The History of Tissue Engineering and Regenerative Medicine in Perspective

1

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Contents

1.1 History

The artificial generation of tissues, organs, or even more complex living organisms was throughout the history of mankind a matter of myth and dream. During the last decades this vision became feasible and has been recently introduced in clinical medicine. Tissue engineering and regenerative medicine are terms for the field in biomedicine that deal with the transformation of these fundamental ideas to practical approaches. Several aspects of generating new tissues and organs out of small pieces of living specimens are now scientifically solved, but at this point it is unknown how much impact these new approaches will have on clinical medicine in the future. In this respect it seems important to recapitulate from where the visions and the work came, in order to speculate or predict where tissue engineering and regenerative medicine will head.

The concept of tissue engineering and regenerative medicine as measures to create more complex organisms from simpler pieces is deeply embedded in the people's imaginary world. A change in the vision, hope, and believe of how to create or regenerate complex organs or organisms can be observed during history as a mirror of the cultural history of mankind. Even the early history of men is related to the idea that independent life can be created without sexual reproduction. Stories from Greek mythology [the creation of persons without sexual reproduction, e.g., the generation of Prometheus (Fig. 1.1)] may be considered as early reports representing the idea of creating living creatures from living or nonliving specimens. The Biblical tale of Eve created from Adam's rib is a further and perhaps the most well-known

Fig. 1.1 The generation of Prometheus

Fig. 1.2 Healing of Justinian

Fig. 1.3 Theophrastus von Hohenheinm

example of this concept [1] (in a modern view a kind of hybrid cloning). A multitude of examples in literature and the arts mirrors the desire of humans to be able to create by themselves living individuals or at least parts of individuals. The envisioned measures to create life are influenced by the social, cultural, and scientific background of individual persons at that time.

The famous painting "Healing of Justinian" (Fig. 1.2) a visualization of the legend of St. Cosmas and St. Damien (278 AD) depicting the transplantation of a homograft limb onto an injured soldier, is one early instance of the vision of regenerative medicine. As humans progressed in the understanding of nature and as they developed more advanced culture techniques they envisioned the generation of living creatures by applying physicochemical or biological techniques. During the transformation from the Middle Ages to the Renaissance in Europe, there was the hope and belief by a number of scientists that through alchemy living organisms could be generated. Theophrastus von Hohenheim, better known as Paracelsus (Fig. 1.3), tried (and failed) to find a recipe to create human life by a mixture of chemical substances in a defined environment.

Johann Wolfgang von Goethe (1749–1832) deals in his fundamental work of literature *Faust* [2] with the relation of an individual (Faust) to knowledge, power, morality, and theology. One central theme in the struggle of Faust to be powerful is the deeply embedded wish to create life. The creation of the artificial being Homunculus in Goethe's *Faust* is a central part of the drama, by which Goethe reveals various transformational processes working in the human soul. In the famous laboratory scene of *Faust (Part II)* he describes the vision of men being able to create life by alchemy (Fig. 1.4), representing the irrepressible human dream of "engineering" life:

Look there's a gleam! – Now hope may be fulfilled,
That hundreds of ingredients, mixed, distilled –
And mixing is the secret – give us power
The stuff of human nature to compound
If in a limbeck we now seal it round
And cohobate with final care profound,
The finished work may crown this silent hour

Fig. 1.4 Depiction of Dr. Faustus and his Homunculus

It works! The substance stirs, is turning clearer!
The truth of my conviction passes nearer
The thing in Nature as high mystery prized,
This has our science probed beyond a doubt
What Nature by slow process organized,
That have we grasped, and crystallized it out.

The description of the creation of Homunculus is also of special concern today, since it is suggestive of many contemporary "Faustian" technologies, such as cloning, genetic, or stem cell techniques in modern tissue engineering and regenerative medicine. With respect to an historical view of tissue engineering, Faust is a representative of Northern European humanity striving for evolution from the scientific and ethical limitations and strictures of the 16th century Reformations to the new aspirations of humanity that Goethe saw developing during the 18th century Enlightenment era. He was attracted to the idea of creating life by adding substances to nonliving specimens, similar to visions of how God created Adam, visualized by the famous painting of Michelangelo (Fig. 1.5). Goethe struggles to weave the personal inner journey of Faust towards some enlightenment (described in the prologue):

I've studied now Philosophy,
And Jurisprudence, Medicine,
And even alas! Theology
All through and through with ardour keen!
Here now I stand, poor fool, and see
I'm just as wise as formerly.
Am called a Master, even Doctor too,
And now I've nearly ten years through
Pulled my students by their noses to and fro
And up and down, across, about,
And see there's nothing we can know!

thereby being in the context of the collective social forces that are undergoing transformation through the historical processes of that time. As Faust deals with nearly all aspects and questions that arise in tissue engineering and regenerative medicine (and that are discussed in the first chapter of this book), it can

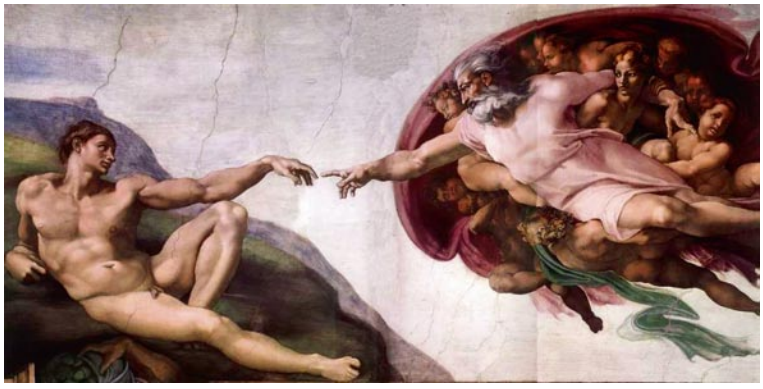

Fig. 1.5 Michelangelo's painting The Creation of Adam

be considered to be a timeless and always relevant consideration on the field of biomedicine.

Later on, as science and medicine progressed, a multitude of stories, reports, paintings, and films dealt with the idea that humans could create life by modern "scientific" measures. A prominent newer example in literature and film is the story of Frankenstein, written by Mary Shelley in 1818 (Fig. 1.6), describing the vitalization of a creature, reassembled from different body parts.

Parallel to the mythological, biblical, and fictional reports, various persons performed pioneering practical work to generate, heal, or regenerate body parts. The emergence of tissue engineering is, through their work, closely connected with the development of clinical medicine (prosthetics, reconstructive surgery, transplantation medicine, microsurgery) and biology (cell biology, biochemistry, molecular biology, genetics).

The mechanical substitution of body parts by nonvital prosthetic devices (metallic and ivory dentures, wooden legs) can be considered as early efforts to use biomaterials in reconstructive medicine. The first attempts to replace teeth in the sense of modern dental implantology seems to go back as early as in the Galileo-Roman period. The anthroposophic finding of a human skull, containing a metallic implant in the jaw [3], is indicative of early attempts of humans to regain lost function by tissue substitution. Leading areas of reconstructive medicine in clinical use were evident in the age before modern dentistry and orthopedics. Ambroise Pare` (1510–1590) described in his work *Dix livres de la chirurgie* [4] measures to reconstruct teeth, noses, and other parts of the body. A common method in the 18th century to replace teeth was the homologous transplantation of teeth in humans. John Hunter (1728–1793) investigated in his pioneering work the effect of transplantation not only at a clinical level (he claimed, that homologous transplanted teeth lasted for years in the host) but also performed animal experimental work on the fate of transplants, thereby setting the basis for a scientific approach on transplantation medicine [5].

A milestone in the modern view of tissue engineering was the use of skin grafts. The use of skin grafts is closely related to the work of the famous surgeon Johann Friedrich Dieffenbach (1792–1847). As he performed animal experimental and clinical work on skin transplantation (described in *Nonnulla de Regeneratione et Transplantatione* [6]), and as he also established ways to use pedicled skin flaps (since most of the clinical skin transplantation treatments failed), Dieffenbach is one of the modern founders of plastic and reconstructive surgery and can also be considered to be an early practitioner in transplantation medicine. Breakthroughs in the clinical use of skin grafts were made by Heinrich Christian Bünger, first successful autologous skin transplantation [7]; Jaques Reverdin (1842–1929), use of small graft islets; and Karl Thiersch (1827–1895), split thickness grafts [8, 9]. The high number of failures were overcome by the observation of Esser (1877–1964) that immobilization of transplants through the use of dental impression materials improves the fate of transplants in facial wound reconstruction. The clinical efforts reached through the combined use of surgical and dental techniques in reconstructive surgery and transplantation medicine led to the evolution of the dental- and medical-based Maxillofacial and Plastic Facial Surgery discipline. The foundation and establishment of this new specialty at the Westdeutsche Kieferklinik in Düsseldorf and the extensive experience in this center with injured soldiers during the

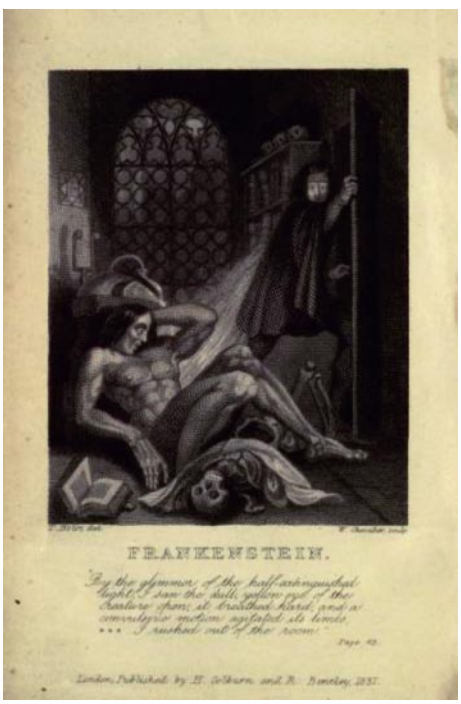

Fig. 1.6 Book cover of Frankenstein (Edition 1831)

First and Second World War led to significant improvements in tissue regeneration and reconstruction in the plastic and reconstructive surgery field. The underlying biological reason for the success of clinical skin transplantation by refining the transplantation approach (the shift from enlarged grafts to small cell-containing particles, the invention of fixation protocols) was in the beginning to a great extent unknown. Enlightenment into the biological mechanisms that accounted for the fate of transplants was provided by the fundamental biological work of Rudolf Virchow (1821–1902). He described in his *Cellularpathologie* [10] that tissue regeneration is dependent on cell proliferation. His work led not only to the investigation of tissue healing through cellular effects, but also to the cultivation of cells outside the body (in vitro, first suggested by Leo Loeb [11]). C.A. Ljunggren and J. Jolly were the first researchers to attempt to cultivate cells outside the body [12]. The milestone breakthrough in in vitro cell cultivation was reached by R.G. Harrison (1870–1959), demonstrating active growth of cells in culture [13]. Since that time, cell biology and especially in vitro cell culture became the mainstay of what can be considered classical tissue engineering [14].

Underlying in vitro cell culture with subsequent cell transplantation, modern tissue engineering and regenerative medicine is directly connected to microsurgery. Alexis Carrel (1873–1944) can be considered the founder of modern organ transplantation due to his work elaborating the methods of vascular anastomosis [15, 16]. The use of microvascular surgery was primarily performed in organ transplantation and plastic surgery. Whole organ transplantation or transplantation of body parts were made possible by this technique. E. Ullman (first kidney transplantation in animals [17]) and J.P. Merrill (first successful clinical kidney transplantation in identical twins) are directly related to the advances in transplantation medicine [18]. One of the most well-known milestones in organ transplantation medicine was the first heart transplantation 1967 by the South African surgeon Christiaan Barnard. His life—saving transplantation not only had extensive coverage in the newspapers at that time, but also raised an intensive and controversial debate on ethical issues in transplantation medicine [19]. Whereas the indications for microvascular tissue transplantation were extended towards plastic and reconstructive surgery (use of free myocutaneous, osteomyocutaneus, and other vessel containing flaps), and measures to perform microsurgery were refined and standardized, failures in clinical transplantation medicine were mainly based on immune incompatibilities (except for the use of autografts). The success of transplantation medicine, whether cells, tissues, or organs are in use was, and still is, to a great extent dependant on the immune state of graft and host. The science of immunomodulation and immunosupression is therefore still a critical aspect in all tissue engineering and regenerative medicine applications.

The term "tissue engineering" was up to the mid 1980s loosely applied in the literature in cases of surgical manipulation of tissues and organs or in a broader sense when prosthetic devices or biomaterials were used [20]. The term "tissue engineering" as it is nowadays used was introduced in medicine in 1987. The definition that was agreed on was: "Tissue Engineering is the application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathologic mammalian tissue and the development of biological substitutes to restore, maintain, or improve function." The early years of tissue engineering were based on cell and tissue culture approaches. W.T. Green undertook a number of experiments in the early 1970s to generate cartilage using a chondrocyte culture technique in combination with a "bone scaffold." Despite of his inability to generate new cartilage, he set the theoretical and practical concept to connect and coax cells with scaffolds. Innovations in this approach were made by Burke and Yannas through a laboratory and clinical collaboration between Massachusetts General Hospital and M.I.T. in Boston, aimed at generating skin by a culture of dermal fibroblasts or keratinocytes on protein scaffolds, and using it for the regeneration of burn wounds. A key point in tissue engineering was given by the close cooperation between Dr. Joseph Vacanti from Boston Children's Hospital and Dr. Robert Langer from M.I.T. Their article in Science [21], describing the new technology, may be referenced as the beginning of this new biomedical discipline. Later on, a high number of centers all over the world focused their research efforts towards this field. Tissue engineering was catapulted to the forefront of the public awareness with a BBC broadcast exploring the potential of tissue-engineered cartilage which included images of the now infamous "mouse with the human ear" on its back from the

laboratory of Dr. Charles Vacanti at the University of Massachusetts Medical Center. The visual power of the photograph of the "auriculosaurus" helped to transfer the idea and vision of generating new tissues or organs from the imaginary world of human beings to the real world. Since that time, tissue engineering has been considered one of the most promising biomedical technologies of the century [22].

Regenerative medicine seems to be more difficult to define, as this term was used earlier but was less defined in the literature then the term "tissue engineering." It is now viewed by most biologists and physicists as a field where stem cells drive embryonic formation, or where inductive organizers induce a blastema to regenerate a tissue, aimed at reforming damaged tissues and organs in humans. It seems that a rigid definition of regenerative medicine is not constructive while the principle approaches that define the field are still being delineated. Stem cells, being at the center of expectations, hold great promise for the future of regenerative medicine. Stem cell plasticity and cloning, with nuclear transfer, transdifferentiation, and cell fusion as measures to modulate the stem cell differentiation pathway, is now a central issue in regenerative medicine [23]. During cloning, an adult nucleus is transplanted into an egg, which must erase the adult genome's epigenetic marks, so it can re-express every gene necessary to build a new animal. Robert Briggs and Thomas King were the first to demonstrate (based on a similar experiment proposed by Hans Spemann at the University of Freiburg as early as in 1938) how to clone frogs by replacing the nuclei of eggs with cells from tadpoles and adult intestinal epithelium. The further major step in cloning research was the cloning of two lambs (Megan and Morag) from embryonic cells in 1996. Cloning of the sheep Dolly [24] was, from a public awareness point of view, the key event in stem cell research. Ian Wilmut at the Roslin Institute near Edinburgh and his colleagues at PPL Therapeutics in East Lohian reported on February 27th, 1997 in *Nature* that they had produced a lamb named Dolly (Fig. 1.7), born the previous July, that was the first mammalian clone created using the genetic material from an adult cell. As soon as the story hit the front pages (the news was broke by the British Sunday newspaper *The Observer* four days ahead of *Nature's* publication) a public and media maelstrom ensued. Editors and writers of other newspaper went so far as to speculate

Fig. 1.7 Sheep Dolly and her first-born lamb Bonnie

that "It is the prospect of cloning people, creating armies of dictators, that will attract most attention." The creation of Dolly, cloned from an adult udder cell, overturned the idea that in mammals, developed cells could not reverse their fate. The news had a tremendous impact on society, mirrored by the fact that within days the President of the United States, the head of the European Commission, the Vatican, and many others were calling for a review of regulations on cloning research, if not an outright ban. It became obvious that the world was simply not prepared for the debate. The use of modern techniques like cloning or stem cell modulation was considered by a large part of the public to be a modern version of alchemy [25]. Japanese researchers reported in 1998 on the successful birth of eight cloned calves using adult cells from slaughterhouse entrails, raising the possibility that animals could be cloned for the quality of their meat (a situation close to the vision of the creation of Eve). During the last decade, scientists have shown that they are able to clone a variety of mammals including mice, rats, calves, cows, pigs, cats, and dogs. Some envisaged an industry of cloning applications, from the production of medicines in live bioreactors—cloned, genetically modified livestock—to the creation of herds of cloned animals that might one day be used as organ donors. But the low

efficiency of the cloning process, reflecting the problems related to reprogramming a cell's DNA by the content of eggs, has stymied industrial development. New directions focus on the addition of chemicals or proteins to adult cell nuclei, in order to bypass the need for eggs altogether. Egg-free approaches may also enable what many see as the most promising potential application of cloning: the creation of human embryonic stem cells, or cells made from them, that could be used to treat human disease [26].

Ten years later, the ethical debate launched by Dolly and encouraged by science fiction stories has changed. After a decade, mammalian cloning is moving forward with central societal issues remaining unresolved. The recent situation has now been supplanted by a bioethical discussion that is more complex and more focused on the real situation in stem cell biology. One outcome of this discussion is the risk assessment, currently open for public consultation, by the US Food and Drug Administration (see: www.fda.gov/cvm/Clone RiskAssessment.htm). Researchers and physicists speculate that unless there is some unknown fundamental biological obstacle, and given wholly positive motivations, human reproductive cloning is an eventual certainty [27].

1.2 Future Prospects

The future of tissue engineering and regenerative medicine holds the promise of custom-made medical solutions for injured or diseased patients, with genetic (re-)engineering in the zygote or even earlier as one of the most promising and also most debatable aspects of this field. As the field of tissue engineering and regenerative medicine is established as a central discipline in biomedicine nowadays, it seems interesting to speculate where the field will head. As both areas have matured to the point that its research and clinical aspects can be conceptually categorized, various commercial or noncommercial organizations have tried to assess the future of the field. These assessments seem to be important for researchers, policy makers, regulators, funders, technology developers, and the biomedical industry. Among the different assessments, published in open-access or limited access documents (nearly all of them thinking along similar lines), a study performed by the *Tissue Engineering* Journal can be considered to be the most scientific approach [28].

The Journal undertook such an assessment through the evaluation of a questionnaire (using a modified Hoshin process) given to the editorial board of *Tissue Engineering*. The aim of the study was to identify a list of strategically important concepts to achieve clinically relevant solutions for medical problems (up to the year of 2021). The evaluation of the questionnaire revealed some important aspects for the future of tissue engineering and regenerative medicine. One important finding was that highly strategic issues are not at the forefront of the daily work. The most striking example was angiogenic control. The dominance of this issue over all other issues and its low level of present progress propelled it to the top of strategic concepts. The second most important area was assumed to be stem cell science. As the editors assumed that understanding and control of stem cell research may give way for a short conduit in a number of tissue engineering approaches, molecular biology and system biology research seemed to be similarly important in the strategic development of the field. In addition to angiogenic control and stem cell biology, cell sourcing, cell/tissue interaction, immunologic understanding and control, manufacturing and scale up, as well some other issues were considered to be important (in a decreasing manner). With respect to the near and immediate future, the dominant concept that was supported by the largest number of specialists was clinical understanding and interaction. It seemed important that, as the field is oriented towards clinical application, close collaboration between researchers and clinically working physicians is of utmost importance. A close cooperation, based ideally on a deep understanding of the "other's" field, is not only valuable for the establishment of engineered tissue design criteria but also to enhance the potential for the final introduction of such therapies into clinical practice at large. The present book, intended to give researchers and clinicians a common platform, is hopefully one tool to reach this important aim. The future will show when and how which of the multiple approaches in tissue engineering and regenerative medicine will withstand the proof of clinical usage of such therapies over time.

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