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Pre-clinical safety assessment of Tissue Engineered Medical Products (TEMPs)

-An investigation on assays and guidelines for biocompatibility testing-

E.A.E. van Tienhoven, R.E. Geertsma, C. Wassenaar, W.H. de Jong

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RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

Abstract

Tissue engineering aims at the development of biological products that repair, regenerate or replace tissues and/or organs. These products, here referred to as Tissue Engineered Medical Products (TEMPs), can consist of a combination of living cells, natural or synthetic materials and biomolecules. At the moment TEMPs do not fall under any particular Dutch or European legislation. However, with the increasing number of products, the need for consistent guarantees of safety is increasing. Risks related to product use should, before clinical application, be identified on the basis of experience and scientific concepts. This study has focused on risks related to biocompatibility, shown to be among the major risks of implantable products. As TEMPs consist of different components, hazards related to these components as well as to interactions of the components themselves should taken into consideration. This includes toxicity of the final product, uncontrolled cell proliferation and differentiation, uncontrolled material degradation, and immunogenicity of biomolecules. At the moment, there is a lack of validated pre-clinical assays to evaluate the hazards in TEMPs. An entire safety evaluation based on pre-clinical assays is, however, limited due to the biological variation and the species-specific activity of the various TEMP components. Requirements for a safety evaluation can be laid down in standards and guidelines. While safety evaluation of the separate components is partly covered by a variety of documents, guidelines and standards, this evaluation of the final product - consisting of materials, cells and biomolecules – has not (yet) received this coverage. In conclusion, knowledge obtained from transplantations, and the production of medical devices and medicinal products, should be combined, both to develop validated assays and to initiate standardisation procedures to control the safety of TEMPs.

Preface

This report describes biocompatibility aspects relevant for safety evaluation of a group of medical products that combine viable cellular with non-cellular components. These products are intended to replace, repair or regenerate tissues or organs with failing functions.

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Contents

Abbreviations

AATB	American Association of Tissue Banks
ASTM	American Society for Testing and Materials
CBER	Center for Biologics Evaluation and Research
CEN	European Committee for Standardization
CHeF	CEN Healthcare Forum
CPMP	Committee for Proprietary Medicinal Products
EATB	European Association of Tissue Banks
ELISA	Enzyme Linked Immunosorbent Assay
EFI	European Federation of Immunogenetics
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HLA	Human Leukocyte Antigens
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ISO	International Organization for Standardization
OECD	Organisation for Economic Co-operation and Development
rt-PCR	reverse transcript-Polymerase Chain Reaction
TEMP	Tissue Engineered Medical Product
TGA	Therapeutic Goods Administration (Australia)
WHO	World Health Organization

Samenvatting

In de afgelopen decennia is er steeds beter begrip gekomen over de manier waarop cellen elkaar beïnvloeden, de werking van groeifactoren, hoe biomaterialen zich in het lichaam gedragen en hoe stoffen als lichaamseigen of lichaamsvreemd worden herkend. Met deze kennis zijn verschillende vakgebieden, waaronder biochemie, celbiologie, moleculaire biologie en materiaalkunde, in staat gebleken om biologische producten te genereren die weefsel en/of organen kunnen repareren, regenereren of vervangen. Deze benadering wordt aangeduid met het begrip "Tissue Engineering". Op het ogenblik is er een groeiend aantal toepassingsgebieden, waaronder huidtransplantatie bij chronische ulcera en bij kleine brandwonden, vervanging van bot en/of kraakbeen en herstel van weefsels die hormonen en enzymen produceren. De producten bestaan vaak uit een combinatie van levende cellen, biomaterialen en biomoleculen. Deze producten worden ook wel Tissue Engineered Medical Products (TEMPs) genoemd.

De combinatie van levende cellen en materialen genereert producten die op dit moment (nog) niet onder Nederlandse of Europese wetgeving vallen. Deze producten zijn uitgesloten van de medische hulpmiddelen richtlijn (93/42/EEC). Verder wordt er op Europees niveau gedebatteerd of de producten vallen onder de definitie van geneesmiddelen (richtlijn 65/65/EEC).
Onafhankelijk van de wijze van regulatie is het belangrijk dat risico's die verbonden zijn aan het medisch gebruik van deze nieuwe categorie producten geïdentificeerd en geëvalueerd worden. Deze evaluatie dient voor elk nieuw product plaats te vinden voordat met de eerste klinische studies wordt aangevangen. Belangrijke algemene risico's die verbonden zijn aan klinische toepassing van TEMPs liggen op de terreinen van ziekteoverdracht van donor naar ontvanger, biocompatibiliteit en gebrek aan klinische effectiviteit. Echter, doordat TEMPs relatief nieuw zijn, zijn deze terreinen nog niet duidelijk in kaart gebracht.

Met dit rapport hebben we geprobeerd om meer inzicht te geven in een van de bovengenoemde algemene risico's, namelijk die op het terrein van de biocompatibiliteit. Biocompatibiliteit kan omschreven worden als de mogelijkheid van een product om optimaal te presteren met de juiste gastheer respons. Vele karakteristieken van niet alleen het product, maar ook de biologische respons van de gastheer hebben dan ook invloed op de biocompatibiliteit van het product. Om tot een goed overzicht te komen van deze risico's is er gekozen om eerst een theoretisch overzicht te geven van de risico's die verbonden zijn aan de individuele componenten, dus aan levende cellen, biomaterialen en biomoleculen. Belangrijke gevaren ontstaan uit de manipulatie van de levende cellen (met name ongecontroleerde celgroei en differentiatie), de aanwezigheid van degradeerbare materialen (met name schadelijke degradatieproducten) en de dosering van biologische moleculen (met name immuunsysteem-activatie).
Het is echter niet voldoende om uitsluitend de risico's die verbonden zijn aan de individuele componenten te evalueren. Er bestaan complexe interacties tussen de verschillende componenten en deze interacties moeten dan ook betrokken worden in de risico-identificatie. Hier gaat het dan voornamelijk om de invloed van biomaterialen op celkarakteristieken en op de afgifte van biomoleculen.

Na de theoretische beschrijving van risico's moet de daadwerkelijke aanwezigheid en de grootte van de risico's bepaald worden. Dit kan door het uitvoeren van preklinische testen. Ook hier geldt dat voor de individuele componenten testen beschikbaar en gevalideerd zijn. Voor *in vitro* en *in vivo* testen die toepasbaar zijn op het eindproduct is dit veel minder het geval. Het ontwikkelen en valideren van deze testen is noodzakelijk om tot gedegen veiligheids-evaluaties te komen. De keuze van relevante *in vivo* testen wordt bemoeilijkt doordat de humane cellen in de diermodellen een xenogene reactie induceren. Dit kan voorkomen worden door het gebruik van immunogecompromitteerde muizen, maar dit zal niet altijd haalbaar zijn. Om toch ook preklinisch een *in vivo* evaluatie van het product uit te voeren is het verstandig om een model te ontwikkelen dat de humane situatie illustreert.
Tot slot dienen we ons bewust te zijn dat een veiligheids-evaluatie wordt bemoeilijkt door vragen als - welke testen zijn klinisch relevant, welke pass/fail criteria zijn toepasbaar, en welke risk/benefit ratio is aanvaardbaar?

Voorwaarden voor de veiligheids-evaluatie kunnen worden vastgelegd in standaarden en richtlijnen. Er zijn standaarden en richtlijnen beschikbaar aangaande de individuele componenten van TEMPs, echter deze dekken niet alle aspecten op het terrein van de biocompatibiliteit. Zo wordt het gebruik van degradeerbare materialen nog niet in Europese standaarden beschreven en komt ook de bewerking van cellen niet aan de orde. Ook de interacties tussen cellen, biomaterialen en biomoleculen en de interactie van het eindproduct met de ontvanger komen niet aan de orde.

Om tot een coherente aanpak te komen van veiligheidswaarborging van TEMPs, is de ontwikkeling van klinisch relevante gevalideerde testen en de initiatie van specifieke standaarden en richtlijnen toegesneden op TEMPs dan ook noodzakelijk.

Summary

In the past few years there has been a growing understanding of how cells interact with each other, how growth factors influence the differentiation of cells, how biomaterials interact with the human body and how allogeneic responses are defined. This knowledge has contributed to the development of biological products which can repair, regenerate or replace human tissue and organs. This application is usually called "Tissue Engineering". Fields of application are e.g. skin transplantation, replacement of bone and cartilage and repair of tissues that produce hormones and enzymes. The products consist of viable cells and biomaterials whether or not in combination with biomolecules. These products will be referred to as Tissue Engineered Medical Products (TEMPs).

The combination of human cells and materials creates a product that is not (yet) covered by Dutch or European legislation. Such a product is specifically excluded from the European definition of medical devices, which is described in the Council Directive 93/42/EEC. Moreover, at the European level, there is a debate whether TEMPs should fall under the definition of medicinal products (Council Directive 65/65/EEC).
Irrespective of the details of regulation, it is important to identify and evaluate the risks specifically related to the medical application of this new category of products. In general, the risk of transmitting infectious diseases from donor to recipient, the risk of inducing bio-incompatibility and the risk of lack of clinical efficacy are of serious concern for TEMPs. However, given the novelty of these products, their hazards and associated potential harm have not yet been clearly identified.

In this report, we tried to give insight into one of these general risks, namely the risk of lack of biocompatibility. Biocompatibility can be defined as the ability of a product to perform with an appropriate host response in a specific application. This definition indicates that not only characteristics of the product but also the biological responses of the host account for biocompatibility of a TEMP. In general, hazard identification should be carried out on the final product. However, the combination of viable cells, biomaterials and biomolecules in TEMPs increases the complexity of hazard identification. To come to a meaningful extent of hazard identification, first all relevant hazards were listed related to the separate components, i.e. viable cells, biomaterials and biomolecules. Significant hazards can be introduced by manipulation of cells (uncontrolled cell proliferation and differentiation), use of degradable biomaterials (harmful degradation products), and the doses of biomolecules (immunogenicity).
It is, however, not sufficient to summarise the hazards associated with the separate components. Complex interactions exist between cells, materials and biomolecules, such as effect of biomaterials on characteristics of donor cells and on the release of biomolecules. These interactions need to be considered in the safety evaluation.

Theoretical description of the hazards should be followed by pre-clinical assays to identify and estimate the severity of the harm they could cause. For the individual components most hazards can be identified or analysed by well known validated tests. However, only a few of these assays can be applied to the final product. Moreover, *in vitro* and *in vivo* assays specifically validated for the final product are rare. For safety considerations, it is necessary to develop and validate assays. The choice of relevant *in vivo* assays is hampered because

human cells will induce a xenogeneic reaction in the animal model. This can be circumvented by using immunocompromised mice, although, this will not always be practical. Therefore, it is advisable to develop a model that illustrates the human situation. Finally, the interpretation of every pre-clinical assay for TEMPs is hampered due to questions as - which assay should one choose in order to obtain clinically relevant information, what kind of pass/fail criteria should one apply, and how to assess the risk/benefit ratio?

Requirements for safety evaluation can be laid down in standards and guidelines. Such standards exist for safety aspects applicable to the separate components of TEMPs, however, they do not cover all aspects related to TEMPs and biocompatibility. For example, the usage of degradable materials and the manipulation of cells are not (yet) dealt with in European standards. Also standards focussing on interactions between the components are not available. This implies that there is a need for a more detailed focus on generic safety testing.
In conclusion, in order to achieve a coherent approach to control the safety of TEMPs, the development of validated assays and the initiation of standards and guidelines is essential.

1. Introduction

Tissue transplantation within a single individual and organ or tissue transplantation from one individual to another has evolved into a recognised option in the replacement or restoration of tissues or organs with failing functions. However, mainly driven by the shortage of donor tissues and organs, new solutions have emerged. One of these solutions is the development of products that contain specific populations of viable cells whether or not combined with biomolecules or biomaterials. The solutions are based on the concept of regenerative medicine; making use of the knowledge of how tissues are repaired naturally and using this knowledge for the restoration of structure and function of damaged human tissue that does not regenerate spontaneously[1-5]. Four avenues have been explored so far to create new tissue or tissue functions:

1. implantation of a material, with or without the addition of specific biomolecules, in order to stimulate local tissue growth/regeneration
2. injection of isolated functional viable cells
3. application of viable cells encapsulated in a special device and
4. implantation of viable cells combined with biomaterials whether or not combined with biomolecules.

In this report we focus on products which contain viable cells and (biodegradable) materials combined with biomolecules. We refer to these products as Tissue Engineered Medical Products (TEMPs). It should, however, be realised that this acronym is not widely accepted.

Several advantages of TEMPs have been described[1,3,4,6-8]. By growing cells on a scaffold (synthesised or manufactured from one or more biomaterials), a natural organisation of structurally different cell layers can be provided. It can also guide the regeneration/reconstruction of tissue *in situ*, by proliferation of donor cells and ingrowth of host cells. Moreover, a well-designed scaffold will allow the exchange of nutrients from the host to the cells on the scaffold.
As reviewed in a previous RIVM report, the combination of different natural or synthetic scaffolds with cells derived from different origin potentially creates a wide assortment of products[9]. TEMPs can form an organised epithelial/endothelial cell layer for different tissues, including skin, intestine, blood vessels, tracheas, and urethras and they can replace structural tissue like bone or cartilage. There are also TEMPs of which the action depends on the metabolic function of the constituent cells, like hepatocytes (liver cells) and β-cells of islets of Langerhans in the pancreas. These cells can be encapsulated by a matrix which allows the exchange of products (like insulin) and nutrients, whereas endogenous lymphocytes and immunoglobulins can not reach the implanted cells. Cells considered for use in TEMPs can be isolated from adult or neonatal tissue of autologous (donor and recipient are the same individual), allogeneic (donor and recipient are not the same individual yet are within the same species) or xenogeneic (donor and recipient belong to different species) origin. An exciting new approach involves the use of stem cells for the generation of new tissue[10-12]. The use of these cells (especially stem cells derived from embryonic origin) in tissue engineering is, however, still a matter of debate due to practical and ethical problems.

The combination of human cells and materials creates products that are not (yet) covered by Dutch or European legislation. Such products are specifically excluded from the European

definition of medical devices, which is described in the Council Directive 93/42/EEC[13]. Moreover, at the European level, there is a debate whether TEMPs should fall under the definition of medicinal products (Council Directive 65/65/EEC[14]). A more detailed summary of the (lack of) current regulation on this topic has been published recently[15]. In the absence of European legislation, several European countries have established their own approaches for legislation. For example, the Norwegian Medicines Control Authority has decided that products for wound healing with viable cells prepared from human neonatal foreskin are to be considered medicinal products. In the Netherlands, a new law is under preparation which focuses on the safety and quality aspects of human tissues (Act on Safety and Quality of Human Tissue).

Irrespective of the details of regulation, it is important to identify and evaluate the risks specifically related to the application of any medical product in order to compare risks versus benefits. Although the use of TEMPs for medical treatment involves the acceptance of certain risks, the general public expects a manufacturer to reduce the risks as much as possible before starting clinical trials. Strategies to reduce and/or control these risks should therefore be developed. The RIVM report "Tissue Engineered Medical Products (TEMPs): A prelude to risk management"[16] is an attempt to describe this process in detail for TEMPs. In order to perform an adequate risk analysis, the various hazards (source of harm) need to be assessed by estimating the incidence and severity of the adverse effects likely to occur. The acceptability of the risks involved has to be evaluated against the benefit gained from the treatment with the particular product. If necessary, risk reduction has to be considered.

One of the major risks in the application of implants, i.e. TEMPs, concerns lack of biocompatibility. Biocompatibility is a desired property of a product and at the moment the most widely used definition is "the ability of a product to perform with an appropriate host response in a specific application"[17]. This broad definition indicates that many characteristics not only of the product but also of the biological responses of the host account for the biocompatibility of a TEMP. It includes not only the absence of adverse effects but also an appropriate performance in the host. In this report, we focus mainly on safety considerations rather than on performance efficacy.

Hazards leading to lack of biocompatibility can be introduced during all phases of the life cycle of a TEMP. In this report, an attempt was made to summarise the most important anticipated hazards during the sourcing and production phase. In addition, pre-clinical biocompatibility assays which could give an estimate of the probability of occurrence of that particular hazard were discussed. Furthermore, we give an overview of current standards and guidelines dealing with the safety evaluation of the separate components of TEMPs. Finally, we discuss the usability of these standards and guidelines in the assessment of the final product.

2. Methods

Information on hazards, pre-clinical assays and standards/guidelines was obtained from interviews with experts in the field, scientific articles and meetings, internet and documents released by (inter-)national organisations. The use of xenogeneic cells involves, besides biocompatibility related hazards, a number of serious other scientific, ethical and public health issues, and will therefore not be discussed in this report. For additional information on the use of xenogeneic cells we refer to reports issued by the Council of Europe[18] the Organisation for Economic Co-operation and Development (OECD)[19] and the World Health Organization (WHO)[20].
Hazards related to transmission by potentially infectious agents are of main concern in the selection and development of TEMPs. However, this report does not address this risk as this is a major subject on its own.

3. Hazards and pre-clinical assays

Hazard assessment should be based on experience and scientific concepts followed by pre-clinical assays. Hazards leading to lack of biocompatibility can be introduced during all phases of the life cycle of a TEMP. In this chapter, we try to give an overview of these hazards. In addition, a non-exhaustive list of examples of pre-clinical assays is given. These assays can be used for a single characterisation study during product development, for in process-control or for product release testing. Moreover, the assays which are described are not definitively predictive for clinical outcome, but may give some indication of the risks to be expected.

Due to the biological variability of TEMPs and the presence of a variety of components (viable cells, biomaterials and biomolecules), first a survey is given of hazards and pre-clinical assays relevant for the separate components followed by an elaboration on hazards and pre-clinical assays relevant to the final product.

3.1 Viable cells

Sourcing

Both autologous and allogeneic cells are used in the engineering of TEMPs. An example of a product containing autologous cultured cells is Carticel® (Genzyme Biosurgery, Cambridge, USA). Autologous chondrocytes are cultured *in vitro* for about 4 weeks and implanted into a cartilage defect. Similarly, Isotis (Bilthoven, the Netherlands) is performing a pre-clinical trial with VivescOs® for e.g. hip-revisions. Here, autologous derived bone marrow cells are differentiated *in vitro* into osteoblasts and are then seeded onto a calcium phosphate scaffold. Examples of products using allogeneic cells are Dermagraft® (Advanced Tissue Sciences, La Jolla, California, USA) and Apligraf® (Novartis Pharmaceuticals Corporation, East Hanover, US), both of which are registered as medical devices in the US. Apligraf® is a construct which contains viable allogeneic fibroblasts and keratinocytes isolated from human infant foreskin in combination with Type I bovine collagen[21]. Dermagraft® consists of neonatal allogeneic fibroblasts seeded onto a bioabsorble/polyglactin scaffold[22].

The use of allogeneic cells introduces the risks for rejection based on mismatches on Human Leukocyte Antigens (HLA), blood group and/or Rhesus factor between the donor and the recipient. However, it has been shown that Apligraf® is immunologically inert[21]. The absence of rejection has been proposed to be based on the absence of Langerhans cells in the final cell population and on the absence of HLA class I and II surface proteins on fibroblasts and keratinocytes derived from immature tissue.

Another concern in using allogeneic cells is the risk of transmitting (infectious) diseases from donor to recipient. This risk is among other things depending on the type of organs, tissues or cells which are being donated and on the final application of the product. To reduce this risk, the donor should be screened. This could be done by a standard oral questionnaire and by laboratory techniques.

Recently, scientific research has paved the way to use embryo's as a source for stem cells, which have the capacity to differentiate into a wide variety of somatic cells [10-12]. Although the use of these cells is still a matter of debate due to practical and ethical problems, it is likely that in the future these stem cells will be used in tissue engineering.

Although for some cells several *in vitro* passages can be obtained, freshly isolated human cells have the disadvantage that they are difficult to culture. As an alternative researchers can also resort to cell lines. Cell lines have the potential for rapid expansion *in vitro* and they can be characterised extensively. The use of cell lines also ensures a more constant and reproducible product by eliminating donor-to-donor variability. However, the use of these cell lines is limited by the fact that cell lines tend to lose their differentiated function with time. Moreover, there is a possibility that these immortalized cells will have the ability of uncontrolled proliferation in the patient. To our knowledge, no TEMP containing a characterised cell line is yet on the market.

Processing

To process freshly isolated cells, manipulation steps are required. These can vary between simple expansion of the cells in which they retain their original function (homologous function), and the involvement of manipulations resulting in alterations of the functional and/or genetic characteristics of the cells (heterologous function). The inherent variability of cells of different donors requires a high degree of control to reduce or eliminate risks associated with these manipulation steps. Of main concern is the introduction of an uncontrolled proliferation rate. This should be extensively tested for example with cell doubling times or passage numbers beyond the doubling time or the passage number which is routinely used for the cells used in the final product. In addition, the extent of manipulation may influence the differentiation phase of cells. The assessment of the identity and viability of cells should therefore be established. This could be performed by routine methods such as microscopy and flow cytometry.

In addition, adverse effects could be induced by contamination with other cell types. The harm is depending on the type (allogeneic, antigen presenting cells) and the abundance of the contaminating cells. The level of desired purity depends on the correlation between the purification steps and the reduction of the functionality of the product.

To culture, and if necessary, differentiate the cells, substances are added to the culture medium, like foetal calf serum, growth factors and antibiotics. Residues from these components or contaminations, like endotoxins, could remain associated with cells and could induce an unwanted immunogenic or toxic response after implantation. Careful selection and control of the culture medium and its additives is therefore required.

Hazards introduced by the presence of viable cells in an implant leading to lack of biocompatibility are described in Table 1. Most hazards are well known from clinical transplantation experiences and vaccine production technology. The pre-clinical assays established in these fields can be used for the safety evaluation of the cellular component.

Table 1. Hazards and assay/techniques related to viable cells

	Hazards introduced during the sourcing and the processing phase	**Examples of assays/techniques**
1	Variation in cell characteristics	• identity e.g. visual assessment, expression of cell specific antigens, DNA analysis • potency e.g. protein production, in vivo model • viability e.g. trypan blue assay
2	Contamination with other cell types	• purity e.g. flow cytometry
3	Pre-existing diseases (non-infectious)	• questionnaires about clinical history (in special cases of mother/father) • clinical evaluation of the donor (see above) • macroscopic evaluation of tissue • analysis of chromosomal abnormalities
4	Mismatch HLA	• DNA sequencing • serological HLA typing • mixed leukocyte culture test • flow cytometry
5	Mismatch blood group/Rhesus factor	• PCR • serology (agglutination)
6	Incorrect number of cells at time of application	• manual/automatic cell counting • cell proliferation • total DNA • protein synthesis
7	Uncontrolled cell differentiation and proliferation	• characterisation of biological and morphological changes • implantation in nude mice
8	(Systemic) immunogenicity and toxicity of residues in culture medium	• detection of factors influencing the proliferation or metabolism of aspecific T and B cells. • detection of immunoglobulins or T cells in blood and serum of experimental models • immunohistochemistry of surrounding tissue (neutrophils, fibroblasts) at several time points in experimental models • toxicity studies
9	Changes in cell behaviour during processing, storage or transport thereby loss of functionality	• flow cytometry • apoptosis assay • imaging modalities • metabolism e.g. by enzymatic reactions or rt-PCR • protein excretion pattern e.g. western blotting or ELISA

3.2 Biomaterials

One of the main goals of the scaffold is to provide the necessary structural support for cells. In addition, recent attention has been drawn to the role of the scaffold in mimicking the natural microenvironment of cells. The more similar a scaffold is compared to the natural extracellular matrix, the closer it may get in achieving biocompatibility. This includes not only the absence of adverse reactions, but also the desired interaction with host cells.

The scaffold can be made of (a combination of) synthetic materials, like polyglycolic acid, polylactic acid, polylactic-co-glycolic acid or of natural materials like hyaluronic acid derivatives, elastin, collagen and fibronectin. The use of natural materials involves a high variation in the characteristics of the material. This could have an impact on the quality of the final product. The variation can be reduced if synthetic raw materials are used. On the other hand, natural materials present advantages regarding physiological properties, degradability

by the human body and the inherent capacity to interact with proteins and receptors in the natural extracellular matrix and on cells.

An immunological reaction or any significant host response, like a foreign body reaction, sensitisation and irritation of the materials used and additives, like ceramic fillers, blowing agents and coupling agents, should be determined[23-24]. Moreover, the assessment of local short or long-term effects of the materials on living tissue, at both the microscopic and macroscopic level should be determined. The parameters which could be considered are a) tissue ingrowth, b) extent of fibrosis, c) degeneration, d) number and distribution of inflammatory cell types, e) presence of necrosis.
The materials and the release of substances should be tested for local and systemic toxicity. The degradation products should preferably consist of metabolites with no toxic and immunogenic effects. Toxicity should be tested *in vitro* using appropriate biological parameters and *in vivo*. *In vitro* cytotoxicity assays make use of the biological response of mammalian cells to toxic agents. There are several end-points that can be determined, like cell damage, cell proliferation and cellular metabolism.
In addition, consideration should be given to determine possible genotoxicity, carcinogenicity and reproductive and developmental toxicity. If a scaffold comes in contact with blood, blood compatibility needs to be established by a series of testing on specific categories as thrombosis, coagulation, platelets, haematology and complement. The tests should be selected based on the intended use (*in vitro, ex vivo, in vivo*) and duration of contact.

Pyrogenicity testing should be considered. The main cause for pyrogenic reactions are the presence of bacterial endotoxins. In addition, there is also evidence that some materials contain material-related pyrogens. The most widely used assay to detect bacterial endotoxins is the Limulus amoebocyte lysate (LAL). However, this assay does not detect material-related pyrogens. In addition, the LAL suffers from interferences leading to either false positive or false negative results. Therefore, also other tests need to be considered like the (rabbit) pyrogen test. For both assays the pyrogens need to be eluted from the materials. However, pyrogens tend to be firmly attached to materials. For solid materials, recently, the "Human Whole Blood Pyrogen Assay" was proposed, which is now under validation[25]. This assay is based on the production of the pro-inflammatory cytokine Interleukin 1β after contact with pyrogens.

Furthermore, the material characteristics such as porosity of the scaffold should be determined. The ingrowth of host cells and vascularisation of the scaffold may depend on the porosity of the scaffold. In addition, the behaviour of host cells will be influenced by the transmission of mechanical forces through the scaffold and should therefore be tested[26].
The degradation and resorption process of both natural and synthetic materials should facilitate the regeneration of host tissue. The material should be designed to be resorbed over controlled periods of time to allow the host cells to build a new extracellular matrix of adequate structural strength. The breakdown products should be removed from the body and not accumulate. The metabolism and excretion of the degradation products should therefore be determined.

Degradation is not desirable for all TEMPs. A synthetic, selectively permeable membrane can be used to separate cells from the immune system to circumvent immunological rejection. This technique is for example used in restoring *in vivo* the production of insulin by immunoisolated pancreatic β-cells. In this case, incorrect diffusion properties of the material

can give rise to severe adverse effects. This kind of properties can be determined in *in vitro* assays.
Table 2 lists hazards related to biocompatibility which can be introduced by the presence of biomaterials in an implant. The list of pre-clinical assays has been composed from knowledge obtained in the field of medical devices.

Table 2. Hazards and assays/techniques related to the biomaterials

	Hazards introduced during the sourcing and the processing phase	**Examples of assays/techniques**
1	Variation in source of natural scaffold or inconsistency in production of raw/synthetic materials	• high performance liquid chromatography (HPLC) • gaschromatography-mass spectrometry • spectroscopic techniques (electron microscopy) • differential scanning calorimetry • dynamic mechanical thermal analysis • porosity testing • crystallinity testing • end group analysis • mechanical testing
2	(Systemic) toxicity (acute, subchronic and chronic)	• *in vitro* cytotoxicity tests • systemic toxicity test
3	Irritation	• irritation test
4	Sensitisation (delayed hypersensitivity reaction)	• Guinea pig maximisation test • non-adjuvant Buehler test • local lymph node assay
5	Processing residues with adverse effect when released into the body	• HPLC • gas chromatography • X-ray fluorescence • inductively coupled plasma mass spectroscopy
6	Unintended degradation speed of the scaffold	• *in vitro* degradation tests • implantation test with/without predegradation
7	Pyrogenicity	• rabbit pyrogen test • LAL test • human whole blood pyrogen assay
8	Blood incompatibility	• hemolysis • coagulation e.g. partial thromboplastin time • complement activation • platelet activation e.g. β-thromboglobuline
9	Genotoxicity / carcinogenicity	• Ames salmonella reverse mutation assay • sister chromatid exchange test • mammalian cytogenicity test • gene mutation test • chromosomal aberration test • *in vivo* carcinogenicity studies
10	Insufficient mechanical strength	• compression studies • tensile strength determination • endurance testing
11	Inadequate diffusion properties of encapsulated product	• size exclusion chromatography

3.3 Biomolecules

In 1981, it was shown that not only the insoluble residual part of the extracellular matrix was necessary for osteoinductive capacity, but also the presence of soluble factors[27]. This

knowledge has been used in the field of tissue engineering. It has been shown that although cells attached to the scaffold secrete growth factors, the incorporation (both covalently and non-covalently) of additional extracellular biomolecules into the context of the scaffold could enhance *in situ* the formation of e.g. new bone or cartilage[28-32]. Examples of these biomolecules are bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-β), vascular endothelial cell growth factor (VEGF) and peptides containing the amino sequence Arg-Gly-Asp, which are known to influence cell attachment and spreading. The biomolecules may be either biologically active peptides and proteins purified from naturally occurring sources such as plant/animal organisms, human cell lines/tissues, or produced by recombinant DNA techniques, or synthetic analogues of such molecules.

A physicochemical characterisation includes the determination of the composition, physical properties and conformational structure of the product. Impurity and purity testing should consider process related substances (e.g. host proteins, host DNA) and product related substances (e.g. post-translational modifications).

The goal of pre-clinical safety evaluation is to identify an initial safe dose and to identify organs that are susceptible for the effects. Although most biomolecules used in tissue engineering are normally present in individuals, information should be presented substantiating the safe use of the product. Additional testing may be necessary for evaluation of toxic responses at the intended dosages (or higher) used. Also the short and long term toxicity, genotoxicity and carcinogenicity should be determined carefully. Pre-clinical research of these aspects can be of limited value, because the pharmacological action of proteins may be species specific. However, for growth factors this is not much of a problem as those proteins are highly conserved betweeen species. In addition, proteins from other species often elicit an immune response, which may counteract the effect of the biomolecules. Experiments may then be performed in animals lacking the power to raise an immune response against the product, such as nude mice and rats and/or SCID or SCID-hu mice. If pharmacological and/or immunological problems arise, testing the homologous animal protein in animal experiments would also be advisable. The selection of a relevant animal model is then essential.
Process residues need to be tested for toxicity. The selection of a relevant model depends on the nature of the residues (chemical or protein).

Table 3 lists the hazards related to biomolecules and gives examples of pre-clinical assays.

Table 3. Hazards and assays/techniques related to biomolecules

	Hazard during sourcing phase	Examples of assays/techniques
1	Variation in source	• structural analysis e.g. NMR, circular dichroism spectroscopy • physicochemical properties e.g., HPLC
2	Presence of impurities	• HPLC • NMR • mass spectrometry • SDS page
3	Immunogenicity	• measurement of antibody response • complement activation • inflammatory reactions • expression of surface antigens on target cells
4	Toxicity (local and systemic)	• major physiological systems • receptor/epitope distribution • studies on isolated organs • single and repeated toxicity studies
5	Carcinogenicity/Genotoxicity	• receptor binding • stimulating proliferation of normal or malignant cells, e.g. signal transduction, receptor upregulation

3.4 Interactions between separate components

As mentioned in the paragraphs 3.1-3.3, for each component in the final product the safety should be determined in relation to the intended use of the TEMPs. However, for the design of TEMPs with optimal safety characteristics, it is a prerequisite that not only the separate components need to be assessed but also the interaction between the components should be considered.

Interactions that need to be considered are:
1) effects of biomaterials on donor cell characteristics as well as on the biological activity of biomolecules
2) effects of donor cells on the topography and surface chemistry of the scaffold, on the rate of material degradation, and on the amount and type of leachable substances
3) effects of biomolecules on cell characteristics and scaffold material properties

Effects of biomaterials on donor cell characteristics as well as on the biological activity of biomolecules

The surface chemistry and the shape of the scaffold are important for cell attachment and spreading and patterns of protein expressions[31]. The scaffold should have a suitably designed porosity to provide good nutrient transport, a high surface area to volume ratio to allow sufficient surface area for cell/scaffold interactions[33] and if necessary provide vascularization[31]. The adherence, morphology and proliferation of cells to obtain successful colonisation of the material should be monitored during the development phase using for example imaging modalities and histochemistry. As cells sense even the subtlest changes in topography and chemistry of the scaffold, this monitoring is even more essential when degradable materials are used. In addition, degradation products of the scaffold should be tested for their toxicity to donor cells.

Although cells might have the correct morphology, this does not prove that the cell can perform its intended function in the TEMP. It is this function that will determine to a large extent whether or not the TEMP is successful[34]. To determine the functionality of the cell population, the expression of proteins, like heat shock proteins, cytokines or matrix metalloproteinases can be determined. Methods to collect and investigate proteins released by donor cells attached to the scaffold have been analysed for their usability in TEMPs[35,36]. Also the intracellular signalling pathway after cell adhesion to a material can be investigated[37,38].
The physical structure of the scaffold influences the *in situ* release of the incorporated molecules[39]. The choice of the delivery system is depending on the kind of cell type, the material used, the biomolecules used and the final application. For example, the release of interferon from a scaffold composed of hyaluron was reduced 8-fold as compared with non-hyaluron materials[33]. Measuring the concentration of (e.g. radiolabeled) biomolecules both *in vitro* and *in vivo* may be indicative for (unwanted) release of biomolecules from the scaffold.

Effects of donor cells on the topography and surface chemistry of the scaffold, on the rate of material degradation, and on the amount and type of leachable substances
Material characteristics of the scaffold can change under influence of adhering cells. It has been demonstrated that the cell seeding density influences the construct integrity[40]. The cells are cultured within a scaffold matrix and synthesise their native matrix proteins. These proteins precipitate within the scaffold, thereby organising and remodelling it. It is conceivable that cells also influence the degradation rate of the scaffold materials, as the degradation rate is usually dependent on the surface area that is exposed. Moreover, it is also conceivable that the amount of leachables can change under influence of cells attached. No reports describing these effects were found in literature. However, it is very likely that excreted substances of the donor cells could have an effect on the degradation of the material as has been reported for the interaction of host tissues with the degradation characteristics of materials[41]. In order to enhance tissue growth on the scaffolds a culture system with medium flowing through the cell-seeded scaffolds can be used. An effect of the fluid flow to the *in vitro* degradation kinetics of biodegradable scaffolds has been observed[42]. For these reasons, it is advisable to evaluate the degradation process of the scaffold when cells are being cultured on it. The degradation products could be measured in the supernatant of the culture medium. Also structural properties, which are obviously related to the degradation process of the material should be determined at certain time intervals.

Effects of biomolecules on donor cell characteristics
The coating density and the adhesivity of purified molecules can influence the proliferation and differentiation rate of donor cells[26]. Techniques are nowadays available that can immobilise biomolecules with micronscale precision[43], which is necessary for spatial control of cell engineering. These effects on donor cells should therefore be studied pre-clinically.

3.5 Interactions between the final product and host tissue

Studying all the possible interactions between the components in pre-clinical *in vitro* assays will give information about several safety concerns. Nevertheless, implantation of the final product will lead to different environmental conditions as compared with the laboratory situation. Generally, contact between the product and biological processes *in vivo* would not interfere with the intended aim of the product. The outcome with a complex product such as a TEMP is, however, difficult to predict. For example, dynamic surface chemistry changes of the scaffold are likely to occur in the first seconds of contact with the new environment, and

these changes will influence donor cell characteristics. Moreover, release or leakage of biomolecules from the scaffold could likely increase the activation state of the immune system thereby turning an "inert" scaffold into an immunogenic one. In addition, the release of biomolecules could also attract host cells to the implant thereby changing the local environment.

Implantation studies with the final product in relevant animal models can give information on the abovementioned aspects. These studies, however, are hampered by the fact that the human cellular component will induce a xenogeneic immune response in the experimental animal. This could be overcome by using immunopriviliged sites, immunosuppression methods or performing implantation studies in immunocompromised mice reconstituted with human leukocytes (SCID-hu)[44].

4. Standards and guidelines

Standards and guidelines supporting a regulatory system for TEMPs should contain requirements for performance and safety evaluation of products. Methods should be described identifying and evaluating potential adverse-effects. Depending on the specifications of the standards and guidelines, general techniques or specific assays may be assigned as tools for the identification and quantification of the associated risks. Parties in the field, like manufacturers, government and physicians could reach consensus about the type of testing to be performed and the specifications to be met before a product enters the market. These product requirements can be described in national or international standards. At this moment, standards specifically designed for products containing viable cells are not yet available. The demand for harmonisation of biocompatibility related aspects is, however, increasing as more and more researchers (want to) bring their innovative products on the market. This is also illustrated by the fact that EUCOMED, which represents the interests of the majority of the non-pharmaceutical European medical technology industry, issued a position paper in which they stressed the need at an European level for unequivocal registration procedures based on a risk/benefit approach for these products[45].
In this chapter, similar to chapter 3, firstly documents relevant for the quality and safety evaluation of the separate components are summarised. Most documents relate to biocompatibility safety aspects. However, for clarity reasons also documents with a broader scope are mentioned. Secondly, pathways for the standardisation relevant for the safety of the final product are discussed.

4.1 Human organs, tissues and cells

The Health Committee of the Council of Europe has produced a draft guide on safety and quality assurance for organs, tissues and cells[46]. It promotes the use of standard operating procedures for procurement, preservation and distribution of organs, tissues and cells of human origin used for transplantation purposes. One of the major issues is the quality of the starting (source) material. The document contains detailed information about donor selection, including criteria for the donation of various organs and tissues. Detailed recommendations for donor selection are also issued by branch organisations like the European Association of Tissue Banks (EATB) and the European Federation of Immunogenetics (EFI). The EFI describes standard techniques for the determination of histocompatibility.
In the European Union, the legislation for cell based products is generally described in article 152 of the Treaty of Amsterdam. Here, it is stated that the European Commission (EC) shall adopt measures in order to set high standards of quality and safety for organs and substances of human origin, blood and blood derivatives. This gives the possibility to introduce a legal framework for these issues. For advice on these issues several advising bodies are available to the Commission. At the moment, the EC has not issued a specific directive for organs, tissues and cells.
For blood and blood derivatives, specific regulations related to biocompatibility are set down in Council Directive 98/463[47]. It is stated that the donor and each blood donation should be tested in accordance with the rules which provide assurance that all necessary measures have been taken to safeguard the recipients. The core screening tests mentioned are all related to reducing and eliminating the risk for disease transmission.

Products incorporating human cells are specifically excluded from the medical device directive (93/42/EEC[13]). The directive states that a product is not a medical device if it contains transplants or tissues or cells of human origin or products incorporating or derived from tissues or cells of human origin, except for substances derived from human blood or plasma which are an integral part of the device (amendment 2000/C 245/04). This indicates that biocompatibility related aspects of human cells are not regulated under this directive.

According to the definition of medicinal products and substrates (65/65/EEC[14]) it can be argued that products incorporating cellular components should be treated as medicinal products. Whether this is beneficial and effective is still a matter of debate. However, documents issued by the European Agency for the Evaluation of Medicinal Products (EMEA) and guidelines harmonised among the EU, Japan and the USA issued by the International Conference of Harmonization (ICH) may be used to assess the safety of cells used in TEMPs. The documents relate to the use of a variety of cell types for the production of medicinal products (including biologicals)[48-50]. The manufacture of these products requires appropriate controls on all aspects of handling the cells, e.g. genomic and phenotypic stability of cells during culture and the evaluation of the suitability of reagents of human origin necessary to ensure optimal proliferation of cells. In this context stability is of concern for the consistency of protein production and for the retention of protein production capacity during storage. In addition, the World Health Organization Expert Committee on Biological Standardization published requirements for the use of animal cells as *in vitro* substrates for the production of biologicals[51].

The Food and Drug Administration (FDA) has a regulatory pathway for human cells, tissues and cellular and tissue-based products. The regulation focuses under a risk/benefit approach on 1) prevention of use of contaminated tissues 2) prevention of improper handling or processing that might contaminate or damage tissues 3) ensuring that clinical safety and effectiveness is demonstrated. To develop policies, regulations and guidance documents a Tissue Action Plan has been established. The core team of the action plan is composed of representatives from the Center for Biologics Evaluation and Research (CBER) and from the Center for Devices and Radiological Health (CDRH). In the last years, three new rules have been proposed[52-54]. First, a rule was proposed to require cell and tissue establishments to register with the FDA and submit a list of their human cellular and tissue-based products (63 Federal Register 26744). Second, the suitability determination for donors of these products was described (64 Federal Register 52696). Together with the good tissue practice for manufacturers of human cellular and tissue-based products (66 FR 1508), these rules when finalised would establish a regulatory program to be contained in 21 CFR part 1271.
The Tissue Action Plan has also accomplished the publication of the Guidance for human somatic cell therapy and gene therapy[55], which discusses amongst other things the materials used during manufacturing and the development and characterisation of cell populations. Although not directly dealing with biocompatibility related safety aspects, the FDA also issued a guidance document for products comprised of viable autologous cells manipulated *ex vivo* for structural repair or reconstruction[56]. It describes regulatory requirements for clinical studies needed for market approval.
Moreover, the United States Pharmacopeia (USP) bimonthly journal, the Pharmacopeial Forum, published a USP draft general chapter on cell and gene therapy products[57]. These USP standards are enforceable by the FDA and the governments of more than 35 other countries. This document elaborates on the need for validated assays to identify identity, dose, potency, purity, safety and stability of the products, but does not describes generic assays.

4.2 Biomaterials

A document that is useful in the evaluation of biocompatibility aspects for scaffolds is the EN ISO 10993 series[58]. This standard supports the medical device directive and is issued by the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN). It is divided into 20 different parts and is based on the application site, frequency and duration of the implant to the body. Part 1 describes how to select appropriate tests and the subsequent parts describe specific types of testing and or evaluation.
The applicability of this standard for scaffolds used in TEMPs is at the moment hampered by the fact that it is prepared for solid non-degradable materials, whereas scaffolds used in tissue engineering are mostly intended to degrade in time. Only recently work has started to develop specific guidance for biocompatibility testing of biodegradable materials within the framework of EN ISO 10993. The use of non viable animal tissues for the generation of biomaterials is covered by the medical device directive. The 12442 series standard provides requirements for the evaluation of undesired pyrogenic, immunological or toxicological reactions of these animal tissues and their derivatives[59].

4.3 Biomolecules

A significant body of current European documents covers the quality and safety aspects of biomolecules as several biomolecules are already marketed as medicinal products under Council Directive 65/65/EEC[14]. The requirements for these biomolecules are described in documents issued by the CPMP or ICH. The CPMP and ICH documents focus on pre-clinical safety evaluation of biotechnology derived pharmaceuticals, the selection of relevant animal models for testing toxicity and immunogenicity, and characterisation specifications as purity and impurities and contaminations of the product[60-64]. These documents do not describe generic tests. Besides these CPMP and ICH guidance, the European Pharmacopoeia contains monographs describing quality requirements and descriptions of a large number of general test methods for several biologically active compounds, such as interferon and growth factors.

4.4 Final product

Although the above mentioned guidelines or standards are applicable to the separate components, none of them explicitly take into account the simultaneous presence of a cellular component, a non-cellular material and biomolecules into one single product, and their possible interactions. As there is limited experience with these kind of products several groups and expert committees started discussions and prepared (draft) documents on these issues.

One of the pioneers for standardisation of TEMPs is Division IV of the Committee F04 on Medical and Surgical Devices & Materials under the auspices of the American Society for Testing and Materials (ASTM). About 40 different task groups in this division cover different safety and quality aspects of TEMPs. These task groups include amongst other things terminology, tissue characterisation, biomolecules and normal biology. Using a consensus process characterised by input from academia, manufacturers, government and other interested parties, development of standards for e.g. test methods, terminology and classifications is in progress.
The CEN Healthcare Forum (CHeF) has set up a task group to investigate the needs of stakeholders for the development of European standards in the field of human tissues. This

group recommends the start of standardisation on horizontal subjects related to the field of TEMPs such as terminology, risk management, quality systems, biological safety and microbiological safety. It is proposed that this work could be done by already existing technical committees (TCs) such as CEN/TC 316 -Medical devices utilising tissues, CEN/TC206-Biocompatibility of medical and dental materials and devices-, and CEN/TC 285-Non-active surgical implants-. In addition ISO/TC 150 "Implants for surgery" gathered a task force to discuss the necessity to develop standards specifically designed for TEMPs. Their conclusion was that the ISO should follow closely standardisation activities of other organisations, like the ASTM and the CEN, and would not start activities at its own for the time being (personal communications).

Also the EMEA anticipated the growing number of products containing viable cells by drafting a Points to Consider document on human somatic cell therapy products (viable somatic cell preparations suitably adapted for transfer to an individual)[65]. According to this document these products could fall within the definition of medicinal products if the cells are subjected to a manufacturing process carried out in specialised facilities. The process should in those cases encompass expansion or more than minimal manipulation designed to alter the biological, physiological or functional characteristics of the resulting cells. It is stated that this manipulation can include the combination with a non-cellular matrix. In addition, the resulting cell product should be definable in terms of qualitative and quantitative composition, which may include biological activity. This draft document deals with donor suitability, source and characterisation of other materials used in the manufacturing process (e.g. albumin, immunoglobulin and antibiotics) and cell culture procedures, without describing generic tests. Closely related to this subject is a Note for Guidance on the aspects of gene transfer medicinal products[66]. In this document, it is considered that genetically modified cells may be grown in appropriate matrices. It states that the quality of the final product and its separate components, where appropriate, should be characterised and documented. However, no detailed information has been given.

Finally, the Council Directive 2001/20/EEC[67] establishes provisions regarding the conduct of clinical trials in particular relating to the implementation of good clinical practice. This directive states that written authorisation of the competent authority shall be required before commencing clinical trials involving amongst others somatic cell therapy. This legal framework can now be used to prepare additional guidelines for pre-clinical quality and safety assessment.

5. Discussion

In the past few years, tissue engineering has evolved into a relevant application for the replacement or restoration of tissues or organs with failing functions. The increasing complexity of tissue engineered medical products and their manufacturing processes requires careful consideration on safety and quality aspects. The risk of transmitting infectious diseases from donor to recipient, the risk of inducing bio-incompatibility and the risk of lack of clinical efficacy are of serious concern.

In this report, we focussed on biocompatibility related aspects of TEMPs. Given the novelty of these products, their hazards and associated potential harm have not yet been clearly identified. In general, hazard identification should be carried out on the final product. However, the simultaneous presence of viable cells, biomaterials and biomolecules in TEMPs increases the complexity of hazard identification. To come to a meaningful extent of hazard identification, first all relevant hazards were listed related to the separate components. Significant hazards are manipulation of cells (uncontrolled cell proliferation and differentiation), use of degradable biomaterials (harmful degradation products), and the doses of biomolecules (immunogenicity). It can be concluded, however, that the hazards associated with the final product are not merely a sum of the individual hazards. Complex interactions between the various components, such as the effect of biomaterials on cell characteristics and on the release of the biomolecules, might occur. Safety evaluation in relation to biocompatibility has to take these hazards into consideration.

Theoretical identification of the hazards needs to be followed by pre-clinical assays. For the separate components, scaffolds, cells and biomolecules, most hazards can be identified or analysed by validated tests as experience has already been obtained in the field of medical devices, transplantation and medicinal products. However, assays validated for the final product are rare. The selection of assays for the final product is hampered due to the specific biological activity of TEMPs and the limited clinical experience with these products. It may also be hampered due to limited availability of the final product. In addition, assays *in vivo* are hampered because human cells will induce a xenogeneic reaction in the animal model. Therefore, it is advisable to use immunopriviliged sites, immunosuppression methods or performing implantation studies in immunocompromised mice reconstituted with human leukocytes (SCID-hu). If these approaches are not adequate, it is desirable to develop an animal model that parallels or illustrates the human condition. The time course and the site of implantation have to be determined carefully to assess the safety of the product. To support the safety evaluation, especially in those cases described above where selection of biocompatibility related assays is hampered, process monitoring and demonstration of consistincy should be performed before the final stage of production.
Finally, questions as - which assay should one choose in order to obtain clinically relevant information, which assay should one choose which does not lead to destruction of the final product, what kind of pass/ fail criteria should one apply, and how to assess the risk/benefit ratio?- also hamper the monitoring of the safety of the product. Therefore, more research should focus on the validation of relevant safety assays.

In order to achieve a coherent approach to assure the safety of TEMPs, the use of standards and guidelines is preferable. Such European standards exist for safety aspects applicable to

the separate components, however, they do not cover all aspects related to TEMPs and biocompatibility. For example, the usage of degradable materials and the manipulation of cells are not (yet) dealt with in European standards. In addition, also standards focussing on the interaction between the components are not available. Therefore, additional standards that connect to the European situation have to be developed. These standards could be based on the ASTM-standards. In addition, the implementation of a code of Good Manufacturing Practice for Tissues as has been developed in Australia[68], would be of great value.

In conclusion, the development and evaluation of clinical relevant validated assays and the focus on European standardisation procedures will contribute to the safety assurance of TEMPs.

References

1. Omstead DR, Baird LG, Christenson L, Du MG, Tubo R, Maxted DD et al. Voluntary guidance for the development of tissue-engineered products. Tissue Eng 1998; 4:239-266.

2. Babensee JE, Anderson JM, McIntire LV, Mikos AG. Host response to tissue engineered devices. Adv Drug Deliv Rev 1998; 33:111-139.

3. Marler JJ, .Upton J, Langer R, Vacanti JP. Transplantation of cells in matrices for tissue regeneration. Adv Drug Deliv Rev 1998; 33:165-182.

4. Lanza RP, Hayes JL, Chick WL. Encapsulated cell technology. Nat Biotechnol 1996; 14:1107-1111.

5. Nemecek GM, Dayan AD. Safety evaluation of human living skin equivalents. Toxicol Pathol 1999; 27:101-103.

6. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R et al. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol Bioeng 1999; 64:580-589.

7. Eaglstein WH, Falanga V. Tissue engineering for skin: an update. J Am Acad Dermatol 1998; 39:1007-1010.

8. Reddi AH. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol 1998; 16:247-252.

9. Wassenaar C, Geertsma RE. Tissue Engineered Medical Products (TEMPs) -Historische ontwikkelingen en toekomstverwachtingen. Bilthoven; National Institue of Public Health and the Environment; 2000 October. Report no. 605910 004 (in Dutch).

10. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science 2000; 287:1427-1430.

11. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science 2000; 287:1442-1446.

12. Van der Kooy D., Weiss S. Why stem cells? Science 2000; 287:1439-1441.

13. Council Directive 93/42/EEC of 14 june 1993 concerning medical devices.

14. Council Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by law, regulation or administrative action relating to medicinal products.

15. De Jong M. The conundrum of products of human origin. The Regulatory affairs Journal 2000; 11:283-293.

16. Wassenaar C, Geertsma RE., Kallewaard M. Tissue Engineered Medical Products (TEMPs), a prelude to risk management. Bilthoven: National Institute of Public Health and the Environment; 2001 July. report no. 605148 009.

17. D.F. Williams. The Williams dictionary of biomaterials. 1999. Liverpool University press. Liverpool, UK.

18. Council of Europe. 2000. (draft) State of the art on Xenotransplantation. Strasbourg, France.

19. Organisation of Economic Co-operation and Development (OECD). 1999. Xenotransplantation. International policy issues. OECD Proceedings. Paris, France.

20. World Health Organization (WHO). 1997. Report of WHO Consultation on Xenotransplantation. WHO/EMC/ZOO/98.2. Geneve, Switzerland.

21. Eaglstein WH, Falanga V. Tissue Engineering and the Development of Apligraf®, a human skin equivalent. Clinical Therapeutics 1997; 19:894-905.

22. Gentzkow GD, Iwasaki SD, Hershon KS, Mengel M, Prendergast JJ, Ricotta JJ et al. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. Diabetes Care 1996; 19:350-354.

23. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000; 21:2529-2543.

24. Gomes ME, Reis RL, Cunha AM, Blitterswijk CA, de Bruijn JD. Cytocompatibility and response of osteoblastic-like cells to starch-based polymers: effect of several additives and processing conditions. Biomaterials 2001; 22:1911-1917.

25. Fennrich S, Wendel A, Hartung T. A new application of the human whole blood pyrogen assay (PyroCheck) Altex 1999; 16:146-149.

26. Cima LG, Vacanti JP, Vacanti C, Ingber D, Mooney D, Langer R. Tissue engineering by cell transplantation using degradable polymer substrates. J Biomech Eng 1991; 113(2):143-151.

27. Sampath TK, Reddi AH. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci U S A 1981; 78:7599-7603.

28. Reddi AH. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. Tissue Eng 2000; 6:351-359.

29. Mann BK, Schmedlen RH, West JL. Tethered-TGF-beta increases extracellular matrix production of vascular smooth muscle cells. Biomaterials 2001; 22:439-444.

30. Shakesheff K, Cannizzaro S, Langer R. Creating biomimetic micro-environments with synthetic polymer-peptide hybrid molecules. J Biomater Sci Polym Ed 1998; 9:507-518.

31. Matsuzaka K, Walboomers XF, de Ruijter JE, Jansen JA. The effect of poly-L-lactic acid with parallel surface micro groove on osteoblast-like cells in vitro. Biomaterials 1999; 20:1293-1301.

32. Elcin YM, Dixit V, Gitnick G. Extensive in vivo angiogenesis following controlled release of human vascular endothelial cell growth factor: implications for tissue engineering and wound healing. Artif Organs 2001; 25:558-65.

33. Vercruysse KP, Prestwich GD. Hyaluronate derivatives in drug delivery. Crit Rev Ther Drug Carrier Syst 1998; 15:513-555.

34. Kirkpatrick CJ. New aspects of biocompatibility testing: where should it be going? Med Device Technol 1998; 9:22-29.

35. Van Kooten TG, Klein CL, Kirkpatrick CJ. Western blotting as a method for studying cell-biomaterial interactions: the role of protein collection. J Biomed Mater Res 2001; 54:385-389.

36. Attawia MA, Nicholson JJ, Laurencin CT. Coculture system to assess biocompatibility of candidate orthopaedic materials. Clin Orthop 1999; 365:230-236.

37. Krause A, Cowles EA, Gronowicz G. Integrin-mediated signaling in osteoblasts on titanium implant materials. J Biomed Mater Res 2000; 52:738-747.

38. Alpar B, Leyhausen G, Gunay H, Geurtsen W. Compatibility of resorbable and nonresorbable guided tissue regeneration membranes in cultures of primary human periodontal ligament fibroblasts and human osteoblast-like cells. Clin Oral Investig 2000; 4:219-225.

39. Bezemer JM, Grijpma DW, Dijkstra PJ, van Blitterswijk CA, Feijen J. A controlled release system for proteins based on poly(etherester) block-copolymers: polymer network characterization. J Control Release 1999; 62:393-405.

40. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, Freed LE, Vunjak-Novakovic G. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol Bioeng 1999; 64:580-589.

41. Gogolewski S. Bioresorbable polymers in trauma and bone surgery. Injury 2000; suppl 4:28-32.

42. Agrawal CM, McKinney JS, Lanctot D, Athanasiou KA. Effects of fluid flow on the in vitro degradation kinetics of biodegradable scaffolds for tissue engineering. Biomaterials 2000; 21:2443-2452.

43. Patel N, Padera R, Sanders GH, Cannizzaro SM, Davies MC, Langer R et al. Spatially controlled cell engineering on biodegradable polymer surfaces. FASEB J 1998; 12:1447-1454.

44. Boehncke WH. The SCID-hu xenogeneic transplantation model: complex but telling. Arch Dermatol Res 1999; 291:367-373.

45. EUCOMED Human Tissues Task Group. Industry position on human tissue product regulation. Brussels, Belgium.

46. Council of Europe. 2000. (draft) Guide on safety and quality assurance for organs, tissues and cells. Strasbourg, France.

47. Council Directive 98/463/EC of 29 june 1998 on the suitability of blood and plasma donors and the screening of donated blood in the European Community.

48. Committee for Proprietary Medicinal Products (CPMP). 2000. Position statement on the use of tumourigenic cells of human origin for the production of biological and biotechnological medicinal products. London, UK.

49. International Conference on harmonization (ICH) Topic Q5D. 1998. Note for guidance on quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological/biological products. London, UK.

50. International Conference on harmonization (ICH) Topic Q6B. 1999. Specifications: Test procedures and acceptance criteria for biotechnological/biological products. London, UK.

51. World Health Organization (WHO). 1999. WHO Expert Committee on Biological standardization. 47th Report.

52. Food and Drug Administration (FDA). 1999. Suitability determination for donors of human cellular and tissue-based products (proposed rule). FDA, 21 CFR 210, 211, 820 and 1271. Federal Register 64(189):52696-52723.

53. Food and Drug Administration (FDA). 2001. Current Good Tissue Practice for manufacturers of human cellular and tissue-based products. Inspection and Enforcement (proposed rule). 21 CFR 1271. Federal Register 66(5):1508-1559.

54. Food and Drug Administration (FDA). 2001. Human cells, tissues, and cellular and tissue-based products; Establishment registration and listing (final rule). 21 CFR 207, 807, 1271. Federal Register 66(13):5447-5469.

55. Food and Drug Administration (FDA). 1998. Guidance for human somatic cell therapy and gene therapy. Rockville, USA.

56. The Food and Drug Adminstration (FDA). 1997. Guidance for the submission of chemistry, manufacturing, and controls information and establishment description for autologous somatic cell therapy. Rockville, USA.

57. Cell and gene therapy products. Pharmacopeial Forum 2000; 26:56-124.

58. International Organization for Standardization (ISO). 1994. Biological evaluation of medical devices. EN ISO 10993.

59. European Committee for Standardization (CEN). 2000. Animal tissues and their derivates utilized in the manufacture of medical devices, CEN EN 12442.

60. International Conference on harmonization (ICH). 1997. Topic S6. Note for guidance on preclinical safety evaluation of biotechnology-derived products. London, UK.

61. Committee for Proprietary Medicinal Products (CPMP). 2001. Note for guidance on plasma-derived medicinal products. London, UK.

62. Committee for Proprietary Medicinal Products (CPMP). 2000. Development pharmaceutics for biotechnological and biological products- Annex to note for guidance on development of pharmceutics. London, UK.

63. International Conference on harmonization (ICH). 1995. Topic Q5B Note for guidance on quality of biotechnological prodcuts; analysis of the expression construct in cell lines used for production of r-DNA derived protein products. London, UK.

64. International Conference on harmonization (ICH). 1995. Topic Q5C Note for guidance on quality of biotechnological products: Stability testing of biotechnologicla/biological products. London, UK.

65. Committee for proprietary medicinal products (CPMP). 2001. Points to consider on the manufacture of human somatic cell therapy products (for adoption). London, UK.

66. Committee for Proprietary Medicinal Products (CPMP). 2001. Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products. London, UK.

67. Council Directive 2001/20/EC. 2001. On the approximation of the laws, regulations, and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

68. Therapeutic Goods Administration (TGA). 2000. Code of Good Manufacturing Practice- Human Blood and Tissues. Woden, Australia.

Appendix 1 Mailing list

1. Directorate-General of the National Institute of Public Health and the Environment
2. Chairman of the Health Council of The Netherlands
3. prof. dr. J.A. Jansen, Department of Biomaterials, College of Dental Science, University Medical Center Nijmegen, Nijmegen, The Netherlands
4. dr. J.L.C. van Susante and dr. P. Buma, Department of Orthopaedics, University Medical Center Nijmegen, Nijmegen, The Netherlands (2)
5. dr. P.B. van Wachem and dr. H. Leuven, Department of Cellbiology and Biomaterials, Faculty of Medical Sciences, Groningen University, Groningen, The Netherlands (2)
6. prof. dr. P.Wuisman, dr. I.C. Heyligers and dr. J. Klein-Nulend, Department of Orthopaedics, Vrije Universiteit Hospital, Amsterdam, The Netherlands (3)
7. dr. P. Brown, Genzyme Biosurgery, Naarden, The Netherlands
8. dr. W.J.A. Dhert, Utrecht University, Faculty of Medical Sciences, Department of Orthopaedics, Utrecht, The Netherlands
9. prof. dr. D. F. Williams, Department of Clinical Engineering, University of Liverpool, Liverpool, United Kingdom
10. dr. J. van Loon, Laboratory for Medical Device Evaluation, Bioscan, Bilthoven, The Netherlands
11. CEN Healthcare Forum (CHeF) Tissue engineering, CEN, Brussels, Belgium (20)
12. Vice-Chairman of the Division TEMPs of the American Society for Testing and Materials, Rockville, USA (3)
13. Chairman of the Working Group Tissue Engineering of the Scientific Committee for Medicinal Products and Medical Devices, EC DG SANCO, Brussels, Belgium (7)
14. EUCOMED, Woluwe St. Lambert, Belgium (3)
15. Dutch Standardization Body, Delft, The Netherlands (3)
16. Head of the Cluster Body materials, GMT, VWS, Den Haag, The Netherlands (4)
17. Head Inspector of Pharmacy and Medical Technology, IGZ, Den Haag, The Netherlands (3)
18. Depot Nederlandse Publikaties en Nederlandse Bibliografie, Den Haag, The Netherlands
19. dr. E. Putman, drs. P.M.J.M. Jongen and dr. R.M. van der Plas, RIVM
20. Director of Sector 3-4, RIVM
21. Head of the Laboratory for Medicines and Medical Devices (LGM), RIVM
22. Head of the Laboratory for Pathology and Immunobiology (LPI), RIVM
23. Head of the Department of the preclinical assessment group of medicines evaluation board (GMB), LGM, RIVM
24. Head of the Department of Biologicals (BTG), LGM, RIVM
25. dr. ir. H.J.G.M. Derks, cluster Medicinal Products and Medical Devices, RIVM
26. dr. M. Lambert, LGM, RIVM
27. Authors (4)

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