Review

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Regenerative Medicine

A 3D-bioprinting exemplar of the consequences of the regulatory requirements on customized processes

Computer-aided 3D printing approaches to the industrial production of customized 3D functional living constructs for restoration of tissue and organ function face significant regulatory challenges. Using the manufacture of a customized, 3D-bioprinted nasal implant as a well-informed but hypothetical exemplar, we examine how these products might be regulated. Existing EU and USA regulatory frameworks do not account for the differences between 3D printing and conventional manufacturing methods or the ability to create individual customized products using mechanized rather than craft approaches. Already subject to extensive regulatory control, issues related to control of the computer-aided design to manufacture process and the associated software system chain present additional scientific and regulatory challenges for manufacturers of these complex 3D-bioprinted advanced combination products.

Keywords: 3D-bioprinting • additive manufacturing • ATMP • autologous • combination • customization • EMA • personalized • regulation • US FDA • validation

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Worldwide, groups are increasingly turning their attention to additive manufacturing or 3D printing technologies and approaches to fabricate 3D medicinal products and devices. These range from relatively simple customized implants or prosthetics printed from single metals or polymers, to 3D-cell-based, functional living constructs with biological and mechanical properties suitable for clinical restoration of tissue and organ function at increasing levels of complexity [1–4]. However, despite recent significant and exciting scientific and medical advances, the industrialization of these 3D-printed customizable medicinal products faces notable regulatory challenges under existing frameworks.

Working with a leading ear, nose and throat consultant, the multidisciplinary team within the Centre for Innovative Manufacturing in Regenerative Medicine at Nottingham University is developing a 3D-printing process for the production of a prosthetic implant for the total replacement of the nose following oromaxillary nasal trauma after tumor removal. Using thermoresponsive materials and a new 3D-printing platform, the implant can be printed from a 3D image of the patient's original nose or other alternative, providing a strong, weightbearing and novel noncontracting biofabricated scaffold incorporating precisely positioned autologous cells. As part of a collaborative project with researchers at Nottingham University, we have used this well-informed but hypothetical exemplar of a 3D functional living construct to investigate the regulatory challenges posed by 3D-bioprinting and its ability to create customized cell-scaffold products, which are personalized by geometry and constituent cells.

To determine which Regulations or Directives may apply, we first examine the legislation which describes the frameworks that regulate advanced combination of medicinal products in the EU and the USA. Broadly defined as products comprising two or more regulated components, we examine the factors influencing their classification and map the emerging regulatory landscape to illustrate the key principles and processes for

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their premarket review, authorization and postmarket regulation. Next, we use this case example to shed light on the key scientific and regulatory challenges that may have significant ramifications under the existing legislation for manufacturers intending to apply computer-aided 3D-bioprinting approaches to the manufacture and industrial production of these complex, customized 3D products.

The intent is to provide evidence of general applicability to products with similar characteristics and a framework to elucidate the criteria for establishing the regulatory readiness of the 3D-printing technology when tested against current and emerging regulatory frameworks in the EU and the USA. This is now being recognized by important stakeholders as a likely barrier to their clinical use. The test case example takes the regulatory viewpoint from the perspective of a manufacturer making and assembling both the cellular and scaffold component parts in the same production facility using an industrial process (note: there is no clear regulatory definition for an 'industrial process' by which the advanced therapy medicinal product (ATMP) regulation attempts to distinguish commercial enterprise from 'hospital'-based preparation of therapies for single patients on a one-off basis). The case does not cover distributed manufacturing strategies or product development pathways under fast track approval programs (USA) or named patient and hospital exemption (EU) regulatory schemes, although these are also anticipated to raise other regulatory issues for the technology. The purpose of this distinction is to examine the changes necessary within the existing regulations if such products are to reach patients on the scale required to achieve their full potential for patient well-being.

Methodology

The contents and coverage of the EU and USA legal frameworks for the regulation of therapeutic products intended for human use were analyzed to identify the applicable medicinal product categories and which of the legal instruments and associated guidelines included combined medicinal products. Characteristics and features critical for determining the regulatory classification of cell-based medicinal products were identified. The legislation was reviewed using associated guidelines and standards to interpret the provisions of the requirements in the development process and identify the regulatory input across each stage of the product development pathway.

Classification of medicinal products under EU & USA regulatory frameworks

The rules governing the placement of human medicines on the market in the EU and the USA are broadly divided under public health and core pharmaceutical and medical device legislation. Pharmaceuticals, biologics and medical devices are subject to different regulatory requirements that govern premarket applications, manufacturing practices and postmarket reporting of adverse events. In the USA, the Code of Federal Regulations (Title 21 CFR), based on the Federal Food, Drug and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act), establishes the legal framework within which the US FDA regulates the distribution and sale of medical products. These legal instruments provide the precise product definition and legal basis for classification of products as drugs, biologics, medical devices or combination products. Each of the product types is regulated by a different office within the FDA, either the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER) or the Center for Devices and Radiological Health (CDRH). In the EU, a relevant product can only be regulated as either a medicinal product (whether drug or biologic) or a medical device, as classified by the applicable and legally separate sector-specific legislation that differ markedly in terms of procedures to be followed to place the product on the EU market. In broad terms, this legislation is enacted by the EMA under EU regulations and a number of legally binding directives that are addressed to the National Competent Authorities (NCA) of the member states.

However, while the principal definitions in the EU and USA legislation have set out the key elements in characterizing medicinal products as single entities with respect to the presentational (properties related to intended use) and the functional aspects, the emergence of ever more sophisticated products and medical approaches continues to challenge the existing demarcation between these regulated product categories. For manufacturers and regulators of products which combine components or show features from more than one of these product categories this has made the classification process increasingly more complex. Understanding how these combination products fit the existing regulatory frameworks from the earliest stage of development will be crucial to unlocking the potential clinical advantages of the technologies. It will help manufacturers determine the applicable regulatory pathway and identify the appropriate product development strategy and risk management plans for navigating the route to their intended geographical market(s).

How are cell-scaffold combination products regulated?

In the USA, combination products as legally defined entities under 21 CFR 3.2(e) [5], are broadly regulated by the FDA as products comprising two or more regu-

lated components (i.e., drug–device, device–biologic, drug–biologic or drug–device–biologic) that are physically, chemically or otherwise combined or mixed and produced as a single entity. As defined, the term also includes components that are co-packaged or packaged separately but have labeling that requires use with another component to achieve the intended use (not relevant to this test case example). In the EU however, there is no general definition or specific regulation for a combination product. As a general rule, combination products are currently regulated in the EU as medicinal products (including ATMPs) or medical devices under the respective sector-specific legislation [6,7]. Classification remains an area of discussion at the European level with recent publications on the classification of ATMPs and borderline products [8,9].

For a combination product that consists of or contains cells or tissues and a scaffold matrix, the classification and thus the legislation into which the product fits is determined by the way in which the finished product achieves its intended medical purpose, the claimed mode of action (MoA), the characteristics of the active substance and the way in which it is combined in the finished product. This means the manufacturer would need to determine a number of critical characteristics that define the product under development before being able to classify the finished product as follows:

- Does the cellular or tissue part of the product meet the definition of a biological medicinal product as defined in Directive 2001/83/EC (EU) [6] or the definition of a Human Cells, Tissues, and Cellular and Tissue-Based Product (HCT/P) under 21 CFR 1271(US) [10]?
- If so, is the cellular or tissue part presented has having properties liable to act on the human body when combined with the scaffold/matrix?
- Does the scaffold/matrix form an integral part of the finished product?
- How is the finished product (i.e., ready for clinical use) intended to function/work in the recipient?
- How is the principal/primary intended MoA of the finished product achieved?

This five-step question framework provides the basis for analyzing the features of the product and process that influence the classification of the 3D-bioprinted cell-scaffold nasal implant and how this dictates the regulatory input into the development process for such cell-based combination products under EU and USA regulatory frameworks.

Features influencing the classification & regulatory governance of the 3D-bioprinted nasal implant

The manufacturing process for the centralized industrial production of the autologous 3D-bioprinted nasal implant product is shown in Figure 1. The figure shows a cross-functional map of the whole production process envisaged for the manufacture of the active substance and the finished product, where the manufacturer, as the holder of the manufacturing authorization (as defined in Article 40 of Directive 2001/83/EC) [6], is making both component parts in the same production facility. In identifying the major processing steps and process flows involved from biopsy to patient delivery, the map provides the basis for determining where the regulatory legislation and associated scientific guidance might apply across each stage of the development pathway (as indicated by the swim-lanes).

The process begins with surgical retrieval of a patient biopsy sample from autologous ear and rib cartilage, harvested under local anesthesia in the primary care facility. Once acquired, the tissue sample, which represents the source of autologous chondrocyte cells, is transported to the facility of the manufacturer for processing under Good Manufacturing Practice (GMP). On arriving at the facility, the tissue is transferred to a highly controlled environment (Grade A or Class 100) for further processing. The cells are isolated from the biopsy tissue and expanded *ex vivo* for several weeks to achieve the required numbers of clinically relevant cells in 2D monolayer culture. In an industrialized manufacturing process, the expanded live cells are encapsulated within a UV cured synthetic gelatin methacrylate (GelMA) hydrogel (as an example of an available 'printable' and biocompatible synthetic polymer). Under a Grade A environment, the cell-laden hydrogel is co-printed with a clinically approved (i.e., used in CE marked, surgically deployed devices) biodegradable polycaprolactone (PCL) matrix using a commercially available, computer-aided bioprinting system (regenHu 3D-printer) via a two-stage process.

Broadly described, image data derived from computed tomography (CT) scans or magnetic resonance imaging (MRI) of the patient's nose is transformed (software segmentation) using computer-aided design (CAD) software into an accurate digital 3D model of the nose. Machining process templates are used to automatically apply predefined programming methods, setups, machining operations and tool selections to new patient cases. At this stage, computer-aided manufacturing (CAM) software is used to automatically update the shape definitions and other customization features (e.g., stiffness and diffusion properties) of the implant according to the surgeon's specification

(the surgical plan), thereby tailoring the implant to individual patient requirements. The 3D-printing machine then assembles the cell-laden hydrogel and PCL components into the finished (or intermediate) cell-scaffold product.

Postproduction, the tissue construct is maintained *in vitro* in a perfusion bioreactor to allow time for cell-mediated cartilage extracellular matrix (ECM) remodeling (e.g., collagen and fibrous protein production; [11]) and maturation toward a solid construct (and potentially patient-matched testing pre-implantation) before transfer back to the primary care facility for implantation into the same patient. Alternatively it may be remodeled intraoperatively and matured *in situ* once implanted into the patient and begins to integrate with existing bone. The reconstructive surgical step is likely to include final shaping and suturing by the surgeon and the use of pedicle flaps to re-establish adequate tissue perfusion to the implant postoperatively to permit further remodeling and integration.

The following sections will explore these product and process features as they relate to how the EMA and the FDA might regulate these cell-based combination products in the EU and the USA, respectively.

The EMA's approach to regulating combination products in the EU

Classification & premarket review

Considering the properties and MoA of the individual cellular and other material parts and the way in which these as distinct components are combined in this case example, Figure 1 shows that autologous cartilage tissue derived chondrocyte cells are expanded *ex vivo* in culture before being co-printed as living cells with a synthetic scaffold matrix to form the finished product for use in humans. Under EU legislation, where the product contains viable cells or tissues, the pharmacological, immunological or metabolic action of these cells is considered to be the principal MoA. The cells are intended to be used for the same essential function (homologous use) but as expanded cells they are considered to be substantially manipulated according to annex 1 of the ATMP Regulation (EC) No 1394/2007 [15]. The derived living cellular part of the combination therefore qualifies as an active substance of a biological medicinal product according to EU Directive 2001/83/EC [6] and falls within the scope and additional provisions of the ATMP regulation [15].

For the substantially manipulated cellular part of the combination, provided it is not genetically modified as defined under Directive 2001/83/EC [6], the specific requirements for somatic cell therapy medicinal products (sCTMP) under Directive 2001/83/EC [6] or tissue-engineered products (TEP) under Regulation (EC) No 1394/2007 [15] apply. The decision on whether a product fulfills the requirements of an sCTMP or a TEP is taken on the basis of the principal MoA and the intended function claimed by the manufacturer. In this context, it is important to ascertain whether the product is for treatment, prevention or diagnosis of a disease and exerts its activity via a pharmacological, immunological or metabolic action or whether the intended MoA of the product is regeneration, repair or replacement of cells/tissues. In this case example, the principal MoA is provided by the viable chondrocyte cells, linked to inducing the regeneration of cartilage tissue at the site of implantation. According to the definition in Article 2(1)(b) of Regulation (EC) No 1394/2007 [15], this would specifically qualify the product as a tissue engineered product.

In this instance, because the cells are combined with the synthetic scaffold matrix at the time of manufacture and it retains its original form and function in the finished product, the scaffold is considered as a starting material and as an integral part of the finished product [6]. Here, the principal function of the bioresorbable scaffold matrix is to provide a physical and permissive support for the attachment and growth of cells and to reproduce the required form and shape of the nose at the site of implantation. Tissue regeneration is accomplished by the living cells as the scaffold matrix undergoes progressive degradation while new tissue regenerates to replace missing cartilage. Referring to the EU definition of a medical device, this places the MoA for scaffold matrix firmly within the scope of the European medical device legislation [6,7,16,17].

This being the case, the candidate ATMP classification can be identified. On the basis that the product consists of substantially manipulated chondrocyte cells, is presented as having properties for regeneration and repair of a human tissue, and contains as an integral part, scaffold material that fulfills its function as a medical device when deployed in the patient, the product falls within the definition of a tissue engineered combined ATMP, as defined in article 2(1)(d) of Regulation (EC) No 1394/2007/EC [15].

However, although it is important to get clarification of the regulatory requirements as early as possible, in practice the manufacturer may not have sufficient data and/or scientific knowledge in the early development stage to substantiate the principal MoA and thereby identify the candidate ATMP classification. As the underlying science improves, the manufacturer may need to clarify whether the product under development still meets the scientific criteria that define combined ATMPs so that questions of evolving borderlines between other noncombined ATMP categories and even non-ATMPs can be resolved [7,8].

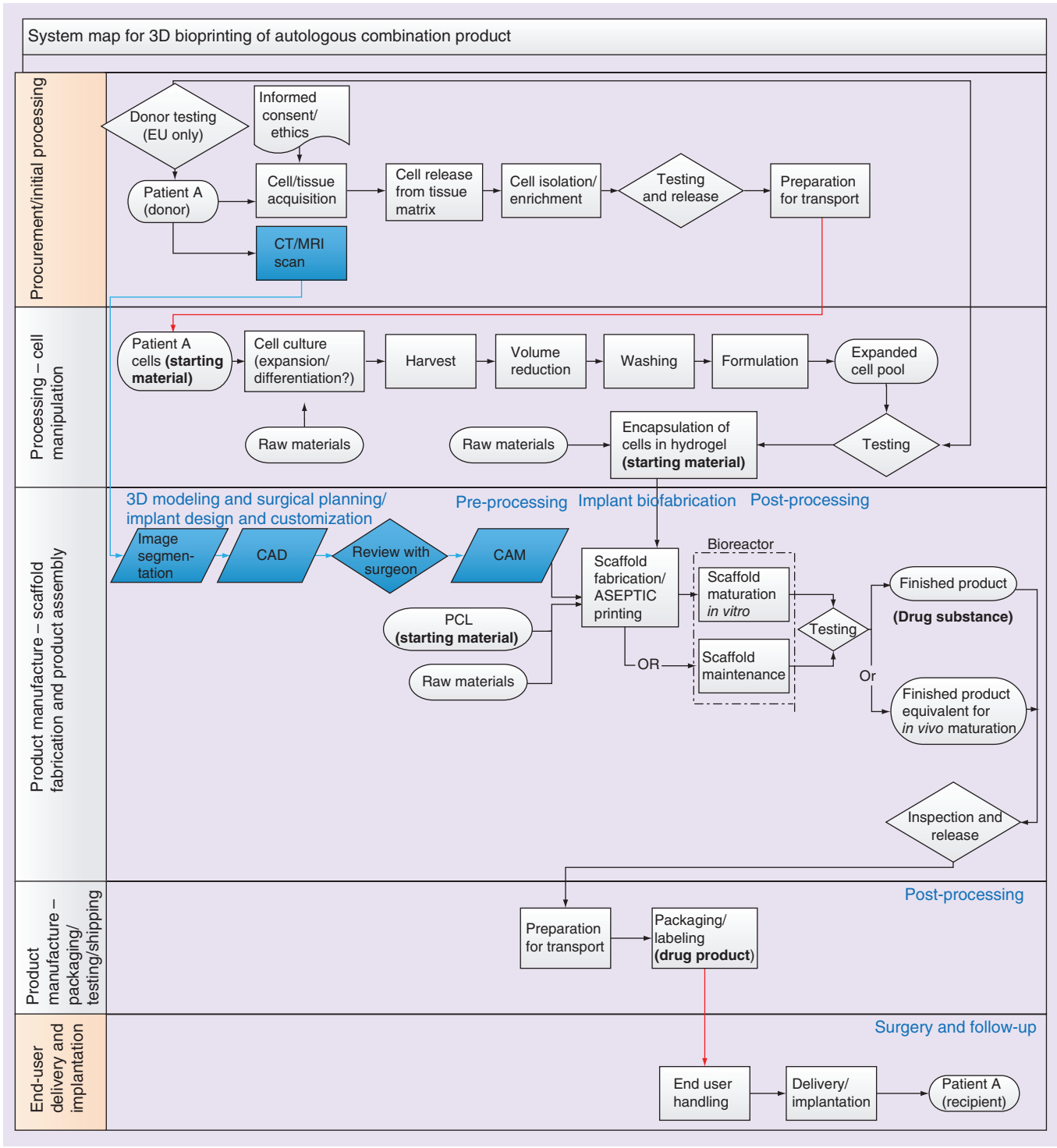

Figure 1. Cross-functional flow diagram of the manufacturing process for the centralized industrial production of a personalized autologous 3D-bioprinted combination product for total replacement of the nose after tumor removal. Cell culture and bioprinting activities are co-located in the Good Manufacturing Practice (GMP) manufacturing facility. The swim-lanes show the key activities and highlight transfer (red arrows) between the GMP manufacturing facility (unshaded swim-lanes) and the primary care facility (shaded swim-lanes). Blue boxes and arrows delineate activities involved in the computer-aided bioprinting process. The qualified person assesses release criteria and adherence to GMP/quality system regulation standards before release of the finished product batch. The starting materials, drug substance and drug product are defined according to both EMA and US FDA guidance documents [12,13]. These elements define the quality expectations for subsequent clinical trial and manufacturing authorization submissions [14].
CAD: Computer-aided design; CAM: Computer-aided manufacturing; CT: Computed tomography; PCL: Polycaprolactone.

For instance, to make sure that the device part of the combination is acting as a device rather than an excipient or an active substance of the final product or to make sure the function of the matrix does not become decoupled from its structural properties at the time of implantation. The Committee for Advanced Therapies (CAT) provide scientific advice on whether a product meets the scientific criteria which defines ATMPs [18].

Once the candidate ATMP classification has been clarified and confirmed, the manufacturer will need to continue dialogue with the CAT as processes and process knowledge evolve between the research and industrialization stages. To build a robust regulatory strategy and a postapproval change management plan, the manufacturer will need to anticipate process changes and consider long-term plans for transfer of the discovery or pilot processes to clinical-grade GMP production. For instance, plans that involve modification to the manufacturing process or changes to product composition, such as changes to the source and/or level and type of manipulation of the starting material, the type and characteristics of the biomaterial (new or novel material is likely to require Class III regulatory approval), use of additional cellular material or addition of adjuncts, such as growth factors or antibodies that enhance cell attachment for example, will all raise similar questions with regard to borderline status and ultimately impact the regulatory classification decision.

From an EU perspective, an ATMP which comprises an integral combination of a medical device, as defined in Directive 90/385/EEC [16] or 93/42/EEC [17] (both amended by Directive 2007/47/EC), and a cellular part in which the cells are viable (or are exclusively nonviable but with an action that is primary to that of the device part), is regulated as a combined ATMP under Directive 2001/83/EC and Regulation (EC) No 1394/2007 [6,15]. According to the procedural advice on the evaluation of combined ATMPs' [19], the product as a whole is subject to evaluation by the CAT and centralized market authorization by the EMA in accordance with the provisions of Regulation (EC) No 726/2004 [20]. As a starting material, combined as an integral part with the manipulated cells, the device part (and/or associated packaging and instruction leaflets) should be CE marked for the intended use (issued by the Notified Body (NB); a certification organization designated by the NCA to carry out the conformity assessment procedures) or it should conform to the Essential Requirements laid down in Directive 2007/47/EC (the amending medical device legislation) [7]. This information is required as part of the market authorization application (MAA), which will include information on the intended function of the medical device and demonstration of compatibility of the device with other components of the product. Where available this would also need to include the results of an assessment of the medical device part of the combined ATMP by the NB in accordance with the medical devices legislation. If not, the EMA will seek an assessment from the NB unless the CAT advises otherwise.

The national and centralized provisions under this core ATMP legislation, in addition to the relevant medical device Directives, form the regulatory framework that controls all critical aspects of the development, manufacture, approval and subsequent postmarket vigilance for combination ATMPs. This includes regulatory input into setting the quality and safety standards for donation, procurement and testing of human cells or tissues under the EU Tissue and Cells Directive (Directives 2004/23/EC, 2006/17/EC and 2006/86/EC). This also includes standards for the manufacture of products in compliance with the principles of GMP (Directive 2003/94/EC; Eudralex Volume 4) and for conduct of clinical trials in the EU (Directive 2001/20/EC) in accordance with the principles for Good Clinical Practice (GCP) laid down in Directives 2005/28/EC and 95/46/EC [21]. Summarized in Figure 2, this legislative framework provides clarity on the EU Regulations and Directives that should be applied at each stage of the combination ATMP development pathway. From a scientific perspective, technical guidance documents have been issued and interaction platforms are available to define and make this EU regulatory framework more accessible (see Eudralex website, [57]). Recognizing combination ATMPs as a distinct product category, the CAT has issued specific procedural advice for their classification and evaluation [18,19] but little specific guidance is currently available other than that incorporated in the general cell-based medicinal product or ATMP guidelines [12,15]. For the medical device part, national and international standards will play a much more significant function in demonstrating compliance with the Essential Requirements defined in the medical device Directives [22].

The FDA's approach to regulating combination products in the USA

Classification & premarket review

In the USA, as a product containing living human cells that are intended for implantation or transplantation into a human recipient as part of a synthetic scaffold matrix, the cellular part qualifies as an HCT/P, as defined in 21 CFR Part 1271.3(d) [10]. In this case example, as more-than-minimally manipulated cells (as defined in 21 CFR Part 1271.1(f)), the derived cellular part of the product falls within the scope of the biologics regulation under Section 351 of the PHS Act

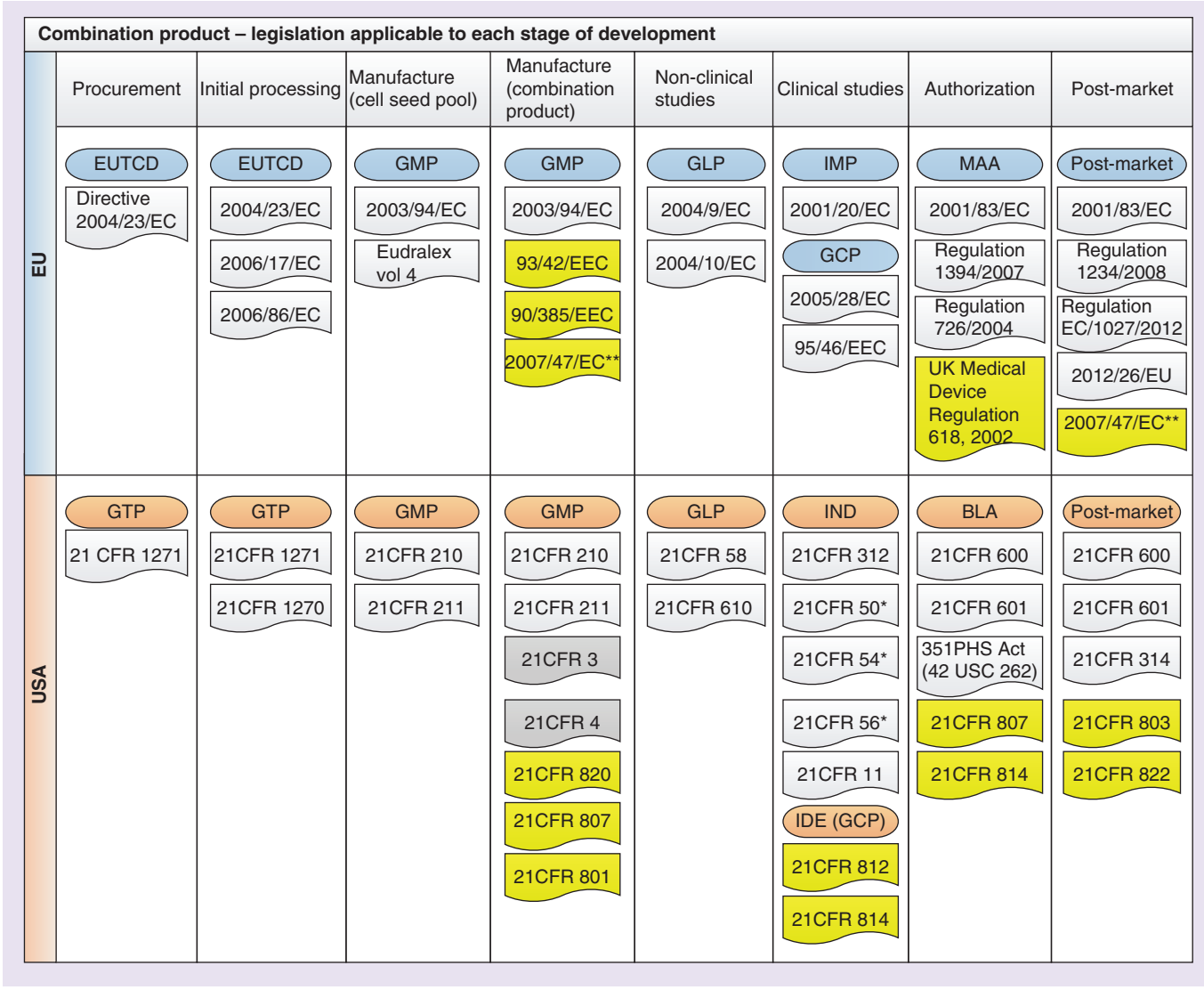

Figure 2. Summary of the main EU and US regulatory legislation for a combination product that comprises an integral combination of a medical device (scaffold) and a cellular part consisting of viable substantially manipulated cells. The figure indicates which legislation applies at each development stage, using a specific synthetic 3D-bioprinted cell-scaffold nasal implant product as a case example. The legislation may overlap stages for example between the legislation identified for cell processing and that for GMP manufacture. In EU, manufacturers must demonstrate compliance with applicable regulation/directives for advanced therapy medicinal products (ATMPs) and medical devices. The manufacture of products regulated as an ATMP is authorized by the National Competent Authorities of the Member State concerned. For clinical trials occurring within their borders, the remit of the National Competent Authorities also includes assessment of applications for clinical trial authorization and the associated manufacturer's license for investigational ATMPs, noting that a new EU Clinical Trials Regulation No 536/2014 becomes applicable in 2016 to replace Directive 2001/20/EC. The pharmacovigilance guidance for human medicinal products (Eudralex Volume 9A) is replaced by the Good Pharmacovigilance Practice (GMP) guidelines released by the EMA [23], but until the availability of the respective GMP modules, Eudralex Volume 9A remains the reference [24]. All EU Directives and Regulations (as amended) can be accessed via the Eudralex website [57]. In the USA, the US FDA is responsible for all facets of regulating combination products. The FDA provides an option for a streamlined hybrid approach to GMP compliance and postmarket reporting when both constituent parts are manufactured at the same facility. This determines which CFRs apply – provisions for manufacturers following CFR 820 and/or CFR 210/211. All US Regulations can be accessed via the FDA website [58].
Key: **: amending Medical Device Directive; Light blue swim-lane: applicable EU ATMP directives and regulations; Red swim-lane: applicable US CFRs; Yellow boxes: applicable Medical Device Directives or Regulations applicable to combination products; Grey boxes: specific US regulations applied to combination products.
BLA: Biologics License Application; CFR: Code of Federal Regulation; GCP: Good Clinical Practice; GMP: Good Manufacturing Practice; GTP: Good Tissue Practice; IND: Investigational New Drug; MAA: Market authorization application.

and 21 CFR Part 1271 [10]. The manipulated living cells are physically combined with the synthetic scaffold matrix to create a finished product with a medical purpose. As defined in Section 201(h) of the FDC Act, the function of the clinically approved (i.e. used in FDA approved, surgically deployed devices) bioresorbable scaffold matrix places it firmly within the scope of the USA medical device legislation (primarily under applicable parts of 21 CFR 800-898).

Under the USA regulatory system, a biologic product which is physically combined with an integral constituent part that would independently be regulated as a medical device and produced as a single entity is defined as a combination product under 21 CFR 3.2(e) [5]. In accordance with Section 503(g) of the FD&C Act (21USC 353(g)) the FDA (via the Office of Combination Products; OCP) classifies these combination products according to the primary MoA through which the product achieves its therapeutic effect. This is similar to the situation in the EU, but in this case determines which of the CBER, the CDER or the CDRH will lead the premarket review process and determine the regulatory pathway most appropriate for the product. Under 21 CFR 3.7 [5], by submitting a 'Request for Designation' (RFD) to the OCP, the manufacturer can obtain a formal determination of the combination product's primary MoA and identify the lead agency Center assigned to the product's premarket review and postmarket regulation. In the case of the nasal implant, the FDA would assign the product to the CBER as the lead agency because the cellular constituent part (the HCT/P component) provides the greater contribution to the overall therapeutic effect of the combined product, as previously described. The product would therefore be regulated as an HCT/P within the scope of the biologics legislation under Section 351 of the PHS Act and 21 CFR 1271 [10]. Based on an assessment of the safety and effectiveness of the constituent parts and their interaction, the product would be reviewed under a single Biologics License Application (BLA) in accordance with the provisions set out in 21 CFR parts 600, 601 and 610 [25–27].

This core legislation forms the regulatory framework that the FDA uses to control all critical aspects of the development, manufacture, approval and subsequent postmarket vigilance for biologic-device combination products. This includes regulatory input into setting the quality and safety standards for donation, procurement and testing of human cells or tissues under GTP (21 CFR 1271). This also includes standards for the manufacture of HCT/Ps in compliance with the principles of GMP (21 CFR 210/211) and for conduct of clinical trials in the USA via an Investigational New Drug (IND) application under 21 CFR 312 and in accordance with the principles for GCP laid down in 21 CFR parts 11, 50, 54 and 56 [21]. Summarized in Figure 2, this legislative framework provides clarity on the USA regulations that should be applied at each stage of a biologic-device combination product development pathway. The FDA has issued multiple technical guidance documents to help guide HCT/P manufacturers and define the US regulatory framework (see FDA website). Unlike the EMA, the FDA under a more general definition (21 CFR 3.2(e); [5]) identifies combination products as a distinct category of product that could be subject specialized regulatory controls. To that effect the FDA has issued or proposed new rules aimed at providing greater clarity on how current GMP and postmarket safety reporting requirements are applied to combination products. Their implementation has implications for manufacturers of biologic/device combination products intended for distribution and sale in the USA.

Good Manufacturing Practice & Quality System requirements for combination products under EU & US regulatory frameworks

The final rule on cGMP requirements for combination products was implemented by the FDA in July 2014 [28,29]. As defined under 21 CFR 4 (Subpart A), the rule applies to all 'single-entity' and 'co-packaged' combination products, including legacy products already being manufactured prior to the rule's effective date. Under the final rule, as part of their compliance with cGMP, a manufacturer of single entity combination products comprising both an HCT/P and a medical device has two options for demonstrating compliance with applicable quality system requirements. The manufacturer could demonstrate compliance with each applicable regulation in its entirety by implementing a quality system that fully incorporates the cGMP regulations under 21 CFR 210/211 [30,31] and the device Quality System Regulations (QSR) in 21 CFR 820 (Figure 2) [32]. Alternatively, the manufacturer could elect to follow a 'streamlined approach'. This allows operational compliance with either 21 CFR 210/211 or 21 CFR 820 rather than both, provided that they also incorporate a specified subset of provisions from the other GMP framework (Box 1).

For example, as a single entity combination product comprising an HCT/P and a medical device, the manufacturer of the nasal implant operating in compliance with 21 CFR 210/211, would also be required to incorporate the applicable provisions from 21 CFR 820, as specified in Box 1. They would also need to demonstrate compliance with the relevant provisions of 21 CFR 600-680 and 21 CFR 1271 (Figure 2) [33]. This 'streamlined approach' is only available when both the HCT/P and device constituent parts are being

Box 1. Key provisions for a streamlined approach to demonstrating compliance with the current Good Manufacturing Practice in 21 Code of Federal Regulation 210/211 or the device Quality System Regulations in 21 Code of Federal Regulation 820.

Manufacturers following 21 CFR 210/211

- Key provisions of QSR to be implemented:
  - 21 CFR 820.20 Management responsibility
  - 21 CFR 820.30 Design controls
  - 21 CFR 820.50 Purchasing controls
  - 21 CFR 820.100 Corrective and preventive action
  - 21 CFR 820. 170 Installation
  - 21 CFR 820.200 Servicing

Manufacturers following 21 CFR 820

- Key provisions of drug GMPs to be implemented:
  - 21 CFR 211.84 Testing and approval/rejection of components, drug product containers and closures
  - 21 CFR 211.103 Calculation of yield
  - 21 CFR 211.132 Tamper-evident packaging for over-the-counter human drug products
  - 21 CFR 211.137 Expiration dating
  - 21 CFR 211.165 Testing and release for distribution
  - 21 CFR 211.166 Stability testing
  - 21 CFR 211.167 Special testing requirements
  - 21 CFR 211.170 Reserve samples

CFR: Code of Federal Regulation; GMP: Good Manufacturing Practice; QSR: Quality System Regulation.
Data taken with permission from [30–32].

manufactured at the same facility.

Under the existing regulatory frameworks in the EU, when both the ATMP and medical device constituent parts are being manufactured at the same facility, the manufacturer is required to demonstrate compliance with each applicable regulation. The manufacturer must apply for assessment of the quality system (established under ISO13485 for example; [34]) with the NB under Annex II and V of Directive 93/42/EEC [17]. The manufacturer is required to operate in compliance with the principles of GMP for medicinal products laid down in Directive 2003/94/EC [35] and Annex 2 of the EU GMP guidelines [36], in addition to the relevant provisions of Article 5 of the ATMP Regulation (EC) No 1394/2007 (Figure 2) [15].

Post-authorization vigilance systems for combination products under EU & USA regulatory frameworks

In 2015, the FDA is set to issue a final rule to clarify the postmarket safety reporting requirements for combination products. The rule will create Subpart B of 21 CFR Part 4 [37]. Intended to minimize duplicative reporting requirements, it will clarify that a combination product is subject to the reporting requirements associated with the type of MAA under which the product was approved or licensed and to certain additional requirements dependent on the type of constituent parts of which it is comprised. For example, the developer of a biologic/device combination product approved under a BLA would be subject to the postmarket adverse event reporting requirements for biological products under 21 CFR 314.80(c) and 600.80(c), but may also be required to comply with the device requirements should an adverse event occur that is attributable to the device constituent part under 21 CFR. 803.20 or 21 CFR 803.53(a) (Figure 2) [23,38,39].

In the EU, the manufacturer of a combination ATMP authorized through the centralized procedure would be subject to the postmarket reporting requirements for both the ATMP and the medical device constituent parts should an adverse event occurs. The combination ATMP would be subject to common rules for postauthorization surveillance (pharmacovigilance) of medicinal products for human use under Directive 2012/26/EU and Regulation (EU) No 1027/2012, in addition to the provisions of Regulation (EC) No 1394/2007 (Figure 2) [15,40,41]. The combination ATMP would also be subject to the medical device vigilance system requirements contained within Directive 2007/47/EC (the amending MD legislation) and applied under the guideline in MedDEV 2.12/1 [42]. Under the forthcoming revision of the medical device regulation, manufacturers will be subject to more extensive requirements for pre- and post-market assessments, especially for high-risk (Class III) devices [43].

Regulation of postmarket modifications to combination products in the EU & USA

In the USA, the requirements for making changes to a product or its manufacturing process approved under a BLA are described in 21 CFR 601.12

(Figure 2) [24]. The FDA has developed specific draft guidance which outlines the process for determining which type of submission to provide for a post-market change to a constituent part of a combination product under one application (BLA, NDA or PMA) [44]. The guideline does not address the type or amount of comparability information to include in the submission.

In general, all but 'minor/nonsubstantial' changes require prior approval by the FDA. If the nasal implant was filed under a BLA as a combination product for example, changes in the biological constituent part or design modification to the device part (or changes to the production process, quality controls, equipment, facilities or responsible personnel) that have substantial potential to adversely affect the critical to quality attributes (CQAs) of the product as they relate to safety and effectiveness would require submission of a 'BLA Prior Approval Supplement' [44].

In the EU, no such guidance is currently available, although guidance on post-approval procedures to clarify the interaction between the CAT and the NB's for combination products is under development. Currently, manufacturers making all but minor post-approval modifications to the manufacturing process (e.g., materials changes, improvements, scale-up or new facilities), to a constituent part or to the combination product as a whole, may require prior approval from the EMA under the centralized MA. Such amendments may involve changes to the product information or changes to the technical dossier submitted by the MA holder. The procedures for the approval of such amendments have been set out in Regulation (EC) No 1234/2008 [45]. Coordinated by the EMA, this regulation differentiates between minor variations (Type IA and Type IB variations, e.g., minor quality changes), major variations (Type II variations, e.g., most manufacturing changes) and certain other major variations in the MA (so-called extensions) that necessitate a new MA procedure, for example, new indication. In addition, 'significant/substantial' changes to the approved device part or its design that could affect the conformity with the Essential Requirements or the conditions prescribed for use of the device must be approved by the NB according to Annex II and III of Directive 93/42/EEC (Figure 2) [17]. This might include changes relating to materials or the manufacturing process, facility or equipment that have the potential to affect the safety and performance of the device part. Substantial changes to the manufacturer's approved Quality System are also reportable to the NB under Annex II, V and VI of Directive 93/42/EEC (Figure 2) [17].

Regulatory challenges for 3D-bioprinting of customizable advanced combination medicinal products

As described earlier, under current EU and USA legislation, cell-based medicinal products in which engineered cells are combined with a material scaffold that provides physical support for the growth of new tissue, are regulated as biologics in the USA and as combined ATMPs in EU. This section considers the hurdles that will raise novel challenges for manufacturers of customized 3D-bioprinted autologous versions of these products under the existing regulatory systems.

Implications for the cell-based combination product manufacturer

As described, the USA regulatory system offers manufacturers of combination products containing biologic and device constituent parts some flexibility in terms of how compliance to the varying requirements can be achieved. In contrast to the EU, a more adapted approval process aims to avoid unnecessary duplication and redundancy by providing ways to streamline the overlapping aspects of development and the requirements for manufacture. Although the regulatory process in the USA is not without organizational and operational implications for the manufacturer in terms of increased quality system costs and longer implementation times [46], in the EU, where the regulations for combination ATMPs appear less flexible as discussed below, the implications may be more far reaching.

In the EU, the regulation requires the device component to receive a separate license (CE mark) in addition to review of the MAA for the cell-based component. If the manufacturer of the ATMP also manufactures the scaffold or device or if the device component, as an integral part of the ATMP, is not manufactured as a separate entity (as in this case example), this may create a significant regulatory burden for manufacturers. This will especially apply to manufacturers of device components considered to be Class II or Class III, particularly if they are only familiar with one set of the regulations. This was the widely perceived view of stakeholders in the European Commission's public consultation on the ATMP Regulation in 2012, in which a single assessment process for combined ATMPs was supported to reduce uncertainty and avoid potential interpretative disparities between different notified bodies (NBs) or between the NB and the CAT/CHMP [47,48].

There is a lack of clarity on how regulatory inputs for the development of these combination products are aligned under the current delineation of the regulations. There is also uncertainty about the potential impact on the development and authorization of combined ATMPs by the current proposals to amend

the medical devices Directives in the EU [43]. This is expected to change the role of the NBs and NCAs and impose additional requirements for post-marketing studies for devices and the materials that may be part of them. Under Directive 2001/83/EC [6], the manufacturer of the combined ATMP is already subject to additional requirements to demonstrate the safety, interaction and biocompatibility of all structural components. This raises questions about the grounds on which the EMA consider evidence of conformity of the medical device part sufficient or indeed relevant to the role of the device and the evaluation of the combination product as a whole. This especially applies to the use of existing data for base materials that have been previously deployed in CE marked devices and the extent it can be used to support the safety and effectiveness assessment. This particularly applies to 3D-printed product types such as this case example, in which the role, form and function of the materials used are different to that in the original CE marked device and may be changed when printed, during post-processing/remodeling or when used in combination with cellular material. Without such clarity, there is potential that the device data requirements could be disproportionate to the role of the device in the combined ATMP. This may not lead to better quality products and could confer significant costs and delays in marketing approval for some combination products.

Current regulatory environment for 3D-printed medicinal products

Additive manufacturing or 3D-printing is an emerging production alternative in healthcare enabling manufacturers to produce medical products that are accurate and precise in geometry and mechanically complex [1]. 3D-bioprinting allows the design and build of anatomically matched functional 3D tissue engineered constructs by simultaneously placing living cells into defined spatial locations within customizable scaffolds using computer-assisted design and manufacturing models derived from CT scans or MRI of the patient's anatomy. However, despite recent progress in bringing 3D-printed medical devices to clinical use, industrial manufacturers of 3D-bioprinted tissue engineered combination products face many unique scientific and regulatory hurdles that result from the ability to create customized products, in addition to the challenges that 3D-printing will pose for conventional manufacturing paradigms.

As combination products, they are already subject to extensive control via implementation of a range of legal instruments and an extensive array of technical guidance and standards (Figure 2). Manufacturing requires regulatory-compliant production facilities that have design and device controls and an appropriate clean manufacturing environment. The manufacturing process requires a quality system (GMP, QSR). Furthermore, the biological process applied to harvest, transport and use the biologic material is regulated, as are the starting materials as part of the medicinal product. The question is how do autologous products that are patient-specific ('batches of one'), made potentially on demand using an industrialized manufacturing process and comprise an integral scaffold or device component that can be customizable in the production process (and consequently the end result) fit the existing regulatory frameworks?

With the potential functional capabilities and limitations of 3D-printing not yet fully understood, there is currently no distinct legislation or clear direction from regulators regarding the approval of medicinal products manufactured using 3D-printing technology. However, while a lack of regulatory coverage for the safe use of 3D-printed patient-specific devices can be seen in the current EU framework, even under the new proposals for medical device regulation [43], the FDA has set up an additive manufacturing working group as a path forward, at least for 3D-printed medical devices [49]. Supported by the FDA's laboratories for 'Functional Performance and Device Use' and for 'Solid Mechanics', the working group aims explore ways of improving the evaluation of customized 3D-printed devices and to establish parameters and standards for materials and other critical product safety aspects. Guided by a 2-day additive manufacturing workshop that was held in October 2014 to explore the technical challenges associated with 3D-printed devices, the FDA has outlined its commitment to developing a policy for regulating the additive manufacturing field and to creating regulatory guidance on 3D-printing within the next 2 years [50].

As an established manufacturing method in medical planning and anatomical modeling 3D-printing has already had a transformative effect on hearing aid manufacturing [2–4]. The approach has since been used to build patient-specific implants and prosthetics in the dental, orthopedic and cranio-maxillofacial fields. A number of these 3D-printed medical devices have been approved by the regulator for emergency or compassionate use in difficult clinical situations [2]. Recently however a number of 3D-printed devices have received 510(k) clearance through the existing medical device regulatory pathway in the USA. Oxford Performance Materials (OPM) for example, has received 510(k) clearances for two 3D-printed devices over the past 2 years; the OsteoFab Patient-Specific Cranial Device (cleared in February 2013) and the OsteoFab Patient-Specific Facial Device (cleared in July 2014). That the predicate devices had not been manufactured using

3D-printing did not prevent the FDA from determining that the new devices were 'substantially equivalent' to the predicate devices.

This shows that currently there is scope for FDA approval of this technology while retaining the ability to create patient-specific devices without individual approval of each item produced. Providing the processes can be validated and the specified CQAs and performance criteria are conserved, this seems to fit 3D-printing as a method for mass production of customized devices rather than for production of custom-made devices [51]. In the USA, it therefore appears that devices constructed using 3D-printing technology are currently subject to the same regulatory review standards as devices constructed using traditional formative or subtractive manufacturing practices, in other words, based on the equivalence of properties when considered as an 'engineering material'. However, the FDA acknowledges that the formal complexity of these 3D-printed products is pushing the boundaries of current regulatory practice and that further clarity on safety and quality control issues will be required to match advances in the 3D-printing technology and its application to higher risk product categories.

Regulatory considerations for use of 3D-bioprinting technology to manufacture customizable tissue-engineered combination products

As defined, the regulatory routes for tissue engineered combination products are complex but relatively well defined (Figure 2). Howver, they do not address the differences between products (or their structural parts) manufactured using 3D-printing technology and those manufactured using conventional manufacturing techniques. The use of 3D-bioprinting technology and the customizable nature of these 3D functional living constructs impose constraints on the chemistry, manufacturing and control (CMC) elements of their development process from design to manufacture and add considerable challenges for product quality assurance and testing [1,22,50]. Taking the perspective of a manufacturer making both constituent parts in the same production facility, the specific areas where existing EU and USA regulations raise challenges for manufacturers of customized autologous 3D-bioprinted tissue engineered products are summarized in Table 1.

The regulatory challenges that surround the adaptive manufacturing processing and characterization of the autologous cellular component are common to all combination products containing engineered cells. 3D-printing will further challenge these already contested requirements for autologous testing [47,48]. In considering 3D co-printing of the nasal implant, the different elements within the computer-aided design to manufacture chain identify where the specific product and process related quality and safety expectations differ compared with the situation where device part is conventionally manufactured or preformed before seeding with living cells. As illustrated in Figure 3, the main differences lie in software system chain control and the ability to manage and test multiparametric and customizable features of the patient-matched product ('batch of one') as they relate to potential variation in the CQAs of the product and the impact that these and their interactions may have on product performance after surgery. In the following section, we examine whether these differences raise new regulatory science questions when tested against current and emerging regulatory frameworks in the EU and the USA.

Specific issues & requirements for process validation & control

The customizable nature of 3D-bioprinted products means that it is crucial to build a control strategy that relies not only on end product testing but also on control of starting and raw materials, design control and manufacturing process validation (and associated controlling software). This will likely require multiparametric and risk-based approaches to evaluate the sources of error or failure and the relationships between process and build parameters and the final product CQA's. This will help establish the critical process parameter limits and controls that should be monitored during the production and that when validated will provide assurance of the product's design and build specification at release.

Construction of an accurate solid 3D-printed model: specific issues & requirements for validating the software system chain

The anatomically matched 3D product is designed and built from CAD and CAM models derived from CT scans or MRI data using a variety of printing technologies (Figure 1). This defines the software system chain that drives each step in the model construction; the software algorithms used to acquire the image of the patient's anatomy, the software algorithms that analyze and transform (segment out) the image data into an accurate digital 3D model, the software algorithms that approximate the solid and surface representations of the segmented model and the software that applies the base model (predefined programming methods, setups, machining operations and tool selections), updates the customization features of the implant according to the surgeon's specification and drives the 3D printer system that builds the 3D-printed product layer by layer.

The challenge from a regulatory perspective is to design and create optimized microstructures within anatomic geometries that can be manufactured and individually customized in a reproducible and controllable manner so that finished product can be verified to meet design control requirements under medical device regulations [52]. The interoperability and capability of the software system chain that drives each step therefore needs to be sufficiently robust to reproduce accurate models that can be transfigured to achieve the specified form, function, formation and fixation characteristics in the final patient-matched product [46]. However, there are multiple translation and compilation steps between multiple parameterized software applications, each with different error sensitivities. The accuracy of the model may be compromised by errors or geometric distortions introduced during each of the building steps. These may be due to the selection of nonoptimal parameters within them, the presence of image artifacts, microstructure resolution limitations of the printing technology and/or additional errors arising from microporosity and anisotropy in the microstructure features introduced during the manufacturing process [52,53]. Beyond these purely geometric issues, the propagation of these errors has potential consequences for the mechanical strength of the finished product, the compatibility of the bioprocesses that occur within it, for example, cell ingrowth and mass transport and even the maintenance of sterility. This can lead to differences between the manufactured and the designed scaffold.

In terms of image acquisition, the manufacturer will need to verify the accuracy and resolution of the anatomic image, accounting for the effects of image artifacts and the potential variation between scanners. For the digital design, including the dimensional (geometric shape and its feature) and technical specification (tolerance, surface finish, etc.), there may be significant regulatory issues related to the CAD-CAM step and the use of manual or computer-aided process planning (CAPP) to translate the design information into the process steps and instructions (operation sequence, machining parameters, tool setup, QA checkpoints, etc.) to manufacture the product. While CAPP systems are moving toward being generative, they still require large amounts of human expertise and manual interaction depending on the complexity of the product being produced. This applies to the nasal implant where the interactions between surface and solid representations will affect the complexity of the decision rules for process planning, particularly those that demand both automated and operator design decisions in the customization of the base model to fit the device to the patient.

The manufacturer will need to verify that the printer controlling software can accurately translate the print parameters into the final fabricated product (Figure 3). In this regard, optimal design approaches can be applied to attain scaffolds with a controlled microstructure that have for example, the physical properties that balance the often conflicting requirements for mechanical rigidity and cell in-growth and maintenance that are needed to support regeneration (remodeling and bioresorption *in vivo*) at the implant site, while also enabling the flexibility for surgical handling. Using 3D-printed prototypes, these computational modeling and simulation approaches can be applied to investigate how the design to manufacture chain affects the reproducibility of the optimized design characteristics in the final manufactured product. For validation purposes (software and hardware), being able to relate the design parameters and feature sizes from the optimized design to errors, flaws and discrepancies propagated in the final manufactured structure will be crucial to defining appropriate design limits and tolerances, as well as in-process and quality controls [52]. This is likely to require advances in existing computational design techniques to gain greater control over the topographical features of the scaffold component. Beyond this, unless the 3D-printing parameters can be locked in or appropriate nondestructive metrology tools are available, the manufacturer may need to run a standardized test part or analog (with or without surrogate cells) to verify that changes, for example to the bioprinting machine (or to different machines), the material parameters, the interoperability of different component software versions in the system chain or even the operator, do not adversely affect the CQAs of the finished product.

Specific issues & requirements for assuring biocompatibility

Complex 3D geometries and open access microstructures enabled by 3D-printing create challenges to both biocompatibility and sterility. Even if the selected 3D-printing material is 'clinically approved' as part of a market approved or CE marked device, the manufacturer needs to establish its compatibility with biological materials and the printing process [1]. This requires an understanding of the effect of the bioprinting process on the material as even small changes in the chemical, physical (structure and degradation) and mechanical properties can affect the integrity and biocompatibility of the structural component and ultimately the performance of the finished product when implanted [54].

Equally important are the effects that any product or process-related impurities (extractables and leachables)

Table 1. Specific areas within the design to manufacture chain where existing EU and USA regulations raise issues for manufacturers of customized autologous 3D-bioprinted (co-printed) tissue engineered products. Represents the perspective for a manufacturer making both constituent parts in the same production facility.

Specific challenge area	Issues
Bridging the regulatory gaps in the development pathway	
Combination Product Regulations in the EU and USA	Lack of harmonization. Different regulatory approval approaches and demands in the USA and the EU may increase regulatory burden for manufacturers selling into both markets
	Increased compliance costs for quality system implementation, need for highly specialized internal skill sets and additional post-marketing requirements for pharmacovigilance and comparability assessment especially in the EU
	Lack of clarity on how the regulatory inputs are aligned under current delineation of regulations in EU. Separate regulatory assessment of medical device and advanced therapy medicinal product in EU may create duplication and redundancy
	Changes to the manufacturing process (or post-process) or product composition may change the way in which these products might be classified and thus the legislation into which the product fits, raising questions of borderline status
	Limited specific scientific guidelines/standards currently available in EU compared with the USA
	Persistent issues underpinning regulatory product classification associated with a poor understanding of possibly multiple modes of action that contribute to the intended therapeutic effect
	Current EU medical device regulations may result in interpretative disparities between the notified bodies in terms of how devices may be classified, raising questions of borderline status
	Little clarity on the grounds on which the CAT/CHMP consider evidence of conformity of the medical device part sufficient or relevant, particularly where materials have been previously deployed in CE marked devices
	Uncertainty about the potential impact of the current proposals to amend the medical devices Directives in the EU
	Uncertainty as to what extent the surgeon may modify the implant in the clinic. Where does product regulation end and medical practice begin?
	Inherent difficulties in conducting clinical trials for customized products and involving a surgical intervention. How to employ a surgical comparator?
	Current regulatory framework gives incentives to manufacturers to use medical devices already licensed rather than developing new and better targeted devices
Translating 3D-bioprinting to biofabricated product	
3D printing technology	Currently no distinct legislation, clear direction or guidance from regulators regarding the approval of medicinal products manufactured using 3D-printing technology
	Differences between the manufactured and the designed scaffold may be introduced by the manufacturing process itself and due to resolution limitations of the technology
	Interactions with process raw materials and cleaning materials applied to the printer may lead to undesirable surface modification
Software, process control and validation	Model accuracy may be compromised due to the propagation of errors through multiple translation and compilation steps between multiple parameterized software applications in order to generate to a process plan and consequently a customized product
	Uncertainty about where in system chain software validation is required
	Interoperability of different component software versions is uncertain and raises questions on re-validation requirements

Table 1. Specific areas within the design to manufacture chain where existing EU and USA regulations raise issues for manufacturers of customized autologous 3D-bioprinted (co-printed) tissue engineered products. Represents the perspective for a manufacturer making both constituent parts in the same production facility (cont.).

Specific challenge area	Issues
	Potential for errors and discrepancies in the design input translation related to the computer-aided design-computer-aided manufacturing step and the complexity of automated and operator decision rules used in the customization of the base model
	Limitations of current computational modeling can lead to suboptimal designs and difficulties in defining appropriate design limits and tolerances. This has implications for verifying that the finished product meets the design inputs
Product and process testing and validation	
Chemistry, manufacturing and control (CMC) testing of the scaffold component	Unless printed analogs (with or without surrogate cells) can be used, co-printed scaffolds can only be tested as part of the intermediate and final combination product
	Not possible to remove product or process related leachable particles or extractables by conventional cleaning or extraction approaches. Biocompatibility testing may require new scientific and regulatory approaches
	Potential changes to the chemical, physical and mechanical properties of the device material and its interaction/compatibility with the cellular part due to the bioprinting process itself
	Traditional end sterilization techniques cannot be applied
CMC testing of cells prior to assembly	Application of standard cell therapy characterization and test methods is limited by time constraints and the amount of material available in the autologous setting
	Impact of donor-derived variability on the cell culture manufacturing process and availability of seed pool for co-printing
CMC testing of the cell-scaffold construct	Limited validated methods for characterization and imaging of cells within the intact construct, both *in vitro* and *in situ* after implantation
	Full characterization and testing of the final product is hindered by time and sampling constraints related to lot size ('batch of one') and potential for manufacture on demand
	Construct may not be in its final form when administered to patients as *in vivo* remodeling can occur. In this case, any final product specifications determined through *in vitro* testing may not be predictive of clinical safety and efficacy
	Difficult to define potency/performance requirements and specifications because they will be specific to the patient and the intended use likely to involve multiple modes of action
	May not be possible to sample and detect adventitious agents that may be retained deep within potentially complex internal porous microstructures
	Lack of appropriately validated nondestructive metrology tools and standards to address the inspection of physical, geometric and mechanical properties that are specific to the product type and its intended use
	Increased production costs attributed to the requirement for multiple levels quality control throughout the manufacturing process

or degradation products (e.g., generated in-process due to the effects of heat, shear, inadequate line clearance or hold times) have on the cell component during manufacture, or post-processing or after implantation. If the combination product is manufactured as a single entity, whereby live cells are co-printed with a biodegradable synthetic polymer rather than seeded into a preprinted scaffold, it is not possible to remove leachable particles or organics by conventional cleaning or extraction approaches. Assurance of biocompatibility may therefore take substantial additional resources to address unless applicable data have been established for use of the material in other approved medical devices, for example, PCL has a history of use in long-term implantable devices [1].

The manufacturer will need to assess the risk of the presence of harmful or toxic impurities and provide assurance that either the rate or total amount of leaching is at an acceptable level once implanted. Some conventional toxicity testing of raw materials and of the device part alone, using exhaustive extraction approaches as described in ISO 10993 [55] for example, may be necessary before beginning preclinical studies. According to Hollister and Mur-

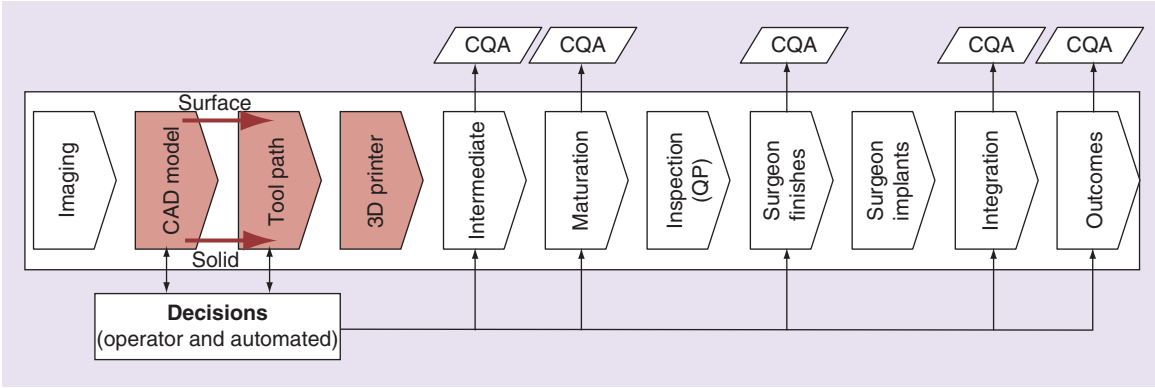

Figure 3. Simplified diagram of the design to manufacture chain for the production of the 3D co-printed nasal implant product. Indicates the key decision processes (highlighted in red) that influence the solid and surface representations of the 3D model and introduce downstream risk points (arrows) to the production of the finished product. The decision processes and associated risk points indicate the processing and post-processing activities of concern to product quality and safety from a regulatory perspective.
CAD: Computer-aided design; CQA: Critical to quality attribute; QP: Qualified person.

phy, these tests could cost between US$0.5 and 3 million [46]. Otherwise, testing may have to rely on the long-term biocompatibility studies to detect the effects of the structural component on cellular behavior and survival (e.g., cell adhesion studies, growth studies) and assess the potential for an adverse biological reaction to occur as a result of exposure to the material once the final product is implanted, perhaps via a conditional adaptive or accelerated licensing process.

Specific issues & requirements for assuring sterility

The control strategy aims to show the ability to reproducibly manufacture a safe product that is sterile and free of unwanted contaminants. Where it is not possible to sterilize the end product, sterility assurance processes become a function of the starting and raw materials, the cleaning processes applied to the 3D-printing machine between runs, the solid and surface microstructures within the geometries of the finished product and environmental conditions in which the bioprinting and any post-processing takes place.

In the case of the nasal implant, like all cell-based products the maintenance of sterility is important throughout the entire process. Of particular concern would be the ability to sample and detect adventitious agents that may be retained deep within potentially complex internal porous microstructures, which may not be reachable by the immune system and that could be released as the material degrades *in vivo*. The manufacturer may need to apply conventional risk-based bracketing designs to sterility testing, such that analogs of the device part can be tested at the minimum and maximum tolerance limits for features such as internal and surface porosity or other critical design parameters, accounting also for *in vivo* biofilm forming capability which may create routes of infection once implanted [50].

Specific issues & requirements for product validation & release: how much testing is required?

When considering design control, validation and release testing for products that are both patient-matched ('batches of one') and made potentially on demand, complete characterization of the finished product that may or may not (where *in vivo* remodeling can occur) be in its final form when implanted into the patient is unlikely to be possible. Beyond optimal design for manufacturing approaches, this places greater emphasis on more extensive preclinical and *in vivo* safety testing, in-process controls and the use of other adaptive approaches to providing sufficient information on risk versus benefit.

Broadly, there are three groups of metrics that will likely define the registered specification on which market authorization for these types of customized implants will be based. These relate to the bulk properties, the surface properties and the critical dimensions of the product. Sufficient evidence will be needed to verify that the final product will perform to the required standards within the range that these metrics may cover alone or in combination. In the case of the prosthetic nose for example, the following physical and patient-matched parameters are some of the critical metrics that may serve as a basis for a testing and validation strategy:

- Bulk properties: the infrastructure, whether homo- or heterogeneous, will need to be validated for the mass transport necessary for cell maintenance and

remodeling rates *in vivo* in order to ensure structural integrity and long-term patient outcomes. This will require the solid mechanics that describe how the scaffold construct responds to the stresses associated with the product's intended use compared with the native tissue it aims to replace. For example, considering that tissue engineered nasal structures are prone to postoperative contraction and may even develop contractile behavior during the *in vitro* maturation process, it will be important to evaluate the influence of the fabrication process on the stability of the intermediate and final tissue engineered implants;

- Surface properties: edge effects that may occur due to the printing technique may need to be either corrected or allowed for in manufacturing or preimplant finishing activities by the surgeon. The latter requires assurance that activities in the clinic do not compromise the interface between patient and prosthetic, taking into account the number, location and local properties of any specific regions that are built into the prosthetic design solely to provide anchor points or eyelets for surgical fixation. This raises the question as to what extent the surgeon may modify the implant in clinic? The postimplant environment must be conducive to integration if necrosis is to be avoided. Consequently, the preimplant finishing activities will need to occur within a prescribed scope if the prosthetic performance is to be maintained;

- Critical dimensions: there will be a limit to the dimensions over which perfusion and tissue migration can occur without being explicitly addressed by the design of the nasal implant, for example, by the provision of blood flow or angiogenesis. These will need to be established, in particular the limits to effective vascularization must be determined as a function of distance for the prosthetic bulk properties, accounting for variations that may occur due to the patient's health and age.

With many different physical and patient-matched design parameters and permutations, approaches to defining product and process validation strategies may need to rely on improved multiparametric computational modeling and simulation methodologies, applied to enhance process knowledge and image analysis for example. Sampling and quality inspection routines, to support parametric release, for example, are likely to require the manufacture of extra predefined analog units of known dimension, void volume and other critical physical features that can be tested within statistically designed bracketing and matrixing test regimes. Even at the dimensional level, such inspection methods, as well as those applied within process and to the finished product, will need to be underpinned by the availability of applicable and validated nondestructive test systems (from the perspective of both constituent parts) and the development of associated industry standards and guidance. Despite recent progress [56], this is likely to require further advances in the development of validated methods for characterization, tracking and imaging of cells within the intact construct, both *in vitro* and *in situ* after implantation.

Conclusion

This paper highlights the importance of defining the characteristics and features of the development process and the finished product to identifying and understanding where the regulatory legislation might apply across each stage of the development pathway. Careful examination of the existing regulatory paths in the EU and the USA emphasizes that combining synthetic scaffolds with cultured human cells creates a combination medicinal product that will command a high level of regulatory scrutiny, particularly for the manufacturer that makes both constituent parts. Despite recent success in bringing 3D-printed medical devices to clinical use, the existing regulatory frameworks do not adequately account for aspects related to the use of computer-aided 3D-bioprinting as an industrial manufacturing process with the ability to create individual customized tissue engineered combination products using mechanized rather than craft approaches, which are typically easier to validate. In considering the hypothetical exemplar of a customized, autologous 3D-printed combination product, the major regulatory concerns and hence the challenges for the manufacturer are likely to resonate most in the process from design to manufacture, in terms of the software system chain control and validation, the potential variation in CQAs of the final manufactured product, such as mechanical stiffness and strength, biocompatibility and sterility and the consequent impact on product performance after surgery.

Beyond the scientific and technical challenges [1], if 3D-bioprinting is to achieve its promissory vision and expectations, new and proportionate regulatory science approaches are likely be required to address concerns that surround aspects related to how these customizable product types can be tested and validated. In the meantime, with little or no scientific guidance available, if the field is to move forward the research and development community will need early and regular dialogue with regulatory agencies at particular translational bottlenecks in the manufacturing and quality development process. We anticipate that key areas that will require focus are product and process characterization,

in particular validation that the printing process plan has delivered a consistent and viable tissue.

Future perspective

In the EU, the regulatory ambiguity in Regenerative Medicine significantly impacted the field until the implementation of the regulation on ATMPs (Regulation (EC) No 1394/2007) by the European Commission at the beginning of 2009. Along with impending changes to the medical device directive and revision to the EU clinical trials framework in 2016, further refinement can be expected in the ATMP Regulation in the near future. In the USA, with medical device reforms and clarification of the regulations for combination products under the 21st Century Cures legislation, the FDA is also expected to release further guidance for combination products and for 3D printing. These changes are likely to address a number of regulatory gaps that exist for combination products but it is unclear whether they will sufficiently address some of the shortcomings the industry is facing when it comes to 3D bioprinting of customizable combination products or how the regulations will differ between the two regions.

Linked to the current FDA activity in the USA, there is a requirement to escalate the need for proportionate regulation of medicinal products that are customizable to individuals using an 'industrial process' that has a significant software component. If expectations around the 3D-bioprinting field are to materialize, action needs to take account of the momentum of clinical demand and the application ambitions in the field. New value and business models are likely to emerge linked to the need for different organizational and development models and for new medical professionals (next-generation prosthetists) to bridge the regulatory gap for customized combination product manufacturers.

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Executive summary

Regulation of cell-scaffold products under existing frameworks

- The classification of the product defines the regulatory route in the USA and the EU. Where living cells are physically combined with a synthetic scaffold matrix to create a finished medicinal product, the product can be classified as a combination product: if the cells are viable; if the cells have been substantially or more than minimally manipulated; if the cells do not carry out the same function in the recipient as the donor; if the scaffold is an integral part of the finished product; if the scaffold meets the definition of a medical device.
- As defined, where the primary or principal mode of action is provided by the cellular constituent part, these combination products are regulated as biologics in the USA and as a Combined Tissue Engineered advanced therapy medicinal products in the EU (if mode of action is regeneration and repair of a human tissue).
- Unlike in the EU, in USA combination products are regulated as a distinct product category and the US FDA have issued specific rules and guidance that streamlines the regulatory route for Good Manufacturing Practice, adverse event reporting and for making postmarket approval changes

Regulation of customizable tissue engineered combination products manufactured using 3D-bioprinting technology

- As defined, the existing regulatory frameworks do not account for aspects related to customization or to differences between products manufactured using 3D-printing technology and those where the device part is manufactured conventionally.
- The main difference between customized implants co-printed with living cells and tissue engineered constructs where the device part (scaffold) is preformed prior to seeding with living cells lies in the computer-aided design to manufacture process. Specific regulatory challenges relate to software system chain control and the ability to manage and test multiparametric and customizable features of the product as they relate to potential variation in critical to quality attributes of the product and the impact that these and their interactions may have on product performance after surgery.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat. Biotechnol.* 32(8), 773–785 (2014).

•• **Comprehensive review of the application of 3D bioprinting to tissue and organ engineering. Describes the main strategies for printing tissue constructs, the different types of bioprinters and limitations of current technologies.**

2 Ventola CL. Medical applications for 3D printing: current and projected uses. *Pharm. Ther.* 39(10), 704–711 (2014).

3 Morsi YS, Wong CS, Patel SS. Virtual prototyping of biomanufacturing in medical applications. In: *Virtual Prototyping & Bio Manufacturing in Medical Applications.* Bidanda B, Bartolo PJ, (Eds). Spinger, USA, 129–148 (2008).

4 Bartolo P, Kruth J-P, Silva J *et al.* Biomedical production of implants by additive electro-chemical and physical processes. *CIRP Annals – Manufacturing Technology.* 61(2), 635–655 (2012).

5 US FDA. 21 CFR Part 3. Product jurisdiction (1999). www.gpo.gov/fdsys

6 Commission Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (Consolidated version: 16/11/2012). Official Journal of the European Union. L0083, 1–188 (2012). http://ec.europa.eu/health

•• **Consolidated version comprises the main legal framework for cell-based medicinal products in the EU. Contains specific requirements for advanced therapy medicinal products containing devices.**

7 Commission Directive 2007/47/EC of the European Parliament and of the Council amending Council Directive 90/385/EEC on the approximation of the laws of the Member States relating to active implantable medical devices, Council Directive 93/42/EEC concerning medical devices and Directive 98/8/EC concerning the placing of biocidal products on the market. Official Journal of the European Union. L247, 21–55 (2007). http://eur-lex.europa.eu/LexUriServ

•• **The amending medical device legislation comprises the main legal framework for medical devices in the EU.**

8 European Commission. Manual on Borderline and Classification in the Community Regulatory Framework for Medical Devices, MEDDEV Version 1.16, 1–67 (2014). http://ec.europa.eu/growth/sectors

9 Committee for Advanced Therapies (CAT). Reflection Paper on Classification of Advanced Therapy Medicinal Products, EMA/CAT/600280/1–16 (2012). www.ema.europa.eu/docs/en_GB

10 US FDA. Title 21 CFR Part 1271. Human cells, tissues, cellular and tissue-based products (2014). www.accessdata.fda.gov/scripts

•• **Comprises the main legal framework for cell-based medicinal products that are regulated as biologics (351 HCT/P) in the USA.**

11 Gao Y, Liu S, Huang J *et al.* The ECM–cell interaction of cartilage extracellular matrix on chondrocytes. *BioMed Res. Int.* 2014, Article ID 648459 (2014).

12 Committee for Human Medicinal Product (CHMP). Guideline on human cell-based medicinal products, EMEA/CHMP/410869/1–25 (2008). www.ema.europa.eu/docs/en_GB

13 US FDA. Guidance for Industry and Staff. Early development considerations for innovative combination products, 1–17 (2006). www.fda.gov/downloads/RegulatoryInformation

14 Pimpaneau V, Poiseau AD. Designing a CMC strategy for advanced therapy medicinal products in the EU. Scrip Regulatory Affairs, 6–9 (2012). http://voisinconsulting.com/sites

15 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 (Consolidated version: 02/07/2012). *Offic. J. Eur. Union*. L324/121, 1–17 (2012). http://ec.europa.eu/health/documents

•• **Consolidated version comprises the main legal framework for cell-based medicinal products that are regulated as advanced therapy medical products in the EU.**

16 European Council Directive 90/385/EEC of 20 June 1990 on approximation of the laws of Member States relating to active implantable medical devices. *Offic. J. Eur. Union* L189, 17–36 (1990).

17 European Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. *Offic. J. Eur. Union* L169, 1–43 (1993).

18 Committee for Advanced Therapies (CAT). Procedural advice on the provision of scientific recommendation on classification of advanced therapy medicinal products in accordance with article 17 of regulation (EC) no 1394/2007, EMA/CAT/99623/2009 Rev.1 (2013). www.ema.europa.eu/docs/en_GB

19 Committee for Advanced Therapies (CAT). Procedural advice on the evaluation of combined advanced therapy medicinal products and the consultation of Notified Bodies in accordance with Article 9 of Regulation (EC) No. 1394/2007, EMA/354785/2010. 1–15 (2011). www.ema.europa.eu/docs/en_GB

20 Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency (Consolidated version: 05/06/2013). *Offic. J. Eur. Union*. L136, 1–70 (2013). http://ec.europa.eu/health/files

21 PAS 83:2012. Developing human cells for clinical applications in the European Union and the United States of America – Guide. (British Standards Institution 2012). http://shop.bsigroup.com/en/ProductDetail

• **Focused on cell-based medicinal products, this Publicly Available Specification provides a guide to point users in the**

direction of the relevant EU and USA legislation, guidance documents, pharmacopoeial chapters and monographs.

22 Lee MH, Arcidiacono JA, Anastacia MS *et al.* Considerations for tissue-engineered and regenerative medicine product development prior to clinical trials in the United States. *Tissue Eng. Pt B.* 16(1), 41–54 (2010).

23 EMA. Good Pharmacovigilance Practice Guidelines. www.ema.europa.eu/ema

24 European Commission. Eudralex Volume 9A of the rules governing medicinal products in the European Union. Guidelines on Pharmacovigilance for Medicinal Products for Human Use. 1–229 (2008). http://ec.europa.eu/health/files

25 US FDA. Title 21 CFR Part 600. Biological products: General (2014). www.accessdata.fda.gov/scripts

26 US FDA. Title 21 CFR Part 601. Licensing (2014). www.accessdata.fda.gov/scripts

27 US FDA. Title 21 CFR Part 610. General Biologics Standards (2014). www.accessdata.fda.gov/scripts

28 US FDA. Title 21 CFR Part 4. Current Good Manufacturing Practice Requirements for Combination Products (2013). www.gpo.gov/fdsys/pkg

29 US FDA. Guidance for Industry and FDA Staff: Current Good Manufacturing Practice Requirements for Combination Products, Draft Guidance, 1–46 (2015). www.fda.gov/downloads

30 US FDA. Title 21 CFR Part 210. Current Good Manufacturing Practice in manufacturing, processing, packing and holding of drugs (2014). www.accessdata.fda.gov/scripts

31 US FDA. Title 21 CFR Part 211. Current Good Manufacturing Practice for finished pharmaceuticals (2014). www.accessdata.fda.gov/scripts

32 US FDA. Title 21 CFR Part 820. Quality System Regulation (2014). www.accessdata.fda.gov/scripts

33 US FDA. Guidance for Industry. Current Good Tissue Practice (cGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) (2011). www.fda.gov/downloads

34 International Organization for Standardization. International Standard ISO 13485 Medical devices – Quality Management Systems – Requirements for Regulatory Purposes. ISO copyright office, Geneva, Switzerland, 1–57 (2003). http://sic.com.ua/wp-content

35 Commission Directive 2003/94/EC of 8 October 2003 laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. Official Journal of the European Union. L262, 22–26 (2003). http://ec.europa.eu/health/documents

36 European Commission. EudraLex - Volume 4 Good manufacturing practice (GMP) Guidelines. http://ec.europa.eu/health/documents

37 US FDA. Proposed Rule: Post-marketing Safety Reporting for Combination Products (74 FR 50744) (2009). www.gpo.gov/fdsys/pkg

38 US FDA. Title 21 CFR Part 314. Applications for FDA approval to market a new drug (2014) www.accessdata.fda.gov/scripts

39 US FDA. Title 21 CFR Part 803. Medical Device Reporting (2014). www.accessdata.fda.gov/scripts

40 Commission Directive 2012/26/EU of the European Parliament and of the Council of 25 October 2012 amending Directive 2001/83/EC, as regards pharmacovigilance, 1–4 (2012). http://ec.europa.eu/health

41 Regulation (EU) No 1027/2012 of the European Parliament and of the Council of 25 October 2012 amending Regulation (EC) No 6726/2004, as regards pharmacovigilance, 1–3 (2012). http://ec.europa.eu/health

42 European Commission Guidelines on a medical device vigilance system. MEDDEV 2.12/1 (2013). http://ec.europa.eu/health

43 European Commission Proposal for a Regulation of the European Parliament and of the Council on medical devices, and amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/1–194 (2012). http://ec.europa.eu/growth

44 US FDA. Guideline: Submissions for Post-approval Modifications to a Combination Product Approved under a BLA, NDA, or PMA, 1–13 (2013). www.fda.gov/downloads/RegulatoryInformation

45 Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products (Consolidated version: 2.11.2012). *Offic. J. Eur. Union*. L334, 1–32 (2012). http://ec.europa.eu/health/files

46 Hollister SJ, Murphy WL. Scaffold translation: Barriers between concept and clinic. *Tissue Eng. Pt B.* 17(6), 459–473 (2011)

47 Report from the Commission to the European Parliament and the Council in accordance with Article 25 of Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/1–14 (2014) (2004). http://ec.europa.eu/health/files/advtherapies

48 European Commission. Regulation (EC) No. 1394/2007 on Advanced Therapy Medicinal Products: Summary of the responses to the public consultation. 1–8, (2013). http://ec.europa.eu/health/files/advtherapies

49 US FDA. Personalised Medicine Paving the Way for Personalized Medicine: FDA's Role in a New Era of Medical

Product Development. 1–61 (2013). www.fda.gov/downloads/ScienceResearch

50 US FDA. Public Workshop transcripts. Additive Manufacturing of Medical Devices: An Interactive Discussion on the Technical Considerations of 3D Printing, October 8–9 (2014). www.fda.gov/MedicalDevices/NewsEvents

51 Woodlee D. Understanding the US FDA's custom device exemption. *J. Med. Dev. Regulation* 8(3), 3–11 (2011)

52 Coelho PG, Hollister SJ, Flanagan CL, Fernandes PR. Bioresorbable scaffolds for bone tissue engineering: optimal design, fabrication, mechanical testing and scale-size effects analysis. *Med. Eng. Phys.* 37, 287–296 (2015).

53 Pinto JM, Arrieta C, Andia ME *et al.* Sensitivity analysis of geometric errors in additive manufacturing medical models. *Med. Eng. Phys.* 37, 328–334 (2015).

54 Pink J. Risks of Additive Manufacturing: a Product Safety Perspective. European Medical Device Technology (2015). www.emdt.co.uk/daily-buzz

55 International Organization for Standardization. International Standard ISO 10993–10991 Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing Within a Risk Management Process. ISO copyright office, Geneva, Switzerland, 1–21 (2009).

56 Harrison RH, St-Pierre J-P, Stevens MM. Tissue engineering and regenerative medicine: a year in review. *Tissue Eng. Pt B.* 20(1), 1–16 (2014).

57 European Commission - Eudralex. http://ec.europa.eu/health

58 US FDA. www.accessdata.fda.gov