Gustav Steinhoff *Editor*

Regenerative Medicine – from Protocol to Patient

4. Regenerative Therapies I

Third Edition

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Foreword: Regenerative Medicine: From Protocol to Patient

Third Edition

The vision to unravel and develop biological healing mechanisms based on evolving molecular and cellular technologies has led to a worldwide scientific endeavour to establish *Regenerative Medicine*. This field is involving interdisciplinary basic and (pre)clinical research and development on the repair, replacement, regrowth or regeneration of cells, tissues or organs in congenital or acquired disease. Stem cell science and regenerative biology is prompting the most fascinating and controversial medical development of the twenty-first century. It can be envisaged that this development will establish completely new molecular and cellular techniques for medical diagnosis and therapy. An early rush of scientific development was set up more than one hundred years ago by the physiology of blood regeneration (Hall and Eubanks, 1896) and successful vascular surgical techniques for organ transplantation (Carrel and Guthrie, 1905). However, the clinical realization of allogenic blood transfusion lasted until the discovery of the blood group antigens (Landsteiner and Levine, 1928) and successful routine allogenic organ and bone marrow transplantation even until the end of the last century.

Similar to the field of allogenic cell and organ transplantation, it seems that *Regenerative Medicine* again condenses mankind's visions, hopes and fears regarding medicine: hopes of eternal life and effective treatment of incurable disease as well as fears of misuse of technology and uncontrolled modifications of life are polarizing the scientific field. The development and public acceptance of new ethical and regulatory guidelines is a necessary process to support further clinical development. Nevertheless, the vision of a new medicine using the regenerative power of biology to treat disease and restructure the organism is setting the aim for scientific, technological and medical development. Viewing the great expectations to restructure and regenerate tissue, organs or organisms, the current attempts of scientist and physicians are still in an early phase of development.

The field of *Regenerative Medicine* has developed rapidly over the last 20 years with the advent of molecular and cellular techniques. This collection of volumes on *Regenerative Medicine: From Protocol to Patient* aims to explain the scientific knowledge and emerging technology as well as the clinical application in different organ systems and diseases. The international leading experts from four continents describe the latest scientific and clinical knowledge of the field of *Regenerative Medicine*. The process of translating science of *laboratory protocols into therapies* is explained in sections on basic science, technology development, and clinical translation including regulatory, ethical and industrial issues.

This collection is organized into five volumes: *(1) Biology of Tissue Regeneration, (2) Stem Cell Science and Technology, (3) Tissue Engineering, Biomaterials, and Nanotechnology, (4) Regenerative Therapies I, and (5) Regenerative Therapies II.*

Biology of Tissue Regeneration (Volume 1) focuses on regenerative biology with chapters on extracellular matrix, asymmetric stem cell division, stem cell niche regulation, (epi)genetics, immune signalling, and regenerative biology in organ systems and model species as axolotl or zebrafish.

Stem Cell Science and Technology (Volume 2) provides an overview as classification of stem cells and describes techniques for their derivation, programming and culture. Basic properties of differentiation states as well as function in human organism are illustrated, and areas of stem cell pathologies in cancer and therapeutic applications for these cells are discussed with emphasis on their possible use in *Regenerative Medicine*.

Tissue Engineering, Biomaterials and Nanotechnology (Volume 3) focuses the development of technologies, which enable an efficient transfer of therapeutic genes and drugs exclusively to target cells and potential bioactive materials for clinical use. Principles of tissue engineering, vector technology, multifunctionalized nanoparticles and nanostructured biomaterials are described with regard to the technological development of new clinical cell technology. Imaging and targeting technologies as well as biological aspects of tissue and organ engineering are depicted.

Regenerative Therapies I (Volume 4) gives a survey on history of Regenerative Medicine and clinical translation including regulation, ethics and preclinical development. Clinical state-of-the-art, disease-specific approaches of new therapies, application technology, clinical achievements and limitations are described for the central nervous system, head and respiratory system. *Regenerative Therapies II (Volume 5)* contains state-of-the-art knowledge and clinical translation of *Regenerative Medicine* in the cardiovascular, visceral and musculoskeletal systems.

These volumes aim to provide the student, the researcher, the health-care professional, the physician and the patient a complete survey on the current scientific basis, therapeutical protocols, clinical translation and practised therapies in *Regenerative Medicine*. On behalf of the sincere commitment of the international experts, we hope to increase your knowledge understanding, interest and support by reading the book.

After the successful introduction in 2011 with 41 chapters, this work has been actualized and extended for the third edition with into five volumes containing 60 chapters.

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Chapter 1
History of Regenerative Medicine

Raymund E. Horch, Laurentiu M. Popescu, and Elias Polykandriotis

Abstract Generation and regeneration as an answer to disease are far from being a new idea. Philosophers, naturalists and scientists were intrigued by the marvels of regeneration seen in nature. By the middle of the nineties life scientists thought we were only a few years away from bioartificial organs grown in a Petri dish. However, by the dawn of the new millennium it became clear that the mechanistic approach dictated by tissue engineering so far, had neglected issues of vascularization. Processes of angiogenesis were central to homeostasis, bioassimilation and biointegration of tissue engineered constructs. Furthermore, the field of tissue engineering had evolved into something vast, encompassing satellite technologies that were becoming separate science sectors. Advances in genetical engineering, stem cell biology, cloning, biomaterials and biomedical devices to name a few, would come to play a major role of their own – tissue engineering had become a part of a bigger whole. Regenerative medicine is the collective field to shelter these technologies "*…that seeks to develop functional cell, tissue, and organ substitutes to repair, replace or enhance biological function that has been lost due to congenital abnormalities, injury, disease, or aging".*

Keywords Regeenratrive medicine • Tissue engienering • History • Cell culturey • Arterio-venous loop • 3d vascularization • Telocytes

"Those who cannot learn from history are doomed to repeat it", claimed the philosopher G. Santayana in his book "The life of reason" (Santayana 1905). Although this statement reminds somehow of a cliché and its essence is being constantly disputed through the ages, one could hardly find a better example to report upon, other than the case of regenerative medicine. It is widely admitted that the very term of "Regenerative medicine" was coined to express a need for reorientation

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Table 1.1 Scientometric data based on Thomson Reuters released information (August 2009)

	Tissue engineering	Regenerative medicine
Total No of PubMed Papers (starting in..)	14517 (since 1988)	2197 (since 2001)
Year of maximum	–	2008 (ca 600)
Most cited paper	2452 citations	1366 citations
Top 100 papers	At least 200 citations each	At least 40 citations each

(Table 1.1). By the end of the twentieth century, biotechnology firms had maneuvered themselves into a dead-end financially, as well as conceptually (Mason 2007). Furthermore, the field of tissue engineering had evolved into something vast, encompassing satellite technologies that were becoming separate science sectors. Advances in genetical engineering, stem cell biology, cloning, biomaterials and biomedical devices to name a few, would come to play a major role of their own – tissue engineering had become a part of a bigger whole. And it is undisputable that biologicals will be the future (Mason and Dunnill 2008). To quote Paul Kemp: "hype, hubris and hyperbole aside – regenerative medicine will make a real and positive difference…"(Kemp 2006). But where did it all start?

1.1 Regenerative Medicine in the Ancient World

In his *Theogony*, Hesiod (eighth century BC) introduces Prometheus (Fig. 1.1) having created man out of clay and providing him with fire as a source of knowledge. "Hear the sum of the whole matter in the compass of one brief word-- every art possessed by man comes from Prometheus." (Aeschylus 415 BC). By doing that, Prometheus had provoked the wrath of Zeus. He had Prometheus carried to Mount Caucasus (or the Carpathian mountains) where an eagle (often mistaken as a vulture) by the name of Ethon would pick at his liver; it would grow back each day and the eagle would eat it again. His torture lasted 30.000 years until he was freed by Hercules (Fig. 1.1). Interestingly enough, the liver is generally speaking the only of the human organs to regenerate itself spontaneously in the case of lesion.[1] The ancient Greeks were well aware of this, hence they named liver (Greek: hēpar, ήπαρ) after hēpaomai (ηπάομαι), meaning to "repair oneself".

Later on, Aristotle devised two scripts dealing with generation and regeneration in the animal realm. In his "Generation of animals" he related early development with regenerative potential, whereas in "The history of animals" he made observations on regeneration on the limb of salamanders and deer antlers (Aristotle 1984). He propagated that biological form originates from undifferentiated matter and clearly favoured what would later be described as "epigenesis"(Fig. 1.2).

[1] Now the phenomenon of desquamation of the intestinal epithelium and the epidermis has been described. The intestinal epithelium is completely regenerated in 4–5 days. The total regeneration of the epidermis takes 4 weeks. This may mean that for a life expectancy of 77 years, the human epidermis is regenerated 1000 times.

Fig. 1.1 Prometheus. "Prometheus", Gustave Moreau 1868 (Musée Gustave Moreau, Paris). According to some investigators, his torture held for 30.000 years. After having provoked the wrath of Zeus, the eagle Ethon, picked at his liver every night. During the day the liver would regenerate

Fig. 1.2 Aristotleb. Aristotle 's bust. Roman copy from the bronze original by Lyssipos (fifth century B.C.).. (Ludovisi Collection). Aristotle wrote two major works on generation and regeneration in the animal realm. He related early development with regenerative potential and propagated that biological form originates from undifferentiated matter (epigenesis)

In the biblical tradition "the Lord God then built up into a woman the rib that he had taken from the man" (Wenin 2001). The quest for tissue replacement was even more graphically demonstrated in the tradition of Cosmas and Damian. Their practice of medicine and surgery in Asia Minor without fee (hence called 'Anagyroi,' without silver) and their martyrdom in Aegea, in Cilicia made a lasting impression upon the early Church. The grafting by these physician-surgeons of a moor's leg in replacement of a patient's diseased leg, and his surprise at finding himself possessed of two sound legs, his own white, and the other black, has been the subject of numerous paintings the majority of which depict the brothers in long robes, holding surgical instruments, boxes of salves, gallipots, or other medical appliances (Matthews 1968) (Fig. 1.3). Graveyards from the Paracas and Parachamac regions in Peru provide ample evidence that pre-Incan surgeons were performing trephination in great numbers as early as 3000 BC. A survey of more than 10,000 mummies from prehistoric Peru demonstrated that roughly 6 % showed cranial trephination. There is strong evidence that the occasional cranioplasty was also performed. Trephined Incan skulls have been discovered adjacent to shells, gourds, and silver or gold plates (Asenjo 1963).

Fig. 1.3 Saints Cosmas and Damian. "Transplantation of a leg by Saints Cosmas and Damian, assisted by angels", early sixteenth century (Stuttgart, Germany). According to the tradition of Cosmas and Damian these saints grafted a moors leg as a replacement of a patient's diseased leg

1.2 Regeneration in Early Research

Until the middle of the eighteenth century the motive power of biological organisms was thought to be an abstract vital force. Descartes (1596–1650) in his L' Homme postulated that the body works like a machine and biological phenomena are void of a divine meaning but can be explained by means of their physical properties. Lavoisier (1743–1794) postulated further on, that function and viability of organisms depended on chemical processes that could be reproduced in the laboratory. During the same time phenomena of generation and regeneration intrigued scientists and divided them into two distinct camps. Preformationists supported that appendages to be regenerated and organisms to be born pre-existed as miniatures at the site of interest. So, at the base of a severed lizard tail, in their conception a miniature tail was preformed and waited to be "activated" by an amputation. Likewise, in the sperm or in the ovum of the human there existed a miniature "homunculus" that grew into a newborn infant. This theory prevailed until the middle of the eighteenth century being concordant with the mechanistic framework provided at the time and did not come into a direct conflict with the Christian beliefs about divine involvement in the processes of life. On the contrasting end, came the Aristotelean thesis that undifferentiated matter was able to give rise to life. This theory had been actually named "epigenesis" by William Harvey (1578–1657) in his work "on the generation of animals" grossly repeating on Aristotle's works.

In the eighteenth century the process of regeneration in amphibians was matter of intense study. Abraham Trembley (1710–1784) produced several publications on the regenerative phenomena on freshwater polyps. He managed to obtain a clone of 50 polyps from one organism that he had quartered. He performed sections at every conceivable plane, contradicting preformational beliefs of the time (Dinsmore 1991). The question was posed: If the animal soul was the organizing and unifying element of life, how could a newly regenerated form arise? Reaumer and Spallanzani reported about their studies on crustaceans and salamanders respectively (Dinsmore 1991). The latter, being a great methodologist, expanded his research on a number of different organisms including frogs, toads, slugs and snails. He published his findings in 1768 in his work "Prodromo". It was noted by Newth. "In 1768 the snails of France suffered an unprecedented assault. They were decapitated in their thousands by naturalists and others to find out whether or not it was true, as the Italian Spallanzani had recently claimed, that they would then equip themselves with new heads" (Newth 1958; Weaver and Garry 2008).

Until the end of the eighteenth century philosophical and religious debate linked to the science of regeneration was set aside, and epigenesis gained acceptance with the eventual ascendancy of epigenetic embryology.

The last years of the eighteenth century marked a new field of interest for regenerative medicine: organ transplantation. John Hunter (1728–1793) performed allograft transplantations between chickens as well as dental transplantation utilizing xenografts of human teeth to avian hosts. John Hunter was the most prominent

surgeon and anatomist of his time. According to his instructions, his corpse was used for an anatomical dissection by his medical students on the day after his death.

At the beginning of the nineteenth century – following the 1794 description by B.L. in The Gentlemen's Magazine in London of a forehead tissue transposition to restore the nose of a bullock cart driver named Cowasjee, that had been cut off as a punishment (BL 1794) – the English surgeon Carpue was the first surgeon to apply methods of nasal reconstructions known to Indian surgeons for centuries (Carpue 1981 [1816]). Dieffenbach described methods for reconstructions of several components of the face, as well as the anus and the urethra (Goldwyn 1968). Reverdin devised a method for transplantation of skin islets, similar to the later techniques for keratinocyte transplantation (Horch et al. 2001). Transplantational biology was investigated by experimental approaches: In 1824, Franz Riesinger attempted corneal transplants from rabbits to humans, which were not successful (Moffatt et al. 2005). In 1837 Samuel Bigger performed a corneal transplant from a lab gazelle to another Gazelle with full recovery. Later on, Schleiden and Schwann in 1838–1839 postulated the cell theory that was afterwards confirmed by Rudolf LK Virchow through microscopic observations. He stated in 1858 the famous "*omnis cellula ex cellula*". Ultimately, the idea of cells being the elementary units of life being able to replicate themselves by division was born (Coleman 1978; Stocum 2006).

The eminent German pathologist Julius Cohnheim postulated in 1867 what became known as the "Cohnheim hypothesis". He suggested that all of reparative cells taking part in the regeneration of wounds come from the bloodstream (and therefore from the bone marrow) (Wohlrab and Henoch 1988).

At the end of the nineteenth century, Barth observed that upon autologous bone transplantation in hounds the vast majority of cells die and leave a scaffolding behind to be slowly repopulated by new host cells and an adequate neovascular network (Barth 1893).

Another very important advance was new knowledge on descriptive embryology that elicited a revolution in developmental biology. Even Darwin considered embryology as key to providing a special insight into evolution of forms, as seen in his correspondence to his friend Asa Grey.

If the living cell is the key to the tissue engineering of implantable parts and devices, then the advent of mammalian cell culture technology, i.e. the growing of mammalian cells out of the body, represents an event which ultimately opened the door for this field. Modern cell culture dates back to the early part of this century when a French scientist, Alexis Carrel, working at the Rockefeller Research Institute in New York, started a culture from a small slice of heart muscle taken from a chick embryo (Leff 1983). This culture continued for several decades, although along the way the heart muscle cells died out and only fibroblast cells continued to proliferate. Carrel's historic chick-cell culture finally was allowed to expire 34 years after it was started–and 2 years after his own death. Anecdotally and in the retrospect, his "immortal" adult cells might have benefited from interventions from Carrel's staff to keep the cells going and their teacher happy (Witkowski 1980). Now it is known, that according to the "Hayflick limit", cells in culture are not able to replicate more

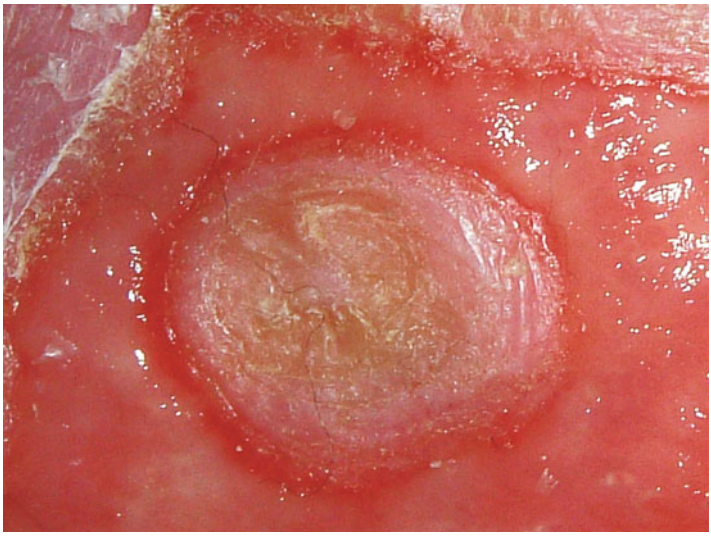

Fig. 1.4 Back from bench to bedside. Back from bench to bedside: small particles of skin inoculated into "biological" environment of wound showing expanding skin islet within a large wound 3 weeks after seeding

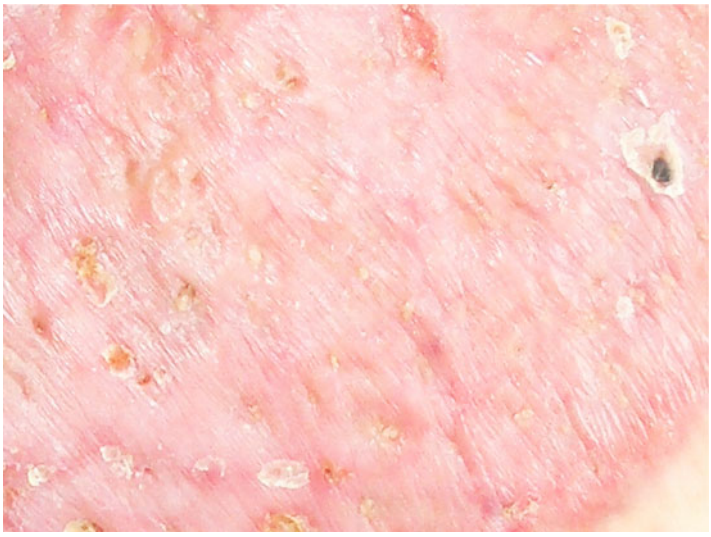

Fig. 1.5 Reepithelialisation. Eight weeks after skin particle seeding. Complete reepithelialisation is accomplished after "in situ culture" utilizing regenerative potentials of the human body

than 40–60 times and they are bound to display signs of senescence with successive passages (Hayflick 2007).

Cell culture has led to research which has paved the way for a number of important breakthroughs in the life sciences (Figs. 1.4 and 1.5). This includes the study of cellular processes, molecular biology and the ability to genetically manipulate cells, and the resulting development of new drugs, with much of recent drug-related research and product development being based on recombinant DNA technology (Nerem 1992). In the early 1970s, Dr. W.T. Green, a pediatric orthopaedic surgeon at Children's Hospital Boston, undertook a number of experiments to generate new cartilage using chondrocytes seeded onto spicules of bone and implanted in nude mice. Although unsuccessful, he correctly concluded that with the advent of innovative biocompatible materials it would be possible to generate new tissue by seeding viable cells onto appropriately configured scaffolds (Figs. 1.6 and 1.7). Several years later, Drs. John Burke, Massachusetts General Hospital, and Ioannis Yannas,

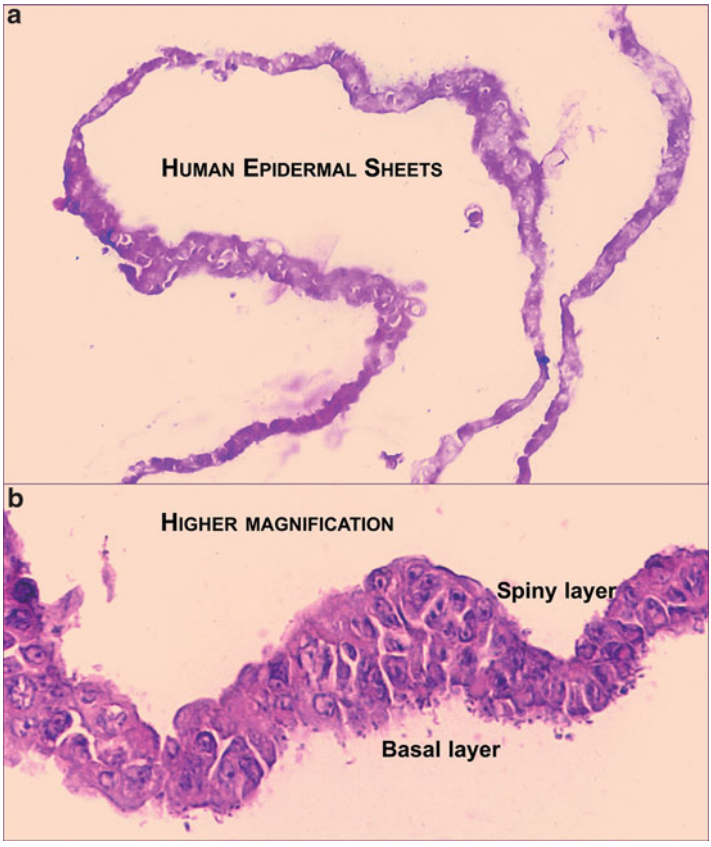

Fig. 1.6 Human epidermal sheets. Human epidermal sheets of autologous keratinocytes, obtained by cell culture (14 days). Light microscopy. (**a**) objective 20×. (**b**) objective 60× (Laboratory of Cellular and Molecular Medicine, Prof. L.M. Popescu, Bucharest)

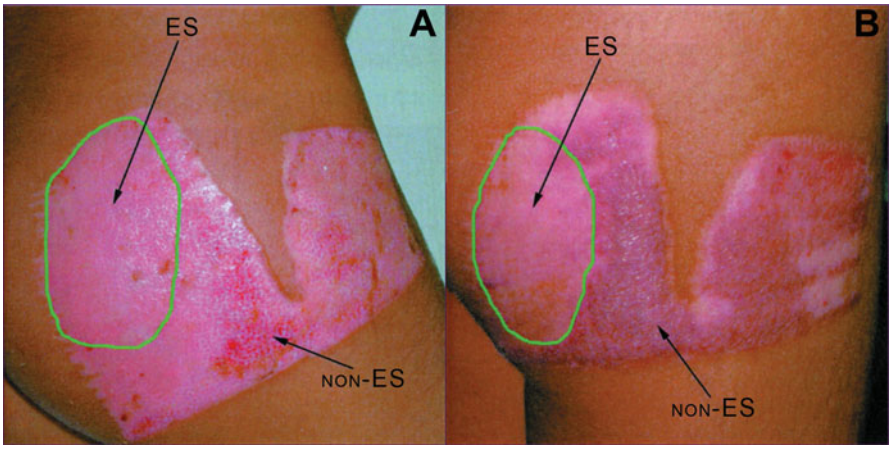

Fig. 1.7 Epidermal sheet treatment. Comparison between the regenerative processes of a donor area with (ES) or without (non-ES) epidermal sheet treatment. (**a**) Ten days after the application of the epidermal sheet. (**b**) Five weeks later (Courtesy of Prof. D. Enescu, Department of Plastic Surgery, Children's Hospital, Bucharest)

MIT, collaborated in studies in both the laboratory and in humans to generate a tissue-engineered skin substitute using a collagen matrix to support the growth of dermal fibroblasts. Dr. Howard Green later transferred sheets of keratinacytes onto burn patients. Dr. Eugene Bell seeded collagen gels with fibroblasts, referring to them as contracted collagen gels. All of these examples represent seeds of the new discipline now known as tissue engineering (Vacanti 2006).

Modern research on embryonic stem cells originates from studies on teratocarcinomas arising from the gonads of imbred mice. These neoplasias displayed a characteristic mixture of different tissues lined up next to each other randomly. By the end of the sixties it was established that they originated from germ cells that were able to give rise to a plethora of different tissues. So the concept of pluripotency of germinal cells was introduced (Kleinsmith and Pierce 1964). From its potential to generate a multitude of different cells, the tumour cell was named embryonal carcinoma stem cell (EC). Research with EC stem cells expanded considerably in the 70s. In a series of experiments, chimeric mice were produced by injecting EC s into early blastocysts (Papaioannou et al. 1975). Interestigly enough, in most of the cases, the tumour cells succumbed to the environment around the developing embryo and they contributed to a perfectly normal mouse pup. Hence it was shown that their genetic code could be "reprogrammed" according to the influence of the environment. Furthermore, the EC stem cells in culture could be constantly kept undifferentiated by frequent splitting or left to differentiate when the culture became too dense and they piled up. However, the EC stem cells were inherently flawed displaying chromosome abnormalities and were unable to differentiate into sperm and egg cells. Since ectopic blastocyst injections were also found to generate teratomas it became soon evident that pluripotent cells could also be derived from blastocysts directly (Damjanov 1993). Soon the next logical step was

undertaken, when Gail Martin (Martin 1981) in USA and Martin Evans (Evans and Kaufman 1981) in England generated in 1981 a stable diploid cell line that could generate every tissue of the adult body, including germ cells. Gail Martin referred to her cells as "embryonic stem cells" and gave them the nickname "ES cells".

The same line of advances had to be repeated for human cells: Human EC stem cell lines could be isolated and cultured from a rare tumor of the male testes, after orchiectomy procedures (Andrews 1988). However, these cells are always aneuploid and usually lack the capacity to differentiate into somatic tissue (Pera et al. 1989). Human ES cells were not available at this time. What was available, were blastocyst-derived embryonic cells from primates including rhesus monkeys and marmosets (Thomson et al. 1996). These cells displayed all favourable characteristics: they were diploid and were able to give rise to all three types of germinal layers, including germ cells. Their phenotype resembled that of the human EC and were distinctly different from the mice ES. All major technological advances for cultivation and characterization of human ES was achieved by the late 90s – but their harvest was not yet possible.

In 1998 a major step was accomplished toward this direction marking the dawn of a new era. Couples undergoing treatment for extracorporeal fertilization donated a surplus of blastocysts for experimental purposes. James Thomson isolated and cultivated a human ES line from these blastocysts (Thomson et al. 1998).

Adult stem cells were also to enter the arena of biomedical research. The idea that bone marrow contained some kind of osteogenic precursor cells started in 1963 when Alexander Friedenstein (Petrakova et al. 1963) showed that by implanting pieces of bone marrow under the renal capsule, it was possible to obtain an osseous tissue. After this he and his co-workers revealed a series of in vivo studies in which the possible existence of stem cells in the bone marrow was shown. Almost 20 years later, Caplan gave these cells the name they have today, Mesenchymal Stem Cells. In 1994, the same author described that these cells, when placed in the adequate culture conditions, could be differentiated into cells with mesenchymal origin and eventually give origin to bone, cartilage, fat, muscle skin, tendon and other tissues of mesenchymal origin, through what he named "the mesengenic process"(Caplan 1994). Since then, a series of researchers have elaborated on the use of hES for the purposes of tissue engineering and regenerative medicine (Guillot et al. 2007).

In summary the idea of utilizing stem cell transplantation for tissue regeneration or even potential organ replacement is by itself fascinating and generates a huge amount of various experimental and hypothetical approaches within the last years. Especially – similar to the principal idea of tissue engineering – the implantation of adult human autologous or embryonic stem cells, which are expanded ex vivo, might circumvent some of the current problems associated with transplantation surgery, particularly in the elderly. This encompasses the hitherto naturally limited availability of organs or tissues as well as the numerous complications that are related to disease transmission and immune rejection.

This is especially true for the complex of so called musculoskeletal degeneration, that is closely associated with the aging process. However, to introduce adult MSC into clinical practice of substituting organs or tissues, it is necessary to vigor-

ously define the capacity of MSC to maintain growth potential and regulated differentiation of such cells into the desirable cell lineage. There is still not enough body of knowledge at the moment with regard to the physiological and pathophysiological parameters of MSC, including environmental conditions such as biomechanical forces, as to fully understand the potential influence on MSC to differentiate and grow into desired tissues, once extracted and cultured ex vivo (Cheung 2010). It is not known how MSC from young individuals behave versus cells harvested from the elderly. Nevertheless, many efforts are underways to gain more insight into the promising field of harnessing the power of stem cells for tissue and organ regeneration (Cheung 2010). Other issues that concern the ethical aspects of human embryonic stem cells need to be further addressed before research and clinical translation will make its break through.

1.3 Tissue Engineering

The origins of tissue engineering are generally traced to the beginning of the eighties in Boston. Funding was received by the Bell Laboratories in Massachusetts Institute of Technology (MIT) for preparing a cell based vascular scaffold. Prior to that, Eugene Bell had published on the use of "living skin equivalents" in Science as early as 1981 (Bell et al. 1981). Lysaght tracked a press release in 1982 stating that "Flow General", one of the funding firms based in Virginia, was pursuing research and development efforts in business segments including tissue engineering and "smart" computer systems (Lysaght and Crager 2009). E. Bell founded in 1985 "Organogenesis Inc." and later on, "Tissue Engineering International – (TEI) Biosciences Inc." both of which are renowned companies in the biotechnology landscape. During the same time, a few doors further in MIT, Joseph Vacanti of Children s Hospital approached Robert Langer with the idea to design custom made scaffolds for cell delivery. Thereupon, they started an extensive collaboration with studies on the properties of functional tissue equivalents (Vacanti 2006). In 1987 a special session was held at the US National Science Foundation meeting in Washington DC, where the denomination "Tissue engineering" (TE) was officially given to the field and organisation of the first conference with focus on "the engineering of living tissue" was initiated, mainly by Y.C. Fung (Nerem 1992). This conference took place in 1988, at Lake Tahoe, California and the first definition of tissue engineering was introduced by Robert Nerem:

> *Tissue engineering is the application of the principles and methods of engineering and the life sciences towards the fundamental understanding of structure/function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve functions.*

The proceedings of this meeting were published a year later as a book titled "Tissue Engineering" (Skalak and Fox 1989). The first peer reviewed article accessible through the NLM database with the term appeared in 1989. It was a report on a

biologically based vascular graft published by Tadashi Matsuda in ASAIO Transactions (Matsuda et al. 1989). Maybe the most cited early review on tissue engineering is a 1993 publication by JP Vacanti and R Langer in the journal Science where the definition is stated again in brief (Langer and Vacanti 1993).

1995 was a turning point of TE, since it was the year of the "auriculosaurus". Charles Vacanti, seeded a polymeric scaffold in the shape of a human ear with cartilage cells and implanted it subcutaneously on the back of a nude mouse. The pictures of this ear-formed bioartificial implant, filmed by a BBC crew, quickly made the round of the world and attracted a huge interest on the new biotechnology. It became a symbol for the emerging field of TE.

In 1996 the Tissue Engineering Society international (TESi) was officially founded by Joseph and Charles Vacanti, and the inaugural meeting took place at the Lake Buena Vista Hotel in Orlando, Florida the same year. The Asian tissue engineering societies were incorporated in TESi by 2000. By the turn of the century Raymund E Horch and G Björn Stark from Freiburg encouraged the foundation of the European branch of TESi the ETES, and they hosted in 2001 the TESi meeting in Germany.

In 1998, a clinical application of tissue engineering became popular by the media. Charles Vacanti, used a biogenic matrix out of coral seeded with osteoblasts, for reconstruction of the skeleton of a traumatized thumb (Vacanti 2006). The first tissue engineering products cleared FDA approval in the same year. Apligraf came from the E. Bell Laboratories and the firm Organogenesis as living skin equivalent. Epicel evolved from Greens laboratory whereas Yannas together with Integra Life Sciences Inc. brought in 2002 an acellular dermis regeneration scaffold by the name of Integra in the market (Kemp 2006). By the beginning of the twenty-first century, there was a wild media hype about these fascinating new technologies with unrealistic expectations both from the public and the biomedical society (Kratz and Huss 2003). Time magazine described with a cover story, tissue engineers as the "hottest job" for the future: "*With man-made skin already on the market and artificial cartilage not far behind, 25 years from now scientists expect to be pulling a pancreas out of a Petri dish*" (What will be the 10 hottest jobs? 2009). Just before 2001 there were over 3000 people working in the sector, with funding exceeding US $ 580 million (Kemp 2006; Lysaght and Hazlehurst 2004).

1.4 The Era of Regenerative Medicine

However, by the middle of the first decade of the twenty-first century, tissue engineering seemed to be going through a crisis. Lysaght noted very graphically in 2006 that "…such highly favourable media treatment has its benefits, but research-minded professionals increasingly recognized a disconnect with the realities. And such disconnects rarely lead to happy endings" or "…Although aggregate development costs exceed $4.5 billion, the field has yet to produce a single profitable product." (Lysaght and Hazlehurst 2004).

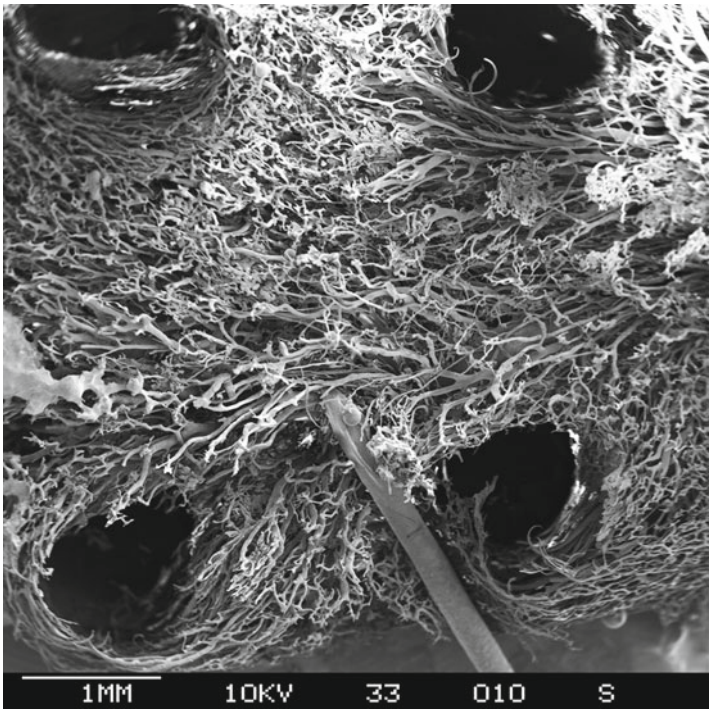

Fig. 1.8 Corrosion casting. A microvascular replica of a bioartificial organoid showing angiogenesis. The neovascular capillaries are "polarized" towards a maximum regenerative stimulus. All these capillaries were formed during the course of less than 1 week

Furthermore, tissue engineering had reached some biological limitations. The mechanistic approach dictated by biomaterial scientists, neglected issues of vascularization. It became clear that angiogenic processes were central to homeostasis, bioassimilation and biointegration of tissue engineered constructs (Vacanti et al. 1998; Mooney and Mikos 1999). Experimental activities were directed to encompass integrative strategies towards generation of autonomously vascularised bioartificial tissue elements (Polykandriotis et al. 2007, 2008; Weigand et al. 2015) (Figs. 1.6 and 1.7). In addition to that, emphasis was being given to cellular therapies, since the era of human embryonic stem cells had arrived. Other satellite technologies had acquired a momentum of themselves, with gene technology reaching the point where a whole mammal could be easily cloned (Wilmut et al. 1997) or genetically manipulated (the Monsanto swine case). Nanotechnology came also into play with generation of new biomaterials (Beier et al. 2009) (Figs. 1.8 and 1.9).

The whole field was consecutively renamed into "regenerative medicine". The terms tissue engineering and regenerative medicine were used in parallel and synonymously to each other, but it is widely accepted that the very change of the name epitomized the beginning of a new era.

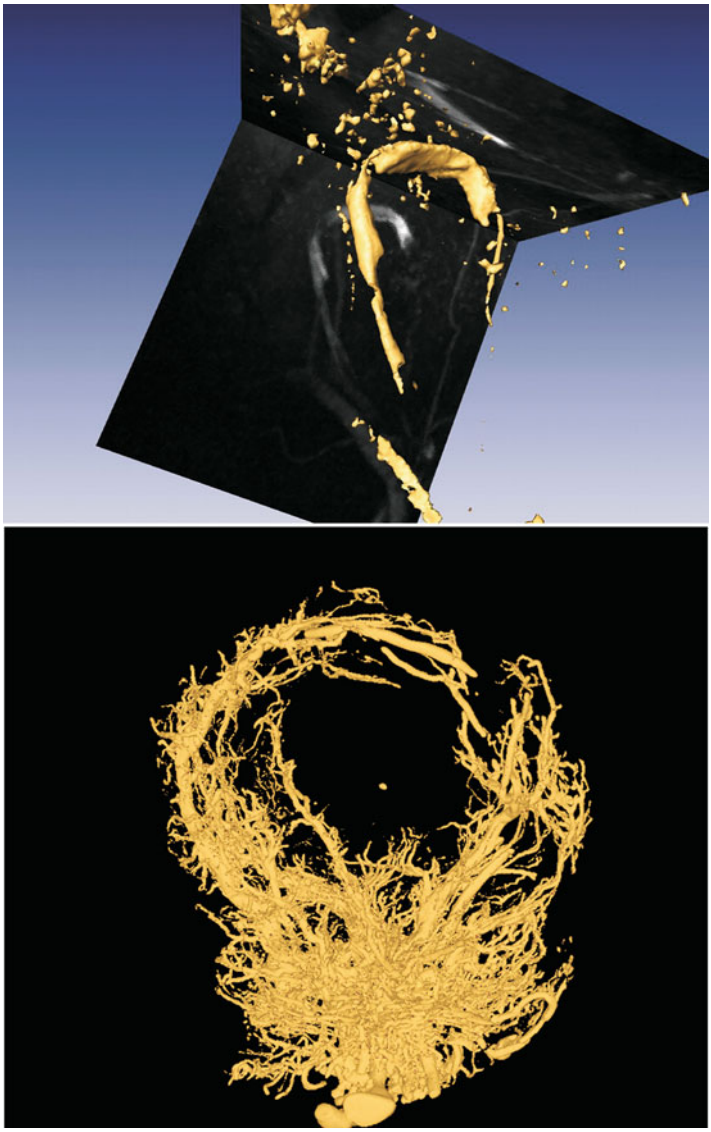

Fig. 1.9 Advanced imaging applications. *Above*: Micro magnetic resonance angiography of a bioartificial organoid grown in a rat. A 4,7 Tesla Brucker bioscan equipment has been used for in vivo monitoring of the nascent biological construct. *Below*: Ex vivo Micro CT study of the same organoid after injection of a contrast medium. Plasticity of the microvascular network produces a parenchyma – like circulation