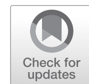

Corneal Tissue Engineering

3

Mohammad Mirazul Islam, Roholah Sharifi, and Miguel Gonzalez-Andrades

3.1 Introduction

Mankind has been always fascinated with the idea of restoring any damaged tissue or organ. In the Ancient Egypt, handmade prostheses were made of hardwood or cartonnage to restore the function of lost toes [1]. Regarding corneal functional restoration, the French ophthalmologist Pellier de Quengsy was the first one proposing in 1789 a replacement of an opaque cornea using a piece of glass surrounded by a silver ring [2]. However, the paradigm of corneal blindness treatment does not change until 1905, when Eduard Zirm performed the first corneal transplant to a patient implanting a donor cornea [3]. Corneal transplant is still the most used and reliable treatment for some corneal diseases [4]. Corneal diseases are one of the most important causes of blindness in developing countries, accounting for 4–8 million people that suffer bilateral corneal blindness [5, 6]. In 2015, only in the USA, more than 48,792 corneal transplants were carried out, which was 53% more than the transplants performed in 2005 [7]. In the USA, donor corneas are readily available for transplantation [8], although the whole world suffers a severe scarcity of donor corneas. This results in 10 million untreated patients worldwide, with an additional 1.5 million new patients every year to the waiting list [9].

Although the cornea is considered as an immune-privileged site of the body because of its avascularity, physiopathological changes such as corneal neovascularization or inflammation disrupt this immune status, which subsequently increases the risk of graft rejection after performing a corneal transplant. Even in non-vascularized and non-inflamed host eyes (low-risk cases), one in three of transplanted corneas eventually leads to rejection [10]. In high-risk cases, such as autoimmune diseases, chemical burns and infections, 50–70% transplants undergo rejection even with high doses of immunosuppressive drugs [11, 12]. Another major complication after corneal transplant is donor-derived infections [13]. Herpes simplex virus type-1 (HSV-1) DNA found from donor corneas before and after corneal transplantation confirmed the spread of HSV-1 through the transplant from donor to recipient [14]. In this context, the microbial testing, the administration and the shipping of a donor cornea can cost $3000 in the USA [15], becoming an unaffordable treatment option for most of the people who live in developing nations. 90% of the visually impaired people live in low-income countries, and most of them (53% of the world population) do not have access to transplantation facilities [16, 17].

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Therefore, there is a great need of finding new therapeutic strategies to address the three major drawbacks of corneal transplantation: the scarcity of donors, the risk of rejection and the transmission of infectious diseases after implantation into the host. In this milieu, corneal tissue engineering (TE) emerges with the ambition of generating artificial corneas or other types of tissue-engineered products that lead to an optimal corneal regeneration, overcoming those major disadvantages of allogeneic corneal transplants. TE was defined in 1988, in a workshop on TE organized by the University of California, Los Angeles, as "the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue functions" [18–20]. Engineering effectual building blocks and assembling them to perform in a unified architecture is the bedrock for the generation of fully functional biological substitutes. Such constructs should also be able to communicate with the other tissues and organs that surround it to coordinate a unified function [21]. This demands profound knowledge in material science, including material interactions with cells and their microenvironment. Especially, this is crucial in ophthalmology where besides physical and chemical properties of the tissues, optical characteristics as well as architectural design dictate the ultimate outcome.

3.2 Corneal Structure-Function Relationships

Firstly, we need to understand the structure-function relationships in the cornea, in order to develop an optimal corneal substitute. The cornea is the outermost part of the eye and plays an important role for vision by transmitting the light to the retina while protecting the interior components of the eye from external aggressions. The cornea is composed of three primary cellular layers, the outermost epithelium layer, a middle stroma containing keratocytes, and an innermost single layer of endothelial cells called endothelium [22]. Two acellular layers separate these cellular layers: Bowman's layer and Descemet's membrane. The extracellular matrix (ECM) of the corneal stroma is mainly composed by collagen and proteoglycans disposed in a highly specific arrangement. This specific matrix arrangement grants the cornea its characteristic transparency and physical structure, which allow an optimal vision and support the intraocular pressure without deforming [23]. ECM is also intimately associated with corneal innervation. Corneal nerves cross through the corneal stroma toward the epithelium. Epithelial innervation plays a vital role in functional activities of the cornea such as preserving the viability and differentiation of the corneal epithelium, apart from their role in tear production and blinking [24]. Moreover, the optimal composition and porosity of the corneal stroma allow the diffusion of nutrients and other solutes from the posterior to the anterior region of the cornea [25, 26]. Corneal nutrition is complemented by the tears [27]. The local immunity of the cornea is conditioned to its avascularity. Antigen-presenting cells like dendritic cells are present in the cornea. These cells are involved in T-cell-mediated immune responses associated with corneal graft rejection. Natural killer cells also participate in the allograft rejection [28, 29]. Graft rejection starts when the host immune system is activated against antigens in the donor corneal tissue through different pathways (for review see Refs. [30, 31]).

3.3 Development of Tissue-Engineered Corneal Substitutes

Once we understand the structure-function relationships in the cornea, we can generate a tissue-engineered corneal substitute to restore, maintain, or improve corneal functions, using different building blocks: cells, scaffolds and bioactive molecules.

3.3.1 Cells

As it was discussed earlier, healthy cellular layers are necessary for the precise function of the human cornea. In case of cell injury or loss, stem cells need to regenerate and repopulate the dam-

aged area. Stem cells are indispensable players for the regeneration of any part of the body. In the cornea, different types of stem cells reside in the limbus area with the capacity to regenerate the corneal epithelium [32] and the stroma [33, 34]. Limbal stem cells (LSCs) can differentiate into corneal epithelial cells after isolating and culturing them from small biopsies of healthy limbal areas [35–37]. Different substrates or carriers can be used to culture and deliver LSCs in cases of limbal stem cell deficiency (LSCD) including human amniotic membranes [38], fibrin substrates [39] and collagen-based materials [40]. This therapeutic approach receives the name of cultured limbal epithelial transplantation (CLET). This expansion of LSCs for transplantation requires certified good manufacturing practices facilities and procedures, which limit the expansion of this therapeutic approach because of the high cost, especially in developing countries [41].

In cases of suffering bilateral LSCD with no healthy limbal area to obtain an optimal biopsy, only allogeneic limbal tissue can be used for performing CLET. To avoid the use of allogeneic cultured cells and its inherent risk of immune rejection, other types of stem cells that do not reside in the limbal area are emerging as possible future cell source for autologous CLET (Table 3.1). Conjunctival epithelial cells from biopsies cultured and expanded *in vitro* on contact lens were used to treat one patient, who improved visual acuity with no recurrence of corneal vascularization [42]. The possibility of treating LSCD using *in vitro* cultivated oral mucosa autograft has been widely studied in at least 20 clinical trials in different countries. Accumulated results from those clinical trials showed that 242 patients received this treatment with a success rate of 72% [43]. Nasal mucosal epithelial cells also showed promising results when transplanted, in two different clinical trials [44, 45]. Cultured human immature dental pulp stem cells reconstructed the eye surface in limbal stem cell-deficient rabbits [46, 47]. Hair follicle bulge-derived stem cells from transgenic mice also showed cornel epithelial cell differentiation in a LSCD model [48]. Human Wharton's jelly stem cells also showed potential to differentiate into corneal

Table 3.1 Human cells other than limbal stem cells evaluated for corneal epithelial regeneration

Source of cells	*In vitro/in vivo*	References
Conjunctival stem cell	*In vivo* (human)	Ang et al. [55]; Sangwan et al. [56, 57]; Subramaniam et al. [58]; Tan et al. [59]
Oral mucosal epithelial cells	*In vivo* (human)	Burillon et al. [60]; Inatomi et al. [61]; Nakamura et al. [62]; Nishida et al. [63]; Takeda et al. [64]; Utheim [65]
Nasal mucosal epithelial cells	*In vivo* (human)	Chun et al. [44]; Kim et al. [45]
Dental pulp stem cells	*In vivo* (rabbit)	Gomes et al. [46]; Monteiro et al. [47]
Hair follicle bulge-derived stem cells	*In vivo* (mouse)	Meyer-Blazejewska et al. [48]
Wharton's jelly stem cells	*In vitro*	Garzon et al. [49]
Embryonic stem cells	*In vitro*	Ahmad et al. [66]; Zhang et al. [67]
Umbilical cord stem cells	*In vivo* (rabbit)	Reza et al. [50]; Reza et al. [68]
Bone marrow-derived MSC	*In vivo* (Rat)	Ma et al. [20]; Rohaina et al. [52]
Orbital fat-derived MSC	*In vivo* (mouse)	Lin et al. [53]
Dermal fibroblast-derived iPS cells	*In vitro*	Hayashi et al. [54]
Corneal limbal epithelial cell-derived iPS cells	*In vitro*	Hayashi et al. [54]

epithelial-like cells on fibrin-agarose-based stromal substitutes [49]. Transplantation of human umbilical cord stem cells in LSCD rabbit eyes resulted in healthy corneal surface with positive marker expression for corneal epithelial cells [50]. Human bone marrow-derived mesenchymal stem cells (MSC) were able to differentiate to corneal epithelial cells *in vitro* and *in vivo*, showing their capability to replace limbal epithelial stem cells [20, 51, 52]. Orbital fat-derived MSC also promoted corneal tissue regeneration through corneal epithelial differentiation [53]. Human adult dermal fibroblast-derived induced pluripotent stem (iPS) cells and human adult corneal limbal epithelial cell-derived iPS cells were

also tested for differentiation into corneal epithelial cells, which revealed that corneal epithelial differentiation efficiency was higher in limbal-derived iPS cells [54].

Keratocytes quiescently reside within collagen lamellae in the stroma of a healthy cornea, synthesizing ECM components, such as collagen and proteoglycans [69, 70]. In the damaged or injured cornea, keratocytes transform into mitotically active fibroblasts [71, 72] and start producing unorganized ECM which ultimately turn into fibrotic tissue, which might lead to vision loss [73]. Keratocytes can be isolated and cultured under specific conditions using corneal biopsies, which can be digested or directly cultured applying an explant-based technique [74, 75]. Keratocytes can also be obtained from MSC isolated from limbal biopsies [33, 34]. They can synthetize aligned collagen and keratan sulfate proteoglycans, being able to reconstitute a fibrotic area in *in vivo* models, without inducing inflammation, vascularization, or rejection [76–78]. iPS cells can be also differentiated to neural crest cells and then cultured on corneal tissue to promote keratocyte differentiation [79].

The corneal endothelium is a monolayer of cells that lines at the posterior corneal surface, which are responsible for pumping out excess amount of water from the corneal stroma and prevent it from swelling [80, 81]. Its failure usually requires a donor endothelial transplant because of the very limited proliferative capacity of these cells to self-regenerate the damaged area. However, under specific conditions, endothelial cells can proliferate and cultured *in vitro*. Shigeru Kinoshita and co-worker described a new promising approach based on the inhibition of ROCK (Rho kinase), which enhances endothelial cell proliferation, promotes cell adhesion, suppresses apoptosis and promotes wound healing. In 2013, they have started a clinical trial to evaluate cultured human endothelial cells in combination with a ROCK inhibitor as treatment for corneal endothelial dysfunction. Recently, they reported their initial results, suggesting that this therapeutic option is safe and effective [82].

3.3.2 Scaffolds

Engineered 3D scaffolds not only can substitute a damaged cornea, providing mechanical and structural stability, but also provide the appropriate microenvironment for the cells to regenerate the tissue. Conceptually, scaffold is an engineered template, which can mimic the ECM of the native tissue and imitate the *in vivo* setting, supporting the cells to proliferate, migrate and create their own microenvironment [83].

The ideal corneal scaffold should (1) be transparent for restoration of vision; (2) be biocompatible and support cellular adhesion, proliferation and migration; (3) have similar biomechanical properties to the human cornea to maintain its shape, critical for an optimal vision, and harmonically respond to the intraocular pressure fluctuations; (4) preserve its smooth surface in order to avoid scattering of light; (5) have biodegradation properties that match the time of tissue remodeling and biointegration; (6) have a refractive index similar to the cornea; (7) possess appropriate porosity and diffusion for nutrients, while serving as a microbial barrier; and (8) be cost-effective in terms of manufacturing process and implementation [84].

The scaffolds explored in ophthalmology for corneal substitution can be categorized into three classes: synthetic, natural-based and hybrid materials. Polyethylene glycol [85], acrylate-based polymers [86], polyesters [87], polydimethylsiloxane [88], polyvinyl alcohol-based polymers [89] and polyamides [90] are the main studied synthetic materials for corneal substitutes. Although these synthetic polymers have tunable chemical and mechanical properties that can be matched to the medical needs, their biomimetic properties required for cell adhesion, proliferation and effectual integration with the host tissue need significant improvement before their translation in the clinical settings. In addition to their non-biodegradable nature, their inability to carry cells and biointegrate during tissue healing and remodeling stands as their main challenge [91]. The continued progress in the engineering of novel biomaterials, along with personalized modifications and the design

of hybrid materials composed of synthetic and natural polymers, might address such shortcomings and can facilitate their widespread applications in the clinic.

On the other hand, natural-based biomaterials present intrinsic biocompatibility and biodegradability along with appreciable degree of biomimetic properties and biological functions. The most studied natural-based corneal scaffolds are protein- or polysaccharide-based scaffolds. Collagen is one of the most studied protein-based scaffolds for artificial cornea. This stems from collagen's biocompatibility, low toxicity, and well-studied structural, physical, chemical and immunological characteristics alongside with maintaining arginine-glycine-aspartic acid sequences in its structure that promotes cell adhesion to the scaffold [92]. Although collagen-based scaffolds demonstrated such promising properties [93–95], their biomimetic characteristics still require an enhancement to totally match those found in specific native tissues [96]. Although improving mechanical and biomimetic properties of hydrogel is an ongoing challenge, engineering new hybrid scaffolds and integrating the biological cues might be a key to unlock their potential as a true corneal substitute. Gelatin [97], fibrin [98] and silk [99] are among the other protein-based biopolymers that have also been explored as possible candidates for corneal substitute. While each class possesses different characteristics, their mechanical properties are significantly inferior compared to those of the native cornea and unable to support integrity of injured cornea. Polysaccharide-based materials (e.g., chitosan [100], chondroitin sulfate [101], dextran [102], hyaluronic acid [103], alginate [104], etc.) have also been explored in corneal tissue engineering. Despite their superior mechanical and optical properties, they fall short in providing 3D microenvironment for effective cell adhesion and proliferation. Therefore, polysaccharide-based materials have not been yet able to offer an effective solution for corneal substitute.

Although both synthetic and natural-based scaffolds offer an initiative window to develop an effective artificial cornea, such scaffolds lack the complexity of the 3D microenvironment of the corneal native tissue in terms of not only physical and chemical properties but also composition gradients, alignment, directionality and microarrangement manifested in the human corneal stroma. The emulation of such biomimetic characteristics is very crucial in tissue engineering and dramatically dictates biointegration of artificial corneas with the host tissue and defines the ultimate clinical outcome. Different strategies have been described in the literature to address some of those challenges such as the use of self-assembly or auto-generation of artificial matrixes *in vitro*.

Peptide amphiphiles (PA) are engineered synthetic molecules constituted from a hydrophilic peptide sequence and hydrophobic long chain, which can self-assemble to generate nanofibers. The non-covalent interaction of such nanofibers via intermolecular forces can lead to the formation of 3D networks. The immense programmability of PA to hold different functional groups enables to generate 3D scaffolds, such as collagen hydrogels, that, in principle, can mimic ordering and complexity of native tissue [105–108]. Integrating such self-assembled structures within various hydrogel offers a precious tool to introduce highly complex multifunctional hydrogels for ophthalmic surgery. Another approach to introduce such nanoscale organization is auto-generation. This concept is based on engineering an *in vitro* culture system that stimulates synthesis of an *in vivo*-like stromal matrix that can lead to the generation of highly organized collagen-based corneal stroma [109, 110]. Moreover, it allows to seed epithelial and endothelial cells on the synthesized scaffold to create a functional, organotypic cornea. In this regard, human mesenchymal stem cells derived from the limbal stroma were cultured in specific culture media, leading to rapid expansion and differentiation into keratocytes and ultimately generating an organized thick lamellar stroma-like tissue containing aligned collagen and keratan sulfate proteoglycans [76]. The constructs synthesized by these cultured cells, however, also present poor mechanical properties and need significant improvement prior to their application in ophthalmic surgery.

Incompetence of bioengineered scaffolds as yet to fulfill the required properties of corneal substi-

tute is the main driving force to study other strategies in parallel such as the use of modified xenografts. Xenogeneic tissues and organs often contain cellular antigens, which can be recognized as foreign by the host tissue and consequently leads to an inflammatory response or an immune-mediated rejection [111]. Decellularization of donor tissue to remove the inhabiting cells and its cellular debris from the ECM of the tissue is a practical strategy to obtain acellular scaffolds of the ordinal tissue and bypass such adverse immune response [112]. Besides intimate resemblance of microarchitecture of xenogeneic corneas with humans, their availability, lower cost and comparable optical and mechanical properties are the main momentum to envisage their application for human corneal substitution. Moreover, it can support construction and host-guest tissue remodeling and bypass the stimulation of inflammation while avoiding scar tissue formation [113–116]. Different animals have been used as source of corneal tissue for decellularization process. Due to availability and the structural similarities between the porcine and human cornea, the domestic pig is the most commonly used animal to obtain decellularized corneal xenografts [117]. Although various chemical and physical techniques have been explored to decellularized animal corneas, they often alter the chemical, physical and biological properties of the ECM via cleaving the collagen fibers and disrupting the matrix ultrastructure or partially eliminating key matrix constituents such as glycosaminoglycans and growth factors and adversely affect its natural properties [112, 118]. Such structural disruptions along with chemical, mechanical and biological variations between porcine and human cornea are the main challenges, preventing their successful translation into the clinic [119].

3.3.3 Bioactive Molecules and Other Environmental Conditions

There are several soluble factors directly involved in the process of proliferation and differentiation of corneal cells. In this context, significant efforts have been carried out to understand the control mechanisms of self-renewal and fate decision of LSCs. There are growing evidences supporting that LSCs are highly regulated by their stem cell niche. LSC niche is a specific microenvironment that comprises cellular and noncellular components that regulate the stem cell pluripotency, proliferation, differentiation, survival and localization. LSC niche is located at the palisades of Vogt of human corneoscleral limbus. Different growth factors also play important role in the differentiation of the stem cell to progeny. In this regard, insulin-like growth factor I (IGF-I) has been identified as the main factor responsible for LSC differentiation into mature corneal epithelial cells after injury. Furthermore, some researchers have demonstrated that IGF-I showed synergistic effect with the neuropeptide substance P in proliferation and wound healing of corneal epithelium [120]. Corneal epithelium also produced fibroblast growth factor and epidermal growth factor (EGF) to support LSC proliferation devoid of affecting differentiation [121]. EGF heparin-binding EGF and amphiregulin have been also shown to stimulate epithelial wound repair by binding to a common EGF receptor [122]. Hepatocyte growth factor is also an important factor expressed by epithelial cells and keratocytes after corneal epithelial injury, which influences the proliferation, migration and apoptosis of corneal epithelial cells [123–125]. Keratinocyte growth factor also plays important role in epithelial wound healing through MAP kinase and PI3K/p70 S6 signaling cascade [126]. Moreover, transforming growth factor-β (TGF-β) expressed by corneal epithelium and stromal cells has mixed effect on corneal cells, inhibiting epithelial cell proliferation [127] and stimulating fibroblast proliferation [128]. TGF-β also showed to influence myofibroblast differentiation of cultured primary keratocytes and corneal fibroblast cell line [129]. Platelet-derived growth factors expressed by differentiated corneal epithelium *in vitro* regulate the proliferation and migration of corneal fibroblast [130]. Nerve growth factor (NGF) is a neurotrophic factor expressed in the corneal epithelium that promotes cell proliferation and wound healing.

NGF improved epithelium restoration in patients with neurotrophic ulcers [131] and after cataract surgery [132]. NGF also showed nerve regeneration in a mechanical nerve injury mouse model established by laser-assisted in situ keratomileusis [133]. Opioid growth factor (OGF) is another growth factor expressed by basal and suprabasal layers of epithelium that binds OGF receptor to inhibit DNA synthesis, cell migration and tissue repair of the corneal epithelium [134]. Important growth factors with their key physiological functions are summarized in Table 3.2.

Not only growth factors but also culture conditions are key in the proliferation and differentiation of corneal cells. Concentration of carbon dioxide in culture conditions critically alters cell differentiation. It was shown that 7% CO_2 in the culture positively influences the differentiation of embryonic stem cell to corneal epithelial progenitor cells [67]. Hypoxic condition is also an important factor that influences the differentiation of limbal stem cells by downregulating Polo-like kinase 3 (Plk3) signaling activity at the transcription level [151]. Co-culturing conditions also positively influence cell growth, as survival and proliferation of LSCs are promoted when these are co-cultured with bone marrow MSC [152].

3.4 Clinical Experiences and International Regulations

Very few tissue-engineered products have been translated into the clinic. Some of the organs where tissue-engineered substitutes have been successfully applied are the trachea [153], blood vessels [154], the urinary bladder [155], and the cornea [4]. Regarding the cornea, some notable mentions are as follows: (1) autologous limbal stem cells were collected from the healthy contralateral eye and expanded on a fibrin substrate and finally transplanted in 112 patients with LSCD. Restoration of a transparent cornea with a restored corneal epithelium was achieved in 76.6% of eyes and 21 patients achieved permanent visual recovery of at least 0.6 [36]; (2) as a phase 1 clinical trial, femtosecond laser cut anterior corneal stroma was decellularized and transplanted in patients with keratoconus. Four out of nine patients received a decellularized stroma seeded with autologous adipose-derived adult stem cells. Haze or scarring was not observed by 3-month postoperative follow-up, and patients got visual improvement after 6 months of the graft [156]; (3) recombinant human collagen (RHC)-based acellular artificial corneas

Table 3.2 List of growth factors that influence corneal regeneration

Growth factors	Key function	References
Epidermal growth factor	Cell migration, proliferation and wound healing of corneal epithelial cells	Zieske et al. [122]; Nakamura et al. [135]
Hepatocyte growth factor	Cell migration, proliferation and wound healing. It inhibits apoptosis of corneal epithelial cells	Wilson et al. [123]; Daniels et al. [124]; Yanai et al. [136]
Keratinocyte growth factor	Epithelial homeostasis and wound healing	Chandrasekher et al. [137]
Insulin-like growth factor	Cell growth, energy metabolism, migration, differentiation, proliferation and survival of corneal epithelial cells	Lee et al. [136]; Trosan et al. [138]; Yanai et al. [139]
Transforming growth factor-β	Inhibition of corneal epithelial cell proliferation. It stimulates stromal fibroblast proliferation	Pancholi et al. [127]; Haber et al. [128]; Andresen et al. [140]; Kay et al. [141]
Platelet-derived growth factors	Migration and proliferation of keratocytes	Denk and Knorr [130]; Kamiyama et al. [142]; Daniels and Khaw [143]
Thymosin-β4	Wound healing in corneal epithelial defects. It decreases inflammation and inhibits apoptosis	Sosne et al. [144, 145]; Dunn et al. [146]
Nerve growth factor	Epithelial and stromal healing, anti-inflammatory effect and recovery of corneal nerves	Lambiase et al. [147]; Lambiase et al. [148]; Joo et al. [149]
Opioid growth factor	Inhibitory effect on corneal epithelial cell proliferation, migration, and tissue organization	Zagon IS et al. [150]

have been transplanted in a clinical trial on ten patients; nine of them had keratoconus and one patient with permanent mid-stromal scar. The implants promoted regeneration of corneal epithelium, stroma, and nerves from host cells. The transplanted cornea remained stable for 4 years without any rejection and without sustained immune suppression. Implanted patients had a 4-year average corrected visual acuity of 0.37 [4, 157]; (4) acellular interpenetrating polymer networks of RHC and 2-methacryloyloxyethyl phosphorylcholine (MPC) have been transplanted in three patients with corneal ulcers and recurrent corneal erosions. The implants provided relief from pain and discomfort, restored corneal integrity, and improved vision in two out of three patients [158]. Another clinical trial has been completed with this materials on January 2017 (CT.gov identifier:NCT02277054). The result showed that all patients improved from pain and discomfort within 1–2 weeks after transplantation. Corneal sensitivity regained, and overall vision improved significantly in half of the study patients, and even if the vision was not enhanced, transplants made the cornea stable for further surgery to improve vision [159]; and (5) there is a randomized, controlled, open-label clinical trial going on in different Spanish hospitals (CT.gov identifier:NCT01765244) to test a fibrin-agarose corneal substitute combined with allogeneic corneal epithelial cells and keratocytes [160].

In the translation of tissue-engineered products into the clinic, the different regulatory agencies have created a regulatory framework that controls and guarantees the correct and ethical use of these therapies in humans, protecting not only the patients but also the clinicians who are applying the treatment. The European Medicines Agency (EMA) includes tissue-engineered products under the definition of advanced therapy medicinal products, which are defined as medicines for human use that are based on genes, cells, or tissue engineering [161]. According to European Parliament regulations and the EMA guidelines [51], "Tissue engineered product means a product that: contains or consists of engineered cells or tissues, and is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue. A tissue engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices." This definition together with other aspects contemplated in the European regulations is shown in Fig. 3.1. Regarding the clinical trials previously mentioned, only decellularized stromas seeded with autologous adipose-derived adult stem cells, autologous epithelial limbal stem cells cultured on fibrin scaffolds, and fibrin-agarose corneal substitutes with allogeneic cultured corneal cells would be considered tissue-engineered products according to EMA guidelines.

The Food and Drug Administration (FDA) is responsible for the regulation of medical products, including tissue-engineered products, in the USA. FDA regulated medical products under the separate categories of devices, biologics, and drugs. According to FDA, human tissue intended for transplantation, such as a donor cornea, is regulated as a human cell, tissue, and cellular and tissue-based product or HCT/P [162]. Tissue-engineered products usually consist in the combination of two or more components that belong to different categories in the FDA regulation, falling into the category of combination products [163]. Tissue-engineered products based on biomaterials in conjugation with cells would fall in this category. Recently, FDA announced new guidance documents for comprehensive regenerative medicine policy, defining the regulatory requirements for devices used in the recovery, isolation and delivery of regenerative medicine advanced therapies (RMATs), including combination products and the description of those regenerative medicine therapies that may be eligible for RMAT designation, including cell therapies, therapeutic tissue-engineered products, human cell and tissue products, and combination products using any such therapies or products, as well as gene therapies that lead to a durable modification of cells or tissues (including genetically modified cells) [164].

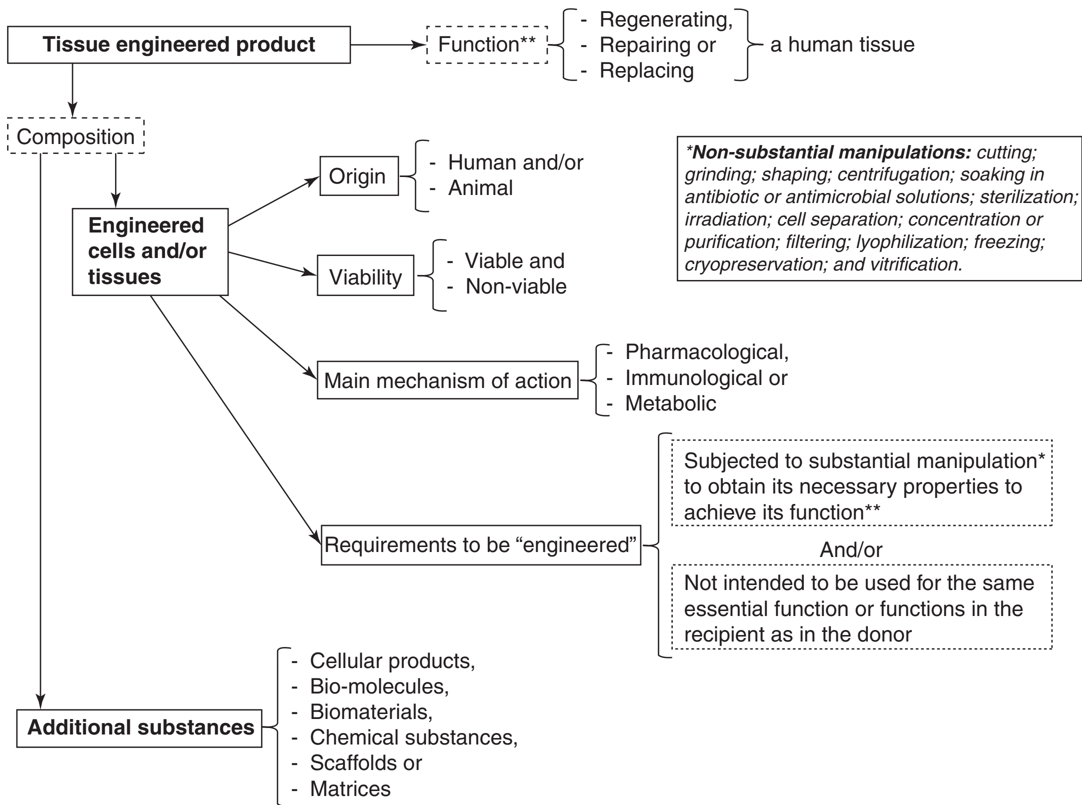

Fig. 3.1 Definition of tissue-engineered product according to the European regulations [51]

3.5 Conclusions

Here we briefly highlight the corneal structure-function relationships and the principles to develop a biological substitute of the human cornea by tissue engineering, including some treatment options for corneal diseases based on specific tissue-engineering strategies. Moreover, we explained the concepts and regulations necessary to understand the future clinical impact of tissue engineering in ophthalmology. The next chapters of this book will elaborately explain the use of cell and tissue-engineering therapies to be surgically applied to different corneal diseases.

Compliance with Ethical Requirement Statements Mohammad Mirazul Islam, Roholah Sharifi, and Miguel González-Andrades declare that they have no conflict of interest. No human or animal studies were carried out by the authors for this article.

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