Gustav Steinhoff *Editor*

Regenerative Medicine - from Protocol to Patient

1. Biology of Tissue Regeneration

Third Edition

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Editor
Gustav Steinhoff
Department of Cardiac Surgery
and Reference and Translation Center
of Cardiac Stem Cell Therapy (RTC),
Medical Faculty
University of Rostock
Rostock, Mecklenburg-Vorpomm, Germany

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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 involves interdisciplinary basic and (pre) clinical research and development on the repair, replacement, regrowth or regeneration of cells, tissues or organs in congenital or acquired diseases. 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. The early rush of scientific development was initiated 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 towards 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 the 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 aims for scientific, technological and medical development. Viewing the great expectations to restructure and regenerate tissues, organs or even organisms, the current attempts of both scientists 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 in the field of *regenerative medicine*. The process of translating the 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 the extracellular matrix, asymmetric stem cell division, stem cell niche regulation, (epi)genetics, immune signalling, and regenerative biology in organ systems and model species such as axolotl and zebrafish.

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

Tissue Engineering, Biomaterials and Nanotechnology (Volume 3) focuses on 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. The principles of tissue engineering, vector technology, multifunctionalized nanoparticles and nanostructured biomaterials are described with regards to the technological development of new clinical cell technologies. Imaging and targeting technologies, as well as the biological aspects of tissue and organ engineering are described.

Regenerative Therapies I (Volume 4) gives a survey of the 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 technologies, clinical achievements and limitations are described for the central nervous system, head and respiratory systems. Finally, *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 healthcare professional, the physician and the patient with a complete account of 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 of the first edition in 2011, this publication has been developed and expanded for the third edition into five volumes.

Department of Cardiac Surgery and Reference and Translation Center of Cardiac Stem Cell Therapy (RTC), Medical Faculty
University of Rostock,
Rostock, Mecklenburg-Vorpomm, Germany
gustav.steinhoff@med.uni-rostock.de
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Gustav Steinhoff

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Contributors

Pascal Bielefeld Neuroscience Program, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Palas Chanda Center for Cardiovascular Regeneration, Department of Cardiovascular Sciences, Houston Methodist Research Institute (HMRI), Houston, TX, USA

Jun Chen National Institute of Biological Sciences, Beijing, China

Natalia Coleman Mathematics Department/Biology Department, New Jersey City University, Jersey City, NJ, USA

John P. Cooke Center for Cardiovascular Regeneration, Department of Cardiovascular Sciences, Houston Methodist Research Institute (HMRI), Houston, TX, USA

Wayne M. Eby Mathematics Department/Biology Department, New Jersey City University, Jersey City, NJ, USA

Piril Erler Department of Biology, Northeastern University, Boston, MA, USA

Johanna E. Farkas Department of Biology, Northeastern University, Boston, MA, USA

Carlos P. Fitzsimons Neuroscience Program, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Silvina A. Fratantoni BioFocus DPI Limited, A Charles River Company, Leiden, The Netherlands

Polina D. Freitas Department of Biology, Northeastern University, Boston, MA, USA

Frank Hirth Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

Elizabeth D. Hutchins School of Life Sciences, Arizona State University, Tempe, AZ, USA

Emilia Kaivosoja Department of Medicine, Institute of Clinical Medicine, Helsinki, Finland

Brian Key Brain Growth & Regeneration Laboratory, School of Biomedical Sciences, University of Queensland, Brisbane, Australia

Yrjö Tapio Konttinen Department of Medicine, Institute of Clinical Medicine, Helsinki, Finland

COXA Hospital for Joint Replacement, Tampere, Finland

ORTON Orthopaedic Hospital, ORTON Foundation, Helsinki, Finland

Kenro Kusumi School of Life Sciences, Arizona State University, Tempe, AZ, USA

Jaakko Levón Department of Medicine, Institute of Clinical Medicine, Helsinki, Finland

Zygmunt Mackiewicz Department of Regenerative Medicine, State Research Institute Center for Innovative Medicine, Vilnius, Lithuania

Shu Meng Center for Cardiovascular Regeneration, Department of Cardiovascular Sciences, Houston Methodist Research Institute (HMRI), Houston, TX, USA

James R. Monaghan Department of Biology, Northeastern University, Boston, MA, USA

Fumitaka Osakada Laboratory of Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya University, Chikusa-ku, Nagoya, Aichi, Japan

Laboratory of Neural Information Processing, Institute for Advanced Research, Nagoya University, Chikusa-ku, Nagoya, Aichi, Japan

Systems Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA

PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

Nik Papaloukas Neuroscience Program, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Marijn Schouten Neuroscience Program, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Rita Sousa-Nunes MRC Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

Vasily Stegajev Department of Medicine, Institute of Clinical Medicine, Helsinki, Finland

Pei Sun National Institute of Biological Sciences, Beijing, China

Alexandra E. Sweeney Department of Biology, Northeastern University, Boston, MA, USA

School of Life Sciences, Queen's Medical Centre, Nottingham University, Nottingham, UK

Masayo Takahashi Laboratory for Retinal Regeneration, Center for Developmental Biology, RIKEN, Chuou-ku, Kobe, Aichi, Japan

Veli-Matti Tiainen ORTON Orthopaedic Hospital, ORTON Foundation, Helsinki, Finland

Hanoch Daniel Wagner Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel

Chenhui Wang National Institute of Biological Sciences, Beijing, China

Gilbert Weidinger Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

Pei Wen National Institute of Biological Sciences, Beijing, China

Chi-Chung Wu Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

Rongwen Xi National Institute of Biological Sciences, Beijing, China

Chapter 1
Extracellular Matrix and Tissue Regeneration

Zygmunt Mackiewicz, Yrjö Tapio Konttinen, Emilia Kaivosoja, Vasily Stegajev, Hanoch Daniel Wagner, Jaakko Levón, and Veli-Matti Tiainen

Abstract Extracellular matrix (ECM) is an important component of stem cell niche areas, which provide residence, regulate stem cell pool size and control stem cell mobilization. ECM is a complex interlinked composite of collagenous molecules, non-collagenous molecules and water-rich mucopolysaccharide ground substance. Cells are integrated to their matrix via integrin and non-integrin receptors, which control adhesion, migration, division, growth, anoikis, transdifferentiation and other cellular behaviour. ECM safeguard cells and tissue architecture and strength, but also growth factor deposits, which proteinases as signalling scissors can release in a site- and process-specific manner. Selected processes, like wound healing, cartilage and heart ECM, and tumor growth are used to exemplify participation of ECM in tissue regenerative processes.

Keywords Extracellular matrix • Stem cells • Regeneration

Z. Mackiewicz (✉)
Department of Regenerative Medicine, State Research Institute Center for Innovative Medicine, Santariškių Str.5, LT-08406 Vilnius, Lithuania
e-mail: zmzmackiewicz@gmail.com

Y.T. Konttinen
Department of Medicine, Institute of Clinical Medicine, Haartmaninkatu 8, 00029 HUS Helsinki, Finland

COXA Hospital for Joint Replacement, Biokatu 6 b, 33520 Tampere, Finland

ORTON Orthopaedic Hospital, ORTON Foundation, Tenholantie 10, 00280 Helsinki, Finland

E. Kaivosoja • V. Stegajev • J. Levón
Department of Medicine, Institute of Clinical Medicine, Haartmaninkatu 8, 00029 HUS Helsinki, Finland

H.D. Wagner
Department of Materials and Interfaces, Weizmann Institute of Science, 76100 Rehovot, Israel

V.-M. Tiainen
ORTON Orthopaedic Hospital, ORTON Foundation, Tenholantie 10, 00280 Helsinki, Finland

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1.1 Introduction

Bone marrow-derived mesenchymal stem/stromal cells (MSC) are nurtured in specialized niche areas by coordinated action via contact with soluble factors, extracellular matrix components and stem or feeder cells, which regulate the size and mobilization of the stem cell pool. MSCs can be recruited from their protected niche locations, be locally activated, or artificially harvested from various anatomical locations to be implanted for distant tissue regeneration. ECM fills the space between cells (interstitial matrix) and is present in tissue (basement membrane) and cellular (synovial lining) interfaces. ECM is a composite of fibrous collagen molecules and non-collagenous proteins embedded in water-rich mucopolysaccharides. Small leucine-rich proteoglycans (SLRP) regulate collagen fibrillogenesis, but also cross-link fibres and cells, and form deposits of growth and differentiation factors (Iozzo and Schaefer 2015). Elastins with its associated molecules, like fibrillin and fibulin, and adhesive glycoproteins laminin and fibronectin are important non-collagenous ECM proteins (Oasmanagic-Myers et al. 2015). Large aggregating proteoglycans or lecticans, like aggrecan, and hyaluronan form the ground substance. Scaffolds or derivatization of scaffolds with ECM molecules are used to regulate proliferation and differentiation of progenitor and stem cells.

Cells are integrated to ECM via at least 24 different non-covalently coupled heterodimeric cellular integrin receptors, which also form an important link in the architecture of cellular actin cytoskeleton. They allow migration and outside-in and inside-out signalling acting together with soluble factors and cell-cell contacts. Discoidin domain receptors, Lutheran Lu/B-CAM complex and α/β-dystroglycan complex are non-integrin matrix receptors. Glycan binding receptors, in particular extracellular C-, R- and I-type lectins and galectins play roles in cell-matrix adhesion and signalling, including MSC recruitment, bone marrow stem cell niche and adult collagen remodelling.

Proteinases modulate the composition of the ECM and are divided based on their catalytic mechanism to secrete neutral serine and metalloendoproteinases (and amino- and carboxypeptidases), and to mostly intralysosomal acidic cysteine and aspartic endoproteinases (Van Doren 2015; Klein et al. 2015; Kessenbrock et al. 2015). They mediate tissue degradative events in normal remodelling and pathological tissue destruction, but are increasingly recognized as signalling scissors.

Matrikines are peptides released by partial proteolysis of extracellular matrix macromolecules, which can participate in regulation of cell activities. There are known the “natural” matrikines, which unchanged participate in signaling from the extracellular ambience and “cryptic” matrikines (matricryptins) that require proteolytic processing to reveal the ligand from ECM protein. Unlike traditional soluble growth factors, most matrikines are characterized by low binding affinity to their receptors. Some of these peptides modulate proliferation, migration, protease production and apoptosis (Maquart et al. 2005; Maquart and Monboisse 2014; Ricard-Blum and Salza 2014; Wells et al. 2015).

Classical examples of processes which reflect stromal stem cell function and some general and some site specific challenges comprise wound healing and tumor growth.

1.2 Stem Cell Niche

Stem cells niche refers to a local microenvironment able to home, house, interact, maintain and mobilize one or more stem cells interacting with them and regulating their fate (Gattazzo et al. 2014; Rezza et al. 2014). The cellular "host" of the niche probably represents a cell, which produces such extracellular matrix (ECM) components, which the stem cell itself may not be able to produce or organize to a niche, but to which it adheres via integrin and non-integrin matrix receptors. Interactive participation of the nurturing "host" cell and stem cell in the process and their responsiveness to external stimuli, such as stem cell mobilizing pro-inflammatory cytokines, makes the niche dynamic. The niche regulates stem cell proliferation and differentiation during the embryonic development but maintains stem cells in a quiescent state in adults, and helps them to get activated upon tissue injury and to disclose the potential of the stem cells to undergo also asymmetric cell divisions (Doe and Bowerman 2001). This may be determined by the orientation of the cytokinesis of the stem cell division, which is in part regulated by the composition of the niche and by integrin mediated anchorage. If the division occurs in a plane parallel to the niche cell-stem cell contact surface, the proximally located parent cell is likely to remain in contact with the niche whereas the distally located daughter cell is displaced from it. This maintains the stemness and the size of the stem cell pool and produces progenitor cells, which loose contact with the niche and their stemness and leave the niche to migrate and/or transit via circulation to a new location to terminally differentiate to specialized cells, respectively (Ellis and Tanantzapf 2010).

Interactive niche-stem cell factors can be classified to three categories: (1) soluble factors (growth factor, nutrients, electrolytes, etc.), (2) direct cell-cell interactions and (3) ECM-stem cell/niche cell interactions. MSC integrin receptors for interstitial type I collagen $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$, may play a role in this respect, but MSC have also been described to contain e.g. α_3, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (Docheva et al. 2007), which may help the stem cells to home, anchor, structure, divide and leave the niche as well as otherwise to perform their other functions as stem cells. However, in spite of the knowledge of the integrins present in MSCs, the actual integrin receptors relevant for the niche cells and for the MSCs are poorly known at present, but several redundant integrin receptors are probably involved (Ellis and Tanantzapf 2010).

Integrin chain specific antibodies coupled to paramagnetic micro-beads and various controlled culture substrate stretching devices have been developed to test the effect of integrin-mediated mechanical forces on cellular phenotype and function

(Sasaki et al. 2007; Pommerenke et al. 1996). Mechanotransduction may help to shape the stem cell niche and regulate the stem cell function (Kuo and Tuan 2008).

Understanding the effects of the above mentioned niche factors on chromatin remodelling and gene expression is essential for proper control of tissue engineering. A delicate balance protects stem cells from depletion, but at the same time prevents excessive, cancer cell-like proliferation. Stem cell niche constitutes the basic unit of stem cell physiology the same way as osteons, chondrons and salivons do in bone, cartilage and salivary gland tissue, respectively.

Niche has implications also for cancer cells and hematopoietic cells. Self-renewing cancer stem cells may reside and renew in cancer stem cell niche composed of a specialized vascular bed of endothelial cells, some sort of mesenchymal cells and ECM components (Nie 2010). Bone marrow derived MSCs in their bone marrow stem cell niche may via their immunosuppressive properties be involved in cancer progression and metastasis (Fulawka et al. 2014). MSCs of bone marrow may also provide the cellular support structure in the niche for hematopoietic stem cells (Battiwalla and Hematti 2009).

To regulate the size of the stem cell or progenitor cell pool, stem cells have a capability pendulate between asymmetric and symmetric cellular divisions (Kfoury and Scadden 2015). Probably the stem cell pool is expanded during embryogenesis, whereas asymmetric divisions allow rapid generation of progenitors upon high demand in various regenerative processes. The process of expansion of the stem cell pool must be controlled at some critical checkpoints to prevent cancer, whereas to latter is also strictly regulated to prevent precocious depletion of the stem cell pool; the cells can probably switch back and forth between these two different modes of proliferation (Oskarsson et al. 2014; Lane et al. 2014). The size of the stem cell pool is probably diminished upon aging via senescence or apoptosis (Jung and Brack 2014).

In spite of their predicted existence, it is a challenge to identify stem cells in their natural surrounding unanimously (Boulais and Frenette 2015). In tissues they cannot be recognized by their ability to differentiate along various specialized cell lineages and demonstration of a palette of markers used for cultured or cloned stem cells by using flow cytometry are not easily adaptable to histological tissue sections at the single cell level in static cytometry.

When cell detatch from ECM it become homeless and die via programmed death mechanism called anoikis (Paoli et al. 2013). Cancer cells develop anoikis resistance.

1.3 Mesenchymal Stromal Cells

Mesenchymal stem cells (MSCs) can be recruited from their protected niche, locally activated, or artificially harvested from various anatomical locations to be implanted as such or in tissue engineering constructs to tissue defects to facilitate repair by expantion, differentiation or perhaps mostly by orchestration of the more simply

programmed resident or immigrant repair cells during tissue regeneration (Sharma et al. 2014; Ikebe and Suzuki 2014; Kfoury and Scadden 2015).

Bone, cartilage and other tissue defects can be repaired by differentiated autologous cells or tissues isolated from the donor site(s) for transplantation, but often it is impossible to obtain enough autologous cells or tissues for such repair procedures and harvesting them from e.g. iliac bone or non-weight bearing femoral head cartilage can be complicated and painful. Allogeneic and xenogeneic cells are better available for clinical use, but immunosuppressive treatment is necessary to overcome immunological. Usage of foetal cells might provide a low immunogenic option (O'Donoghue and Fisk 2004) but raises ethical issues. Induced pluripotent stem cells have huge potential in the field but only the first clinical trials are ongoing.

Tissue injury triggers cellular mechanisms, which regulate homing and engraftment of circulating and local stem cells to fill the void and to regulate the sequential and ordered healing cascades. To avoid scar tissue formation and promote true regeneration with functional tissue, various natural or synthetic scaffolds seeded with stem cells have raised interest. They could be used to augment healing in critical size defects, non-union, non-healing and otherwise clinically threatening tissue defects.

Autologous MSCs have potential to proliferate via symmetric cell divisions and then to differentiate into specialized phenotypes via asymmetric cell divisions. Differentiation is regulated by a dynamic and complex extracellular microenvironment that contains a lot of biophysical and biochemical information, including soluble cues (i.e. growth factors and cytokines), cell – cell contacts, cell – ECM contacts, and physical forces. In addition to the extracellular microenvironment, the chemical and physical properties of a biomaterial control stem cell fate (Kaivosoja et al. 2012).

Vision of MSCs as precursors for resident cellular components of various specialized tissues is often oversimplified. MSCs can prolong the survival of skin and cardiac allografts, ameliorate the course of acute graft-versus-host disease and experimental autoimmune encephalomyelitis, orchestrate tissue repair (Zhao et al. 2010; Das et al. 2013).

MSCs participate in the healing of different tissues damage, however, they are also recruited by cancer cells to aid tumor growth and progression (Sun et al. 2014; Chang et al. 2015).

1.4 Extracellular Matrix

ECM fills the space between cells (interstitial matrix) and is present at tissue (basement membrane) and cellular (synovial lining) interfaces. It is synthesized, maintained and modulated by cells to adapt the growth, evolution, aging, changing mechanical and developmental needs, to meet the reparative needs after micro- and macrodamage to renew and regenerate and to produce new editions of instructive

outside-in signalling matrix in heathy and tumour tissue (Mouw et al. 2014; Bonnans et al. 2014; Picup et al. 2014; Wong and Kumar 2014). ECM is a composite of fibrous collagen molecules and non-collagenous proteins embedded in water-rich mucopolysaccharides. Small leucine-rich proteoglycans (SLRP) regulate collagen fibrillogenesis (fibre thickness), but also cross-link fibres and cells and form deposits of growth and differentiation factors. Elastin with its associated molecules, like fibrillin and fibulin, and adhesive glycoproteins laminin and fibronectin, are important non-collagenous proteins. Apart from SLRPs, large aggregating proteoglycans or lecticans, like aggrecan, and hyaluronan form important components of the ground substance. Scaffolds or derivatization of scaffolds with ECM molecules are used to regulate proliferation and differentiation of progenitor and stem cells. ECM components are widely used for therapeutic purposes (Kular et al. 2014).

Extracellular vesicles – microvesicles are not structural part of ECM. They represent transport mechanism of intercellular communication, usually in ECM milieu. Two types of microvesicles are known: membrane-derived vesicles (EVs) and exosomes. EVs can transfer mRNA and microRNA to target cells, release of apoptotic bodies. The role of microvesicles in tissue regeneration is important (Rani et al. 2015).

1.4.1 Extracellular Matrix: Collagens

ECM fills the void between cells (interstitial matrix) and between tissue interfaces (basement membrane), providing by its toughness structure and physical support to tissue-typical multicellular but dynamically generated (organogenesis) and maintained (remodelling) architecture as well as adhesion substrate and an instructive editable matrix, which literarily is decisive for cellular survival and for the regulation of multiple aspects of cellular behaviour (Aszodi et al. 2006). For most soft (skin, fat, fasciae, muscles, tendons, blood vessels, brain, peripheral nerves etc.) and hard or semi-hard (bone, cartilage, cornea etc.) connective tissues collagen nanofibres form a three dimensional and highly organized scaffolded backbone (Mienaltowski and Birk 2014), whereas the more hydrophilic and permeable ground substance largely composed of proteoglycans and glycoproteins occupies the interfibrillar spaces enabling transfer and filtration of nutrients, oxygen, metabolites and bioactive factors as solubilized, in granules or in EVs. Linker proteins bind these two major components to extensive networks, which provide dynamic and adjustable biomechanical strength, associated with flexibility, to such cell-matrix composite structures. Due to the high biomechanical and instructive demands imposed to the ECM, it undergoes almost continuous mechanotransduction and remodelling by replacing damaged and degenerated tissue elements with new intact and properly organized, to adapt to the varying local functional needs (Aszodi et al. 2006).

Human body contains altogether 28 different collagen types, which are classified to nine different subtypes, including fibrillar collagens, which form the bulk of the interstitial stromal collagens (Table 1.1). Structurally all collagens are characterized

Table 1.1 Classification and types of collagens based on the collagen chain encoding genes, their distribution in tissues and disorders caused mutations

Classification	Type	Own chains	Gene	Exons	Chromo-some[a]	Distribution in tissues	Disorders caused by mutations in genes
Fibrillar collagens	I	αl	*COL1A1*	51	17q21.33–q22	Bone, tendon, ligament, skin	Osteoporosis, scars, Ehlers-Danlos syndrome, osteogenesis imperfecta
		α2	*COL1A2*	52	7q22.1		
	II	αl	*COL2A1*	54	12q13.11	Cartilage, intervertebral disc, vitreous humor	Several chondrodysplasias
			COL2A2	54			Osteoarthritis
	III	αl	*COL3A1*	51	2q24.3–q31	Co-expressed with collagen I in vasculature and skin	Ehlers-Danlos syndrome (type IV), granulation tissue, Dupuytren's contracture
			COL3A1	51	1		arterial aneurysms
	V	αl	*COL5A1*	66	9q34.2–q34.3	Co-expressed with collagen I in lungs, cornea, bone and placenta	Ehlers-Danlos syndrome (types I and II)
		α2	*COL5A2*	54	2q14–q32		
		α3	*COL5A3*	66	19p13.2		
	XI	αl	*COL11A1*	68	1p21	Co-expressed with collagen II	Chondrodysplasias, non-systematic hearing loss, osteoarthritis
		α2	*COL11A2*	66	6P21.1		
		αl(II)					
	XXIV	αl	*COL24A1*	57	1p22.3	Co-expressed with collagen I in bone and cornea	Not known
	XXVII	αl	*COL27A1*	61	9q32	Co-expressed with collagen II in cartilage and epithelia	Not known
	XXVIII	αl	*COL28A1*	32	7p21.3	Peripheral nerves	Not known

(continued)

Table 1.1 (continued)

Classification	Type	Own chains	Gene	Exons	Chromo-some[a]	Distribution in tissues	Disorders caused by mutations in genes
3D network	IV	αl	*COL4A1*	52	13q34	Most basement membranes	Alport syndrome, Goodpasture's syndrome
(BM-collagens)		α2	*COL4A2*	47	13q34	Glomerular and alveolar BM	(COL4A3, COL4A4, COL4A5)
			COL4A2	47	8		Alport syndrome with diffuse oesophageal leiomyomatosis
		α3	*COL4A3*	52	2q34–q37		(COL4A5, COL4A6)
		α4	*COL4A4*	48	2q35–q37		
		α5	*COL4A5*	51	Xq22		Lethality at 14 weeks, progressive glomerulonephritis, renal failure[b]
		α6	*COL4A6*	46	Xq22		Alport syndrome
Microfibril (Beaded filaments)	VI	α1	*COL6A1*	36	21q22.3	Wide tissue distribution, associated with type I collagen, not bone	Bethlem myopathy, Ulrich myopathy
		α2	*COL6A2*	36	21q22.3		
		α3	*COL6A3*	41	2q37		
Anchoring fibril	VII	α1	*COL7A1*	118	3p21.31	Squamous epithelium BM zone	Epidermolysis bullosa
			COL7A1	118	9		
Hexagonal lattice	VIII	α1	*COL8A1*	5	3q12.3	Many tissues, Descemet's membrane of cornea	Corneal endothelial dystrophy
		α2	*COL8A2*	2	1p34.2		
	X	α1	*COL10A1*	3	6q21–q22	Hypertrophic cartilage	Schmid metaphyseal chondrodysplasia
			COL10A1	3	10		

FACITs	IX	α1	*COL9A1*	38	6q12–q14	Associated with type II fibrils in cartilage and cornea	Epiphyseal dysplasia, intervertebral disc disease, osteoarthritis
		α2	*COL9A2*	32	1p32		
			COL9A2	32	4		
		α3	*COL9A3*	32	20q13.3		
	XII	α1	*COL12A1*	65	6q12–q13	Associated with type I fibrils in perichondrium, ligaments and tendons	Disruption of periodontaland skin matrix structures[b]
	XIV	α1	*COL14A1*	44	8q23	Associated with type I fibrils in many tissues	Not known
	XVI	α1	*COL16A1*	67	1p35–p34	Associated with type II in hyaline cartilage and withmicrifibrils in skin	Not known
	XX	α1	*COL20A1*	35	20q13.33	Associated with type I fibrils in sternal cartilage, cornea and tendon	Not known
	XXI	α1	*COL21A1*	28	6p12.3–p11.2	Associated with type I fibrils in vessel walls	Not known
FACIT-like	XIX	α1	*COL19A1*	51	6q12–q14	Rare BM zones, in developing muscles	Abnormal muscle layer in the oesophagus[b]
	XXII	α1	*COL22A1*	63	8q24.23	Associated with microfibrils at tissue junctions	Not known
	XXVI	α1	*EMID2*	13	7q22.1	Testis and ovary	Not known

(continued)

Table 1.1 (continued)

Classification	Type	Own chains	Gene	Exons	Chromo-some[a]	Distribution in tissues	Disorders caused by mutations in genes
Transmembrane	XIII	α1	*COL13A1*	41/42	10q22	Many tissues at a low level	Fetal lethal, cardiovascular and placental defects, tumor formation[b]
			COL13A1	42	10		Progressive muscular atrophy[b]
	XVII	α1	*COL17A1*	56	10q24.3	Skin and intestinal epithelia	Epidermolysis bullosa
	XXIII	α1	*COL23A1*	20	5q35.3	Heart, lung and brain metastatic tumor cells	Not known
	XXV	α1	*COL25A1*	35	4q25	Neurons	Not known
Multiplexins	XV	α1	*COL15A1*	42	9q21–q22	Many BM zones	Mild myopathy, cardiovascular defects[b]
			COL15A1	40	4		
	XVIII	α1	*COL18A1*	43	21q22.3	Endothelial and epithelial BM zones	Knobloch syndrome
			COL18A1	43	10		Vascular abnormalities in the eye[b]

[a]The chromosomal locations and the exones were collected from the Entrez Gene data base

[b]In transgenic mouse models; BM-basement membrane

Modified from Jälinoja (2007), Cosgrove et al (1996), Reichenberger et al. (2000), (Myllyharju and Kivirikko 2001), Fukai et al. (2002), Sund et al. (2001), Kvist et al. (2001), Ricard-Blum (2011) and Eklund et al. (2001)

by the archetypical Gly-X-Y repeat sequences, in which X is often proline and Y either hydroxyproline or hydroxylysine. After synthesis of collagen α-chains, regularly repeated glycins with their minimal side chains (-H) allow three individual collagen chains to wind up around each other into triple helical collagen monomer, tropocollagen, with globular amino- and carboxyterminal propeptide ends. The collagen superhelix domain is, due to its structure, very resistant against non-specific proteinase-mediated degradation. After processing and removal of the globular propeptides individual collagen monomers spontaneously non-enzymatically assemble side by side to near one quarter overlapping supramolecular stacks with the typical cross-striation visible in electron micrographs. Fibre thickness for type I collagen is regulated in part by type III collagen and for type II collagen (Wiliusz et al. 2014) in part by collagens IX embedded in part inside the collagen type II fibre and type XI located on the surface of collagen type II fibre. These still relatively loose fibre stacks mature by covalent cross-linking in a process which involves specific cross-linking enzymes, hydroxyproline and hydrolysin and ascorbic acid without which develops a disease leading to spontaneous bleedings and known as scurvy. The characteristics of cross-linking are importent in healthy tissue and pathology (Snedeker and Gautieri 2014). Collagen network provides substrate for attachment of ground substance and cells and provides the framework for deposition of various bioactive factors.

Purified native or processed allogeneic and recombinant human collagens, especially type I, are composed of nanosize biodegradable biofibres with potential for use in plastic and cosmetic surgery, drug delivery and tissue engineering in form of sheets, pellets, plugs, sponges and other products (Chattopadhyay and Raines 2014). Collagen sponge seeded with bone marrow-derived MSCs can develop healing tissue which to its biomechanical strength to 75 % matches that of the corresponding healthy tissues (Juncosa-Melvin et al. 2006).

Basement membranes support epithelia (Choi et al. 2015) and endothelia casting them to their spatial shapes, such as simple sheets, tubes or relatively sophisticated tubuloacinar, tubuloalveolar and vascular structures. Basement membrane also surrounds some individual cells, like adipocytes, Schwann cells and skeletal muscle cells. Basement membrane components are found in the intercellular cementing substance between fibroblast-like type B and macrophage-like type A synovial lining cells joining them to form synovial lining or intima of the joint cavity, which in spite of its sheet-like structure does not have an actual sheet-like basement membrane.

Basement membrane is a 100–300 nm thick barrier with perforations in the order of ~50 nm permitting free bidirectional movement of small molecules whereas the movement of cells and larger molecules is controlled (Kruegel and Miosge 2010). Certain cells, like the neutrophils, do cross the BM with great efficiency. Chemoattractants and proteolytic events play a role in this process.

It has been thought that type IV collagen polymer network serves as the base platform upon which that laminin network is deposited but laminin polymers may actually serve as a template for the subsequent assembly of the BM (Li et al. 2005; McKee et al. 2007). Collagen IV and laminin forms ternary complexes linked

together by nidogen-1 and -2 (Fox et al. 1991; Kohfeldt et al. 1998). A heparan sulphate/chondroitin sulphate proteoglycan, perlecan is also found as an integral part of this network and is important for its integrity and as a local storage of growth factors (Gohring et al. 1998).

The type IV collagens were first identified by Kefalides in 1966 (Kefalides 1966). Depending upon its location it is synthesized either by fibroblasts, paranchymal cells, epithelial cells, endothelial cells, or by various other cells that are surrounded by the BM. The collagen type IV genes in human encoding its different α-chains are arranged head-to-head in three pairs. Gene encoding type IV collagen α1, α2 (*COL4A1, COL4A2*) and α3, α4 *(COL4A3, COL4A4)* is located on chromosome 13, and on chromosome 2, respectively, while the gene encoding for type IV collagen α5, α6 (*COL4A5, COL4A6*) is located on chromosome X. A common ancestral gene may have been duplicated three times resulting in six evolutionary related genes (Zhou et al. 1994). Sequences and characteristic exon-intron organizations divide them into α1-like group (*COL4A1, COL4A3, COL4A5*), and α2-like group (*COL4A2, COL4A4, COL4A6*). A unique feature of the type collagen IV gene pairs is that they share bidirectional promoters.

All type IV collagen *a* chains are ~1400 amino acids long. They are composed of a ~15-residues long N-terminal 7 S domain, collagenous segments consisting of Gly-X-Y repeats, which are interrupted by 22 short non-collagenous sequences (which provide flexibility and serve as cell-binding sites) and a ~230-residue long C-terminal non-collagenous NC1 domain (Kalluri 2003). Three type IV collagen wind up to a triple helical tropocollagen. According to the currently known combinatorial rules three distinct trimers are formed, α1α1α2, α3α4α5, and α5α5α6 (Boutaud et al. 2000; Hudson et al. 2003; Khoshnoodi et al. 2008).

The collagen monomers further self-assemble to supramolecular networks. First, dimers are formed by head-to-head association of two protomers via their trimeric NC1 domains so that bonding NC1 hexamers are formed. Four dimers then join at their N-terminal cystine- (disulfide bonds) and lysine-rich (lysine- hydroxylysine bonds) containing regions to form a heavily glycosylated 7 S-tetramer. This knot is relatively resistant to collagenase activity and can be isolated from bacterial collagenase treated basement membranes at a sedimentation coefficient 7 S (Risteli et al. 1980). α1α1α2(IV) and α3α4α5(IV) trimers form independent networks, while α1α1α2/α5α5α6 (IV) molecules form combined aggregates (Borza et al. 2001).

Type IV collagen composition of the basement membrane seems to affect cell adhesion, proteolytic susceptibility and ability to interact with other BM components (Kalluri 2003). The α1(IV) and α2(IV) are ubiquitously found throughout the human body, while the other 4 α chains have a more restricted tissue distribution. The α3α4α5(IV) network is found in the glomerular and some tubular basement membranes of the kidney, cochlea, eye, lung and testis, whereas the α5α5α6(IV) trimer is located in the skin, oesophagus, Bowman's capsule of the kidney and smooth muscle cells. A temporal regulation of type IV collagen α-chains expression is seen for instance in the glomelular BM of human kidney. During early embryonic development (day 75), the genes which encode α1(IV) and α2(IV) chains are expressed. As the development proceeds the expression of genes encoding the α3(IV), α4(IV) and α5(IV) chains starts while the levels of α1(IV) and α2(IV)

chains gradually decrease. This switch in gene expression during developmental is critical for maturation of the glomerular BM.

Mutations in the genes encoding either α1(IV) or α2(IV) are embryonic lethal, while mutations in the genes encoding the α3(IV), α4(IV) or α5(IV) chains may lead to human diseases (Hudson et al. 2003; Hudson 2004). Mutation of the *COL4A5* gene results in Alport syndrome. This mutation being mostly inherited and results in glomerulonephritis and hearing loss. Goodpasture's syndrome is an autoimmune disease manifest by rapidly progressive glomerulonephritis and pulmonary hemorrhage. The Goodpasture antigen, which is usually the NC1 domain of the α3(IV) chain, is most exposed in the glomeruli and alveolar basement membranes.

Type IV collagen expression is suppressed by pioglitazone (Ohga et al. 2007; Ko et al. 2008) and methotrexate (Yozai et al. 2005). Tumstatin, the NC1 domain of α3(IV) chain, is an endogenous inhibitor of pathological angiogenesis and suppresses tumour growth via integrin αvβ3, because tumstatin binding inhibits focal adhesion kinase and some other signalling pathways, which inhibits endothelial cell proliferation and induces apoptosis (Maeshima et al. 2001, 2002).

Endothelial cells, basal cell layer keratinocytes, hepatocytes, carcinoma cells, melanoma cells, fibrosacroma cells and many other cells bind via adhesion receptors to multiple sites in the NC1 and/or the triple helix domains of type IV collagen.

1.4.2 Extracellular Matrix: Non-Collagen Proteins

Elastin is a highly elastic stretchable and recoiling strong and elastic fibrous protein of many connective tissue matrices of the body, including in particular large and medium size arteries, lung alveoli, skin and intervertebral discs. It yields under stress but stores energy upon stretching (Baldwin et al. 2013; Green et al. 2014). Polymorphic and soluble tropoelastin monomers is produced and secreted by smooth muscles cells in arteries and by fibroblasts. Around 65 kD size, glycine-, proline-, valine- and alanine-rich tropoelastin monomers are rapidly close to their cellular source covalently cross-linked by lysine oxidase to form elastic di-, tri- or tetrafunctional crosslinks, e.g. desmosine, isodesmosine. This leads to the formation of irregularly organized and randomly coiled amorphous and yellowish elastin-rich networks and sheets. In these structures elastin is surrounded by fibulin and fibrillin sheaths and a pathogenic mutation of fibrillin-1 is linked to Marfan syndrome with e.g. risk for dissection of the aorta.

Heterodimeric laminins glycoporteins form one of the two major non-collagenous networks in the basement membranes (Hohenester and Yurchenco 2013). Laminins affect tissue morphogenesis, maintenance and function by influencing proliferation, migration and differentiation (Jones et al. 2000; Halper and Kjaer 2014). The laminin network is via entacin or nidogen linked to the other major basement membrane network, which is composed of type IV collagen. Laminins (Aumailley 2013) are composed of five different α chains (of which α3 chain has two variants), three different β chains and three different γ chains, which according to currently known combinatorial rules can combine to 17 different trimeric laminin molecules

Table 1.2 Classification of laminins, their abbreviations according to current nomenclature with some alternative names and the genes encoding them

Laminin (LM)	Abbreviation and alternative names	Genes encoding the laminin chains
LM-α1ß1γ1	LM-111, Ln-1	*LAMA1, LAMB1, LMAC1*
LM-α2β1γ1	LM-211, Ln-2	*LAMA2, LAMB1, LAMC1*
LM-α1β2γ1	LM-121, Ln-3	*LAMA1, LAMB2, LAMC1*
LM-α2β2γ1	LM-221, Ln-4	*LAMA2, LAMB2, LAMC1*
LM-α3Aβ3γ2	LM-332/LM-3A32, Ln-5/5A	*LAMA3A, LAMB3, LAMC2*
LM-α3Bβ3γ2	LM-3B32, Ln-5B	*LAMA3B, LAMB3, LAMC2*
LM-α3Aβ1γ1	LM-311/LM-3A11, Ln-6	*LAMA3A, LAMB1, LAMC1*
LM-α3Aβ2γ1	LM-321/LM-3A21, Ln-7	*LAMA3A, LAMB2, LAMC1*
LM-α4β1γ1	LM-411, Ln-8	*LAMA4, LAMB1, LAMC1*
LM-α4β2γ1	LM-421, Ln-9	*LAMA4, LAMB2, LAMC1*
LM-α5β1γ1	LM-511, Ln-10	*LAMA5, LAMB1, LAMC1*
LM-α5β2γ1	LM-521, Ln-11	*LAMA5, LAMB2, LAMC1*
LM-α2β1γ3	LM-213, Ln-12	*LAMA2, LAMB1, LAMC3*
LM-α3β2γ3	LM-323, Ln-13	*LAMA3, LAMB2, LAMC3*
LM-α4β2γ3	LM-423, Ln-14	*LAMA4, LAMB2, LAMC3*
LM-α5β2γ3	LM-523, Ln-15	*LAMA5,LAMB2, LAMC3*
LM-α5β2γ2	LM-522	*LAMA5, LAMB2, LAMC2*

Modified from Patarroyo et al. (2002), Aumailley et al. (2005), Aumailley (2013), Tzu and Marinkovich (2008), Egles et al. (2007)

Table 1.3 Tissue distribution of laminin alpha, beta and gamma chains

α1	Early embryo, neuroretina, adult kidney proximal tubules, salivary and mammary glands
α2	Trophoblast, foetal skin and kidney, adult skin, skeletal and cardiac muscle, peripheral nerve, some capillaries, brain and other tissues
α3	Foetal skin, lung alveoli and bronchi, adult skin, alveoli, bronchi and most other epithelia
α4	Foetal skin and kidney, skeletal muscle, adult skin, cardiac and visceral smooth muscles, nerves, blood vessel endothelia, bone marrow and other tissues
α5	Foetal skin, lungs and kidney, adult skin, alveoli, bronchi, diverse epithelia, kidney, blood vessels, bone marrow, developing muscles and nerves, synaptic membranes
β1	Most tissues
β2	Foetal bronchi and alveoli, kidney, adult neuromuscular junction, blood vessels, kidney glomeruli
β3	Foetal skin and lungs, adult skin and most other epithelia
γ1	Most tissues
γ2	Foetal skin and lungs, adult skin, bronchi and most other epithelia
γ3	Kidney, lungs, reproductive tract, nerves and brain

Modified from Patarroyo et al. (2002), Tzu and Marinkovich (2008)

(Table 1.2). They have a tightly regulated tissue-specific localizations to be able to contribute to the heterogeneity and site-specific regulation of cells and tissues (Table 1.3). This latter aspect should be emphasized, because in spite of the fact that

the effect of soluble regulatory factors can by accident diffuse over and beyond their physiological limits to cause pathology, whereas solid regulatory molecules are from this point of view more site specific and safe.

One important in tissue regeneration adhesive fibrous glycoprotein either locally synthesized or precipitated from the circulation is fibronectin (Stoffels et al. 2013). In spite of local fibroblast-mediated fibronectin synthesis, it does not stain or stains only weakly at the base of chronic, non-healing ulcers (Herrick et al. 1992, 1996). This is probably due to rapid proteolytic degradation of newly synthesized extracellular fibronectin matrix in such inflammatory and proteinase-rich environment (Weckroth et al. 1996). Fibronectin, a major ECM protein, regulates non-canonical Wnt signaling during embryogenesis and in muscle regeneration. It modulates canonical Wnt signaling through modulation of β-catenin (Astudillo and Larraín 2014).

Cartilage oligomeric matrix protein (COMP) is a pentamer with 5 collagen binding "arms", found in cartilage, ligaments and tendons. COMP binds to free collagen type II and I molecules facilitating formation of banded fibres. It is not found in mature fibres, except at the tip/end of eventually growing fibres (Das et al. 2015).

1.4.3 Extracellular Matrix: Ground Substance

Ground substance is amorphous gel-like mass largely composed of proteoglycans, which are formed of an organizing protein core on the surface of the cell or in the interstitium, with attached linear hydrophilic glycosaminoglycan (GAG) bipolymers (mucopolysaccharides). GAGs are composed of 50–1.000 repeat disaccharide units and based on the structure of the disaccharide backbone, chemical bonding utilized between the sugar residues and side chain modifications, such as acetylation and sulphation.

GAGs are divided into (1) hyaluronates composed of D-glucuronate + GlcNAc linkage β(1–3), (2) heparin composed of L-iduronate α (1–4) N-sulfo-D-glucosamine-6-sulphate α(1–4) backbone with variable degrees of sulphation of the L-iduronate (2-O position) and/or glucosamine (3-O or 6-O position, in addition, the N-position of the glucosamine can be sulphated, acetylated or unsubstituted, located in mast cell granules, (3) heparan sulphate composed of D-glucuronate β (1–3) N-sulfo-D-glucosamine-6-sulfate β(1–4) with variable degrees of sulphation of the glucoronate (2-O position) and/or N-acetylglucosamine (3-O or 6-O position, in addition the N-position of the glucosamine can be acetylated, sulphated or unsubstituted). It contains fewer N- and O-sulphate groups and more N-acetyl groups than heparin, but it is heterogenous as it also contains heparin-like segments, found, *e.g.* in cell surface proteins, lung, basement membranes, heparin or heparin sulphate are found in extracellular perlecan (can alternatively contain chondroitin sulphate), cell surface syndecans and glypicans and a small leucin-rich proteoglycan (SLRP) known as prolargin (coded by the *PRELP* gene, standing for proline arginine-rich end leucine-rich repeat protein); SLRPs may in addition to proteoglycans

also contain O-linked oligosaccharides and sulphated tyrosine residues, and one member, integrin-binding chondroadherin, only contains O-linked short oligosaccharides, which form only 1 % of its molecular mass, (4) chondroitin sulphate (composed D-glucoronate β(1–3) N-acetyl-D-galactosamine β(1–4) backbone with variable degrees of sulphation of the glucoronate (carbon 2) and/or N-acetyl-D-galactosamine (carbon 4 in chondroitin-4-sulphate and/or carbon 6 in chondroitin-6-sulphate), *e.g.* cartilage, bone, tendons, ligaments, found in large aggregating proteoglycans or hyaluronan-binding lecticans (hyalectans), like aggrecan (forming 95 % of the proteoglycans in cartilage, bound to hyaluronan core), versican, neurocan and brevican, and some SLRP which contain either chondroitin and/or dermatan sulphate side chains, as found in decorin/small proteoglycan II (1 chain), biglycan/small proteoglycan I (2 chains) and epiphycan (2 chains) in the epiphysis), (5) dermatan sulphate (differs from chondroitin sulphate by also containing L-iduronate α(1–3) N-acetyl-D-galactosamine-4-sulphate β(1–4) disaccharides in its backbone with variable degrees of sulphation of the iduronate (carbon 2) and/or N-acetyl-D-galactosamine (carbon 4 and/or 6), *e.g.* skin, blood vessels, heart valves) and (6) keratan sulphates composed of D-galactose β(1–4) N-acetyl-D-glucosamine-6-sulphate β(1–3) with variable degrees of sulphation of the galactose (carbon 6) and/or N-acetyl-D-glucosamine (carbon 6), *e.g.* cornea, bone, cartilage, nucleus pul- posus, found in some SLRPs, like lumican, keratocan and mimecan (osteoglycin or osteoinductive factor) in the transparent cornea, integrin-binding osteoadherin (osteomodulin) in mineralized tissues and fibromodulin in the cartilage, all with 1–3 N-linked keratan sulphate chains and sulphated tyrosine residues). The sulphate content is highly variable and its molecular components are occasionally substituted with, *e.g.* fucose or mannose.

At the physiological pH most of their sulphate and carboxyl groups of these long molecules are negatively charged making these molecules viscous, highly charged, able to bind water and elastic. These molecules exert swelling pressure checked by the collagen fibres of the matrix. Proteoglycans occur as cell surface and interstitial molecules and provide a cell-friendly hydrogel-like but permeable surrounding for the cells. Perhaps the best recognized role of proteoglycans relates to their ability to bind and deposit growth factors, like most of the 22 now known fibroblast growth factors (FGFs), some of which bind less avidly and can have systemic, endocrine actions, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), vascular endothelial growth factors (VEGF) and endostatin derived from degradation of type XVIII collagen with anti-angiogenic properties. Due to local release and paracrine mode of action GAG-deposited growth regulating factors play important roles in tissue repair. Chondroitin sulphate sulphation motif epitopes are useful in the identification of articular cartilage progenitor cells.

Some hundred bottle brush-like aggrecan molecules, the prototype of lecticans, are in cartilage attached to a hyaluronan core via the globular G1 domain of the aggrecan core molecule located at the N-terminus. This binding to hyaluronan is enforced by a HA-binding link protein. G1 is via an interglobular domain combined with another globular domain, G2, followed by a long GAG-binding region, first one keratan sulphate-rich region (able to bind collagen) and then two chondroitin

sulphate-rich regions, and finally a third globular domain G3 (composed of a splice-dependent complement regulatory protein-like module and an epidermal growth factor (EGF)-like modules, and a constant C-type lectin-module) able to mediate binding to fibulin-1 and-2, fibrillin-1 and tenascin-R, but according to its lectin nature to cell surface glycolipids. Several of the G3 domain ligands are di- or oligomers and could therefore mediate cross-linking of the hyaluronan-lectican complexes to each other. Such cross-linking would be impaired upon age-related fragmentation of aggrecan and loss of the cross-linking G3 domains. Early arthritis is characterized by loss of metachromatic proteoglycan staining, which is due to proteolytic solubilisation of aggrecan by ADAMTS4 (a disintegrin and a metalloproteinase with a thrombospondin motif 4) and ADAMTS5, often at the sensitive interglobular domain. Versican is named for its versatile molecular structure, is produced by vascular smooth muscle cells, fibroblasts, keratinocytes and many other cells. In mesenchymal condensations and developing cartilage versican expression precedes aggrecan expression, which is found together with fibulins as in an attempt to organize the early matrix. Neurocan produced by neuronal cells and brevican produced by astrocytes (with the shortest core protein in this family, occurs also in a glycosylphosphatidylinositol-form) are mainly found in the nervous system and participate in glial scar formation and central nervous system repair (Fawcett and Asher 1999).

SLRPs decorin, fibromodulin and lumican reduce collagen fibre thickness of both type I and II collagens. Fibromodulin may also catalyze lateral growth of type I collagen, whereas perlecan with attached chondroitin sulphate can promote fibril logenesis of type II collagen.

Ground substance contains non-core protein bound and non-sulphated sixth GAG known as hyaluronan composed of D-glucoronate β(1–3) N-acetyl-D-glucosamine β(1–4) backbone and found in *e.g.* synovial fluid, articular cartilage, vitrous fluid of the eye ball. It can be 25–25,000 disaccharide units long and imparts high viscosity to hyaluronan containing body fluids. Due to its water binding ability a hyaluronan domain occupies some 1000-fold the volume of its dry state. GAG-derivatized chitosan membranes increased MSC growth rate about fivefold compared to tissue culture plastic or chitosan alone, but in a GAG-type and concentration-dependent manner. Effects of heparin, heparin sulphate, dermatan sulphate and chondroitin-6-sulphate are fibronectin-dependent, but those of hyaluronan and chondroitin-4-sulphate are fibronectin-independent (Uygun et al. 2009).

1.5 Integrin and Non-integrin Matrix Receptors

Integrins are 24 different non-covalently coupled heterodimeric cellular receptors composed of 16 α and 8 β chains, which form an important link in the integration of cellular actin cytoskeleton with the cellular surrounding, including the ECM. They do not only bind cells to matrix, but also allow exploration, migration and outside-in and inside-out signalling, which acts in concert with soluble and cell-cell signals in the regulation of cell behaviour. Discoidin domain receptors, Lutheran Lu/B-CAM

complex and α/β-dystroglycan complex form important collagen, laminin and/or other ligand binding matrix receptors. Lately lectins, glycan binding receptors, in particular extracellular C-, R- and I-type lectins and galectins have been shown to play roles in cell-matrix adhesion and signalling, including MSC recruitment, bone marrow stem cell niche and adult collagen remodelling.

1.5.1 Integrin-Type Matrix Receptors

Integrins form the major class of cellular receptors for ECM ligands (Heino and Käpylä 2009; Heino 2014), so much so that the other matrix receptors are often summed up as non-integrin receptors. Integrin receptors are heterodimers, which are composed of one of the 16 α and 8 β chains, which all cross the cell membrane only once. They combine in a non- covalent fashion along currently known combinatorial rules to altogether 24 different integrin receptors (Fig. 1.1). All integrin receptors are able to bind to at least two ligands, which leads to overlap and redundancy and cover many important components of the ECM. Alternative processing of α and β chains confers further diversity to the integrin receptor system.

The α-chain of the integrin receptor largely determines its ligand binding specificity, whereas the β-chain mainly participates in the assembly of integrins to specialized clusters known as focal adhesions, which mediate external physical stress from extracellular collagen, fibronectin, laminin and other matrix ligands to cellular actin cytoskeleton, i.e. integrate the cell to its matrix. Integrins are often grouped to subfamilies based on their ligand binding specificity, evolutionary relationships and topological restrictions *(e.g.* leukocyte integrins).

The binding force of one individual integrin-matrix ligand pair is minor compared to other more specialized anchoring transmembrane molecules, but the combined avidity of a myriad of integrins can resist considerable mechanical forces. At the same time this arrangement allows the cell to explore and respond to its extracellular matrix, to bind and to let go, i.e. enable dynamic cellular migration along solid substrates in a process known as haptotaxis. Integrins can form new bonds at the advancing edge of the cells, at the same time when integrin-ligand bonds dissociate at the retracting rear.

Integrins are not passive matrix binders but their expression and binding are actively regulated in a bidirectional inside-out and outside-in signalling, which qualitatively and quantitatively regulates integrin-mediated cell-matrix interactions. Binding to ECM delivers signals regulating migration, proliferation, growth, differentiation and apoptosis, often along the same signal transduction pathways which act in concert with various soluble chemotactic, growth and differentiation factors and their receptors. Thus, integrins both bind cell to its surrounding but also help the cell to respond to it. Integrins are not constantly active but their activity is regulated, in part via other integrins.

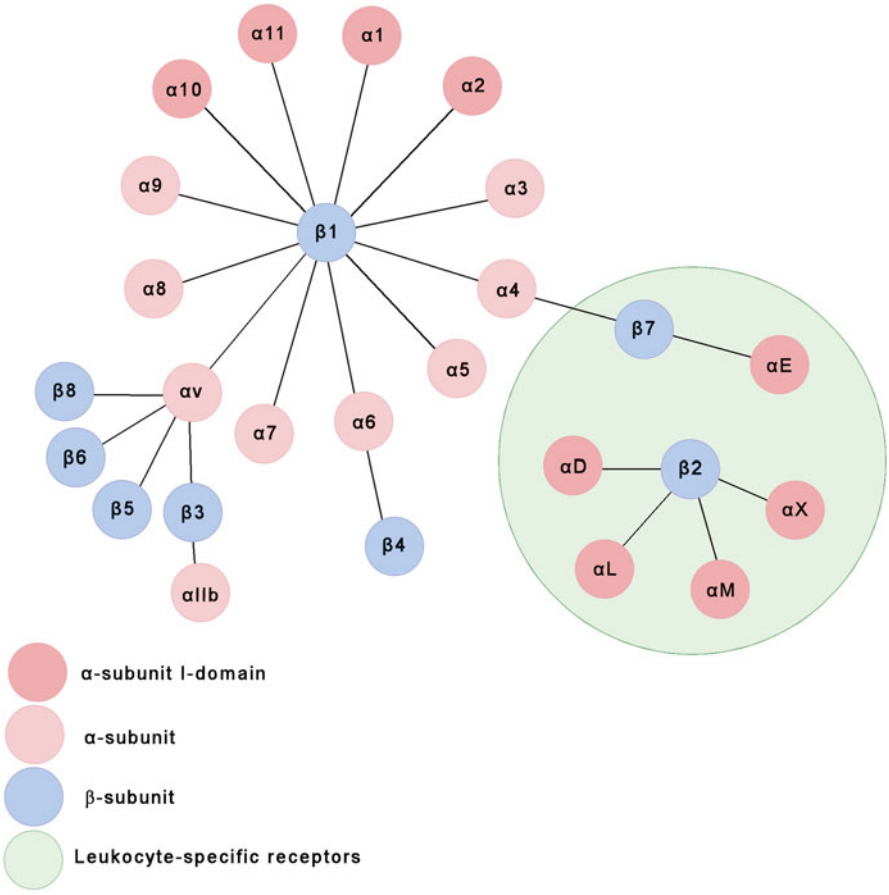

Fig. 1.1 Integrins are heterodimeric receptors composed of one of the 16 known α and 8 β chains. They combine in a non-covalent fashion along currently known combinatorial rules, which are shown in the figure, and which leads to the formation of altogether 24 different integrin receptors

1.5.2 Non-integrin Matrix Receptors

Discoidin domain receptors-1 and -2 (DDR1 and DDR2) mediate in its various isoforms cellular non-integrin binding to collagen and are tyrosine kinase receptors, which regulate cell adhesion, proliferation and ECM. DDR1 has been described in cells in brain, skin, colonic mucosa, kidney tubules, lungs and thyroid gland, whereas DDR2 has been found in heart and skeletal muscle, lung, brain and kidney. Cartilage collagen type II stimulates DDR2 and MMP-13 expression in chondrocytes, which parameters are linked to the severity of osteoarthritis (Sunk et al. 2007).

The Lutheran system Lu/B-CAM comprises Lutheran (Lu) and its alternatively spliced form, basal cell adhesion molecule (B-CAM). They are expressed by red

blood cells, over-expressed in sickle cells, but also expressed by vascular endothelial cells and epithelial cells. In normal cells they are polarized manner and in cancer cells they are over-expressed. They bind laminin α5 containing Lm-511, Lm-521 and Lm-523. Human embryonic stem cells synthesize laminin α1 and α5 chains together with laminin β1 and γ1 chains suggesting that Lms-111 and -511 may be important for the their cell-matrix contacts. Correspondingly, functional adhesions experiments suggested that in particular Lutheran blood group antigen and B-CAM together with Int α3β1 play an essential role for their adhesion to Lm-511, whereas Int α6β1 mediated adhesion to Lm-411 (Vuoristo et al. 2009). Such studies are important because one important role for the non-homologous feeder cell layer may be production of ECM, which is necessary for their interactions with stem cells and for stem cell proliferation and maintenance. It might be possible to culture stem cells without feeder cells and to replace stem cell-feeder cell communication by performing stem cell cultures on appropriate matrix substrate.

Alpha-dystroglycan is extracellular molecule, which binds laminin-α2, agrin and perlecan, whereas the associated transmembranous β-dystroglycan component links the dystroglycan complex intracellularly to dystrophin, which further mediates contact with the actin cytoskeleton. α/β-dystroglycan provides structural integrity and synaptic acetylcholine receptor organization in muscle and other tissues.

Lectins are sugar moiety specific carbohydrate binding non-integrin receptors mediating attachment and aggregation of cells via binding to and cross-linking (at least two sugar binding sites, referred to as carbohydrate-recognition domains) glycoproteins, glycolipids and other glycoconjugates (glycans). Some of them are cell membrane bound. If their glycan ligands locate in the extracellular matrix, they mediate cell-matrix recognition and interactions, but their main task seems to be recognition of various microbial pathogens. Due to their binding specificity, lectin-mediated binding can be regulated by blocking mono- or oligosaccharides, which are useful to study their sugar specificity and have potential as drugs and research tools.

Extracellular lectins include C-type (Ca^{2+}-dependent), R-type (ricin-like), I-type (immunoglobulin domain containing) and galectins (β-galactoside binding), but new extracellular and intracellular lectin families have been recently described. Selectins (endothelial E-, leukocyte L- and platelet and endothelial P-selectins) belong to C-type lectins, which participate in leukocyte recruitment (tethering and rolling). MSCs seem to lack the conventional P-selectin ligands, P-selectin glycoprotein ligand 1 (PSGL-1) and CD24. They may instead express some novel ligand because P-selectin on endothelial cells induces rolling and tethering of circulating MSCs. Chemokines attract and activate MSCs via chemokine receptors to express the very late activation antigen-4 (VLA-4 = Int $\alpha_4\beta_1$ receptor = CD49d/CD29), which firmly adheres the MSC to the vascular cell adhesion molecule-1 (VCAM-1, CD106) on endothelial cell, enabling transmigration to damaged tissues via diapedesis (Fox et al. 2007; Ruster et al. 2006). Selectins may also play a role in the homing to and maintenance of stem cells in the bone marrow stem cell niche. Endo180 on fibroblasts and macrophages, a member of R (ricin-like)-type lectin, contains

fibronectin-like domains, which can mediate binding to *e.g.* collagens I, II, IV and V. It forms a trimolecular complex with urokinase plasminogen activator (uPA) and its receptor (uPAR), but it is not known if its C-type and R-type lectin domains and glycan recognition sites are important in Endo180-mediated cell-matrix adhesion events. Endo180 is also a collagen internalisation receptor, which together with $\alpha_2\beta_1$ integrin receptors mediate specific binding, cellular uptake and delivery of collagens to intracellular, lysosomal degradation. In addition to its major role in the intracellular collagen degradation, endo180 seems to regulate the other major collagenolytic pathway, namely the extracellular and pericellular MMP-dependent collagen degradation pathway (Messaritou et al. 2009). I (Ig-like domain containing)-type lectins contain many members belonging to the siglec group (sialic acid-binding immunoglobulin superfamily lectins) or other I-type lectins. They have been described on various leukocytes, like macrophages, dendritic cells, B cells, neutrophils, eosinophils etc., but one of the best studied I-lectins is NCAM (neural cell adhesion molecule). NCAM can bind heparin/heparin sulphate containing cell surface and matrix proteins and chondroitin sulphate containing neurocan. It can also indirectly bind to collagen via heparin/heparin sulphate bridges (Angata and Brinkman-van der Linden 2002). Galectins (b-galactoside-binding) are expressed on many immune cells and participate in innate and adaptive responses by modulating T-cell apoptosis, proliferation, adhesion, chemotaxis and synthesis of cytokines and other mediators. They are also expressed on keratinocytes (galectin-7), lung (galectin-8) and adipocytes (galectin-12), where they play roles in skin healing, lung cancer and adipogenic signalling/adipocyte differentiation, respectively. Galectin-1 and -3 have been described to modulate cell-matrix interactions (Rabinovich et al. 2002) and galectin-9 to accelerate TGF-β3 induced chondrogenic responses (Arikawa et al. 2009).

Some broad-specificity scavenger receptors of class A, B and C may also bind components of extracellular matrix, *e.g.* CD36 belonging to scavenger receptor type on the surface of platelet can bind it to collagen. Hyaluronan can by bound by hyaluronan cell surface receptor CD44, which has several different isoforms and is found on the surface of chondrocytes and other cells.

1.6 Matrix Modulating Proteinases

Proteinases participate in normal tissue remodelling, but can cause tissue destruction when uncontrolled and excessively active. Proteinases are divided based on their catalytic mechanism to secretory neutral serine and metallo endoproteinases (and amino- and carboxypeptidases) and to mostly intralysosomal acidic cysteine and aspartate endoproteinases. Classification of the proteinases is based on their catalytic mechanisms, which is reflected in the key amino acids necessary for the catalysis. In practice, classification is often based on the use of class specific inhibitors.

1.6.1 Neutral Endoproteinases

Matrix metalloproteinase or MMP family consists of 22 members, subdivided in collagenases, stromelysins and other MMPs (archetypical MMPs); gelatinases; matrilysins; type I and II transmembrane "membrane type MMPs" (MT-MMP), GPI anchored MT-MMPs and secreted MMPs (furin-activatable MMPs).

MMPs have so an extended substrate specificity that they can in practice degrade any protein component of the ECM (Kessenbrock et al. 2015; Klein et al. 2015; Bonnans et al. 2014). MMP activity is regulated at the level of gene transcription (cis-regulatory elements and epigenetic mechanisms), translation (mRNA stability, translational efficiency and probably also micro-RNA-mediated), storage/secretion (*e.g.* pro-MMP8 is stored in neutrophils in the secondary or specific granules), focalization *(e.g.* MT1-MMP/TIMP-2/MMP-2 complexes), activation (of pro-MMP to MMP) and endogenous inhibitors (tissue inhibitor of metalloproteinases, TIMPs). MMPs are subjected to single nucleotide polymorphism, which can modulate their transcriptional efficiency and disease susceptibility. MMPs have a modular structure, which in archetypical MMPs includes a secretory signal sequence (pre-peptide), an activation peptide (pro-peptide), a catalytic Zn^{2+} containing domain, a hinge region and a hemopexin-like domain. In gelatinases the catalytic domain is flanked by a fibronectin-like domain and the MMP structure may contain a furin activation sequence (furin-activatable MMPs), a transmembrane domain (in transmembrane MT-MMPs), a cytoplasmic tail, a glycophosphatidylinositol (GPI) linker (and a GPI anchor), a cysteine array or an immunoglobulin domain, which regulate various aspects of MMP function, such a substrate specificity, activation and membrane-localization. Classification of MMPs is based on their domain arrangement (Fanjul-Fernandez et al. 2010). When neutral pH prevails in ECM only specialized proteinases, first described in the tadpole tail, collagenases, can degrade across the triple helix at ^{775}Gly-^{776}Ile(Leu), which is the specific initial cleavage site. At normal body temperature %- and ^{X}A-degradation fragments formed undergo helix-to-random coil transition to gelatines, which is simply denatured collagen.

MMPs can destroy old or excessive matrix to provide space for cells, such as during vascular invasion, fibroblast or osteoblast migration or chondrocyte alignment. Degradation of cell attachment substrates induces a special form of apoptosis in ECM-dependent mesenchymal cells, so called anoikis, but can also release suppressive effects and stimulate cellular proliferation and differentiation. Due to their effects on non-matrix proteins, such as cell surface molecules or heparin-bound matrix deposited factors and activation of latent pro-proteinase zymogens, MMPs can exert various anti-inflammatory and pro-healing effects. The relatively recently recognized fact that MMPs do not only degrade tissues, but also act as signalling scissors, may explain the failure of more or less generalized MMP-inhibitors (Steinmeyer and Konttinen 2006) in the treatment of tissue destructive diseases, such as cancer growth and metastasis.

As has been learnt from tissue engineering constructs, the pore size and interconnectivity have to be appropriate for the cells to migrate into wound healing scaffolds. With natural scaffolding substances such as fibrin and collagen this does not

pose much of a problem, because the cells are capable to widen proteolytically too tight pores. In contrast, if the matrix is too sparse, cells sense it and produce more matrix to create extracellular substrate for their integrin and non-integrin matrix receptors adequate for adhesion or directed migration. To at least slightly mimic this natural situation tissue engineering scaffolds are often constructed of bioresorbable (biodegradable) materials, which are hydrolyzed and actively degraded to be replaced by natural matrix. Matrix provides solid substrate along which the cells can migrate to assume their optimal positions in the matrix-cell composite in a process known as haptotaxis or contact guidance, guidance of cellular migration via extracellular matrix ligands instead of soluble chemotactic stimuli. This way extracellular matrix can regulated morphogenesis, would healing and vessel growth as well as pathological cancer cells.

Transmigration and invasion of MSCs requires coordinated action of selectins and glycoproteins, chemokines, integrins and adhesion molecules, cellular cytoskeleton and proteinases and their inhibitors, such as MT1-MMP, MMP-2, TIMP-1, TIMP-2 and TIMP-3 (Ries et al. 2007; Steingen et al. 2008).

Serine proteinase form the largest class of mammalian proteinases, which participate in coagulation, fibrinolysis, complement activation, kininogen metabolism and many other cascades as well as tissue remodelling and destruction. Important enzymes in tissue repair are elastase and cathepsin G in neutrophils and monocytes as well as mast cell tryptase and chymase. Neutrophil elastase is synthesized during the promyelocyte stage, stored in the primary or azurophilic granules and released from triggered neutrophils and activated macrophages. It degrades elastin, but also type III and IV collagens, cartilage proteoglycans, fibronectin and laminin. Elastase can activate pro-MMP-3 (pro-stromelysin-1) and degrades TIMPs. Cathepsin G is similarly stored and packaged in serglycin matrix in active form and can degrade matrix, activate some pro-MMPs and degrade TIMPs once released. Also plasminogen activators (tissue type and urokinase type), plasmin, plasma kallikrein are considered to take part in degradation of extracellular matrix (Takagi 1996).

Serine proteinases are inhibited α2 macroglobulin, which utilizes a bait sequence and entrapment, and by specific inhibitors of serine proteinases or serpins. Serpins comprise α_1-antitrypin (α_1-proteinase inhibitor, synthesized mainly in liver, the main inhibitor of elastase), α_1-antichymotrypsin (acute phase reactant, the main inhibitor of cathepsin G), antithrombin III, α_2-antiplasmin, plasminogen activator inhibitors (PAIs) and C1-inhibitor and protease nexins (*e.g.* uPA is inhibited by protease nexin-1). High expression of PAI-1 in MSCs seems to associate with a poor migration capacity (Li et al. 2009).

1.6.2 Acidic Endoproteinases

Cathepsins comprise in man 11 members, cathepsins B, C, F, H, K, L, O, S, W, X and Z. Asparate proteinase family has also many members, including cathepsin D and pepsins, which are produced by the chief cells in the stomach and known for their role in digestion. Acidic proteinases become activated by acid and are active in

phagolysosomes, in Howship's lacunae below the bone resorbing osteoclasts, in the stomach and extracellularly in acidic pH. They participate in the killing and digestion of microbes, ECM and autologous cellular components (autophagy or autophagocytosis). Apart from pH-dependent regulation of activation, cathepsins are inhibited by endogenous cysteine proteinase inhibitors, cystatins, *e.g.* the extracellular cystatin C. An acidic cysteine endoproteinase cathepsin K, the major cathepsin of bone resorbing osteoclasts, can cleave across the collagen triple helix at several sites and may play a role also in the extracellular degradation of matrix, not only in the Howship's resorption lacuna but also around loosening joint implants and other acidic locations (Ma et al. 2006). High levels of cathepsin B (a cysteine endoproteinase) and cathepsin D (an aspartate endoproteinase) are associated with a high migration capacity of MSCs (Li et al. 2009).

1.7 Wound Healing

Wound healing occurs in stages, which comprise haemostasis, inflammation, migration, proliferation and differentiation of fibroblasts and angioblasts, re-epithelialization and scar remodelling. Clot, early, intermediate and mature connective tissue matrices interact with the repair cells via integrin and non-integrin receptors so that chemokinetic, mitogenic and differentiation signals and lytic enzymes can be produces in organized waves following one another. Wound healing provides a good model for the study of regeneration and angiogenesis.

Skin wound healing encompass several stages which include haemostasis via vasoconstriction, adhesion and aggregation of platelets and activation of the external coagulation cascade to form a temporary blood clot and wound matrix filling the tissue defect and attracting blood leukocytes to the wound, and inflammatory protection of the wound site from microbial invasion by neutrophils and monocyte/macrophages, migrating from wound margins along the fibrin- and tenascin-rich temporary scaffold (Badylak 2002; Hodde and Johnson 2007; Agren and Werthen 2007), and removal of necrotic tissue and blood clot in a further proteolytic process, in part orchestrated by lymphocytes via chemokine- and cytokine-mediated mechanisms (Schultz et al. 2005). In addition, these leukocytes produce factors attracting and stimulating migration of fibroblasts to the lesional site via haptotaxis along fibrin, fibronectin and other components of the temporary wound matrix, fibroblast proliferation, fibroblast-mediated synthesis of subepithelial connective tissue dermal matrix or a more permanent wound matrix, and vascular endothelial cell in-growth and angiogenesis, to form so called granulation tissue. Next step is contraction of the open wounds via the action of specialized actin-rich myoepithelial cells, followed by re-epithelialisation by marginal epithelial cells in properly closed (or sutured) wounds and gradual remodelling of the early healing tissue (Clark 1995).

Cells can actively and dynamically assemble and disassemble matrix ligand-integrin receptor attachment areas as platforms to assemble cytoskeletal actin fibres to focal adhesion complexes, which in the subcytolemmal cytoplasm attract and