine basis and in accordance with the necessary quality standards and may only be used within the authorising EU member state.

ATMPs offer a great potential for the development of medical treatments in the future. They can be used for developing personalised therapies for patients and treating conditions for which there is currently no other treatment. However, it would be very important for ATMP manufacturers to clarify the legislation in some respects, as some regulations currently have some scope for interpretation. For example, there is a little variation in HE legislation in different member states. All in all, the current EU legislation provides a comprehensive framework for manufacturing and quality requirements for ATMP products. In the future, further research could be carried out into the member states' own ATMP legislation and the differences between them.

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