# Collagen, from Tissue Culture to Biomaterials, Tissue Engineering, and Beyond

# Collagen, from Tissue Culture to Biomaterials, Tissue Engineering, and Beyond

By

Elena García-Gareta

Cambridge
Scholars
Publishing

Collagen, from Tissue Culture to Biomaterials, Tissue Engineering, and Beyond

By Elena García-Gareta

This book first published 2019

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library



ISBN (10): 1-5275-2246-6
ISBN (13): 978-1-5275-2246-6

To my husband and grandmothers

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# BIOGRAPHY

Dr Elena García-Gareta is an honours PhD/MSc/BSc/BSc scientist with over 14 years research experience in biomaterial development, tissue engineering, cell culture, biochemistry, and analytical chemistry. During her career she has worked in the biotechnology, not-for-profit, and academic sectors. She is currently working as Director of Research at the RAFT Institute devising, directing, and implementing the organisation's research strategy. Since May 2018, she has also been the Acting CEO at the RAFT Institute.

Dr García-Gareta earned a PhD in Biomedical Engineering from University College London in 2012. Wishing to translate advances in tissue engineering into clinical products and therapies, Elena moved to the RAFT Institute (UK), where she initially worked as a postdoctoral scientist on wound healing and bone regeneration. In 2014, she was a Visiting Research Fellow at the Queensland University of Technology in Brisbane, Australia, and in early 2015 she started the Regenerative Biomaterials Research Group at the RAFT Institute. The group develops smart, biodegradable biomaterials that promote healing and aid the body's natural tissue repair mechanisms, with a focus on bone, skin, and fat regeneration. The group also works on novel *in vitro* models of biomaterial testing that predict the biomaterial's performance after implantation *in vivo*. Dr García-Gareta has published over 20 peer-reviewed articles and is co-inventor of 4 patents describing novel biomaterial scaffolds. She is currently editing a book on biomaterials for skin repair and regeneration (Elsevier).

She is a co-inventor of the novel dermal scaffold Smart Matrix®, which is currently undergoing clinical trials and is being commercialised by Smart Matrix Ltd, where she is a scientific consultant. Dr García-Gareta is also a reviewer for some of the most prestigious journals in the fields of tissue engineering and biomaterials.

# PREFACE AND ACKNOWLEDGEMENTS

Collagen is the most abundant family of proteins in the human body and known to be the main component of the extracellular matrix of body tissues, where collagen serves as a solid substratum to cells, providing them with structural and mechanical support. For over a century, scientists have tried to mimic some aspects of this natural collagen support system in *in vitro* tissue culture, and in tissue engineering applications.

The earliest work known on collagen preparations goes back to 1900 when it was reported that extracts of rat-tail tendon could be obtained with dilute mineral acids. The next decades saw important discoveries in this area: collagen could be reconstituted as a transparent gel, and acetic acid solutions of collagen consisted of long and threadlike macromolecules that left a microscopically transparent and brittle cellophane-like sheet when evaporated to dryness. Nevertheless, it was not until the 1950s when Ehrmann and Gey first in 1956 and particularly Bornstein in 1958 described a consistent and reliable method for the use of reconstituted rat-tail collagen as a substrate for tissue cultures. These authors reported cell growth exclusively on the surface of the collagen gel, thus facilitating observation of never-seen-before ultrastructural features of cells and tissues cultured on collagen coated coverslips, tissue chambers, inserts, and dishes.

The work by Ehrmann, Gey and Bornstein on collagen gels was taken further in the late 1970s with the observation by Bell *et al*. that hydrated collagen matrices could be contracted by embedded human fibroblasts, thus reporting the first contractile collagen gel, with great implications in the study of certain phenomena of wound healing. Moreover, collagen gels have since been used as cell culture systems in themselves to study dynamic cell processes or investigate stem cell differentiation.

The lasting legacy of Bornstein, Ehrmann and Gey's work continues with tissue engineering strategies for regeneration of a wide range of collagen-rich tissues. Looking into the future, collagen will play an important role in technologies such as organoids, organs-on-a-chip, tissue-engineered tumour models, or additive manufacturing techniques, which are likely to shape the future of medicine in terms of finding effective ways to treat, for example, cancer, and for developing personalised therapies.

This book builds on a previous commentary that was published in 2014 in the journal Experimental Dermatology (García-Gareta 2014). At the time, I felt that a short commentary did not do justice to the legacy of the work done over fifty years ago by Bornstein, Ehrmann and Gey on collagen preparations and gels for cell culture, tissue repair and regeneration, and more recently personalised medicine. This book describes and explores the implications of the landmark work conducted by these scientists in a wonderful journey through recent scientific history. This book will be of interest to any scientist or historian interested in history of science and development of scientific knowledge, and even to a lay reader with an amateur interest in biomedical science.

The book starts by giving a more detailed introduction and overview into the book than this preface offers (Chapter 1), followed by a description of the structure and function of collagen in tissues (Chapter 2) to understand the tremendous importance of this family of proteins in the human body. Chapter 3 describes in detail the earliest work known on collagen preparations, and in particular the method used by Ehrmann, Gey and Bornstein. Microscopy of fine morphological features of a wide variety of cells and tissue explants is discussed in Chapter 4. The use of collagen as a thin film for tissue culture is reviewed in Chapter 5, along with different collagen solution sources and coating methods, while Chapter 6 explores the use of cell embedded-3D collagen matrices as cell culture systems. Chapter 7 starts by defining and explaining the principles of tissue engineering, and the desired characteristics of the different biomaterials used to produce scaffolds, followed by different tissue engineering strategies involving collagen. Finally, Chapter 8 discusses the role of collagen in the future of biomedicine.

I would like to thank the staff at the RAFT Institute (Mount Vernon Hospital, Northwood, UK), and particularly my team at the Regenerative Biomaterials Research Group, for their invaluable support and encouragement while writing this book. Special thanks to Mr Richard Ellis for patiently and effectively proof-reading this book. I feel incredibly privileged for having a family that loves and supports me and feels proud of me. I would like to especially single out my husband who is always there for me, and who has re-lived PhD memories with me while producing this book.

# LIST OF ABBREVIATIONS

2D: two-dimensional.
3D: three-dimensional.
ALP: alkaline phosphatase.
α-SMA: alpha smooth muscle actin.
ATRA: all trans retinoic acid.
BMPs: bone morphogenetic proteins.
BSS: balanced salt solution.
β-TCP: beta-tricalcium phosphate.
CAD: Computer Aided Design.
CaP: calcium-phosphate.
CAPD: continuous ambulatory peritoneal dialysis.
CBD: collagen-binding domain.
CT: Computed Tomography.
CXCR4: C-X-C chemokine receptor type 4.
DCPD: dicalcium phosphate dehydrate.
ECFC: endothelial colony-forming cells.
ECM: extracellular matrix.
EDS: Ehlers-Danlos syndrome.
EMT: epidermal to mesenchymal transition.
FACITs: fibril-associated collagens with interrupted triple helices.
HA: hydroxyapatite.
HPMCs: human peritoneal mesothelial cells.
HT: high transporters.
HTS: hypertrophic scars.
IGF-1: insulin-like growth factor-1.
ILs: interleukins.
LT: low transporters.
MMPs: matrix metalloproteinases.
MRI: Magnetic Resonance Imaging.
MSCs: mesenchymal stem cells.
NC: non-collagenous.
nHA: nano-hydroxyapatite.
OCP: octacalcium phosphate.
OI: osteogenesis imperfecta.
OPF: oligo (poly-(ethylene glycol) fumarate).

PBS: phosphate buffered saline.
PCL: poly-ε-caprolactone.
PDGF: platelet-derived growth factor.
PELCL: poly(ethylene glycol)-b-poly(L-lactide-co-ε-caprolactone).
PGA: poly-glycolic acid.
PLA: poly-lactic acid.
PLCL: collagen/poly(L-lactide-co-ε-caprolactone).
PLGA: poly-(lactide-co-glycolide).
PVA: poly-vinyl alcohol.
RUNX2: Runt-related transcription factor 2.
SDF-1α: cell-derived factor-1 alpha.
TEVG: tissue-engineered vascular grafts.
TGFβ: tumour growth factor beta.
TSL: Standard Tessellation Language.
VEGF: vascular endothelial growth factor.
WHO: World Health Organisation.

# CHAPTER ONE

# INTRODUCTION

The word “collagen” comes from the Greek “kólla”, which means glue, and the French “-gène”, which means producing. A "producer of glue" is quite a befitting description of collagen, as it is the most ubiquitous and abundant protein in the human body, playing a fundamental role in holding bodily structures together. The name was coined sometime in the mid-1800s as it is the main ingredient of glues produced by boiling animal skin, tendons, and ligaments. Since then, collagen was discovered to be the principal component of the extracellular matrix (ECM) of body tissues, which is the non-cellular constituent present within all tissues and organs.

The ECM provides physical scaffolding for cells and is responsible for releasing essential biochemical and biomechanical signals needed for tissue morphogenesis, differentiation, and homeostasis. The ECM is fundamentally composed of water, proteins, of which collagen is the most abundant, and polysaccharides. Nevertheless, each tissue has a unique ECM with a distinct composition and topology (Frantz *et al*. 2010). For example, tendons’ ECM is mainly composed of collagen type I and smaller amounts of elastin, glycosaminoglycans, and collagen type III; whilst in cartilage, the ECM is 90-95% collagen type II, also containing elastin fibres and proteoglycans. In other tissues, the ECM incorporates an inorganic component, such as in the case of bone and dentin, where their stiffness is due to the inclusion of a calcium-phosphate mineral (Fratzl 2008).

The term “collagen” encompasses a family of, so far, 28 members that are further divided into 8 subfamilies depending on their function and domain homology. However, they all share the famous triple-helical motif that ultimately assembles into fibres (Ricard-Blum 2011; Kadler *et al.* 2007). The hierarchical structure of collagen is key to its prominent mechanical function in various tissues ranging from skin to cornea, bones, or tendons. As the main component of the human ECM, collagen confers not only mechanical stability but also scaffolding as a three-dimensional (3D) substratum for cells to attach to and migrate through. The enormous importance of this family of proteins in the human body is perfectly

illustrated by the genetic and acquired diseases implicating collagen, involving, sadly in many cases, dramatic symptoms.

The earliest work known on collagen preparations was carried out by Zacchariades back in 1900, showing that dilute organic acids can extract proteins from rat-tail tendon (Zacchariades 1900). It took another 5 decades for first Ehrmann and Gey and particularly Bornstein to describe a consistent and reliable method for the use of reconstituted rat-tail collagen as a substrate for tissue cultures (Ehrmann and Gey 1956; Bornstein 1958). The work by these authors was published when scientists were still debating about the formation of collagen: in the late 1950s it was still controversial if collagen fibrils were formed with cellular intervention, or whether the process was entirely extracellular (Ross and Benditt 1961). We now know that procollagen is formed inside the cell and secreted outside, where it is further processed to form fibrils and ultimately fibres (Hulmes 2008). Regardless, the work by Ehrmann, Gey and Bornstein opened the door to tissue culture of a variety of cellular types, including primary, and tissue explants, all of which were observed to exclusively grow on the surface of the thin collagen film. This led to observation of ultrastructural features of cells such as neurons or smooth muscle cells, thus revealing never-observed-before fine morphological attributes that allowed the correlation of ultrastructural features with functional observations.

Over the next decades, and still today, the use of collagen as a thin coating film for tissue culture allowed investigation of cell-ECM interactions, study of cellular events such as migration or apoptosis, or isolation and culture of weakly adherent cells (Rittié 2017). Concurrently, different collagen solution sources and methods of preparation became available commercially and in the scientific literature. Nevertheless, in their native environment cells are embedded in a 3D ECM lattice that is fundamental to the regulation of important cellular events, such as attachment or migration. Therefore, since the 1970s, cell embedded-3D collagen matrices have been used as cell culture systems to study a variety of phenomena, such as wound healing and pathological scarring, dynamic cell processes such as tumour invasion, or stem cell differentiation, as for example occurs in the epidermal layer of skin (Bell *et al*. 1979; Hogg *et al*. 2015).

The lasting legacy of the early work done by Bornstein, Ehrmann and Gey on collagen preparations does not end in tissue culture but continues with tissue engineering strategies for repair and regeneration of a wide range of tissues and organs, such as skin, musculoskeletal tissues, or cornea. To engineer organ/tissue equivalents, a biomaterial that acts as a

scaffold for cells to attach to, proliferate, and deposit the ECM - encouraged by molecular cues that further direct the cells to produce new tissue- is indispensable (Langer and Vacanti, 1993; Lanza *et al*. 2000). As the main component of the natural ECM, collagen often plays a prominent role in the scaffold component, which is in many occasions made of or has collagen as a constituent (García-Gareta *et al*. 2015). Commercially available collagen-based tissue-engineered biomaterials, e.g. for the treatment of dermal injuries or soft tissue repair, have been in the market for a few decades, and next generation collagen-based materials, that for example encourage angiogenesis and innervation, are being developed as we speak (Sorushanova *et al*. 2018; Davison-Kotler *et al*. 2018).

However, the fields of tissue engineering and biomaterials science are much broader than just creating tissue substitutes. Other areas where collagen plays a role, such as organoid cultures, organ-on-a-chip technology, tissue-engineered tumour models, and additive manufacturing techniques, are likely to shape the future of medicine in terms of finding effective methods to treat, for example, cancer, and for developing personalised therapies. Technologies such as organoids and organs-on-a-chip will be routinely used for drug testing and toxicology studies, testing of new therapies for tissue repair and regeneration, and development of personalised therapies where more effective drugs and therapeutic windows for a particular patient will be identified (Dekkers *et al*. 2013, 2016; An *et al*. 2015). Similarly, tissue-engineered tumour models have an enormous potential to find effective treatments against cancer, and to develop personalised therapies by assessing the patients' tumour progression using their own tumour cells (Villasante and Vunjak-Novakovic 2015; Chim and Mikos 2018). Finally, the ever-popular 3D printing offers the attractive feature of being able to produce 3D implants that perfectly fit into the defect to be repaired (Aldaadaa *et al*. 2018; Gopinathan and Noh 2018).

As seen in the previous paragraphs, collagen gels, substrata, and materials are used for a variety of purposes, from substrates for tissue culture, to 3D culture systems in themselves, to biomaterial scaffolds, and to organoids. All this research would not be possible without the work conducted in the 1950s by Ehrmann, Gey and Bornstein, who described the first consistent and reliable method for producing collagen solutions and gels. How this "invention" gave rise to such vast research makes for a remarkable journey through recent scientific history.

# CHAPTER TWO

# FUNCTION, STRUCTURE, AND COMPOSITION OF COLLAGEN IN TISSUES

## Function of collagen

Collagen, and specifically collagen type I, is the most abundant protein in mammals and the human body and a major component of the ECM. The most prominent role of collagen is its mechanical function, conferring mechanical stability, strength and toughness to a variety of tissues that include skin, tendon, ligaments, bone, dentin, blood vessels, and cornea. Other functional roles of collagen in tissues are morphogenesis, repair, angiogenesis, cell adhesion, cell migration, and scaffolding.

The mechanical properties of collagen-rich tissues are adapted by modifying the hierarchical structure of collagen, where the basic building block is the collagen fibril. Exceptions are mineralised tissues such as the aforementioned bone and dentin, where their stiffness is achieved by the inclusion of a mineral component.

### *Collagen in skin*

Skin is the largest and fastest-growing organ in the human body. Skin is composed of two basic layers: the superficial thin epidermis (0.1-0.15 mm) and the deeper thicker dermis (1.5-3 mm) (Fig. 2.1). While the epidermis acts as a surface barrier layer to prevent microbial penetration and fluid loss, it is the dermis that plays a key role in thermoregulation, sensation, and healing.

Epidermis is the outer covering of the skin with keratinocytes being the most abundant cell type (95%). Other cell types found in the epidermis are melanocytes, Langerhans cells, and Merkel cells. Keratinocytes produce the different epidermal components through an organised differentiation process called cornification. During this process, as keratinocytes move from the innermost layer of the epidermis (stratum basale) to the outermost layer (stratum corneum), they differentiate into corneocytes

(dead keratinocytes), which lack intracellular organelles and give the layer a brick-type organisation that is essential for its barrier function. During the terminal differentiation process, keratinocytes produce keratins, essential structural cytoskeletal proteins that converge and terminate at the plasma membrane forming attachment plates desmosomes, thus protecting epithelial cells from mechanical damage (Menon 2002; Houben *et al*. 2007).

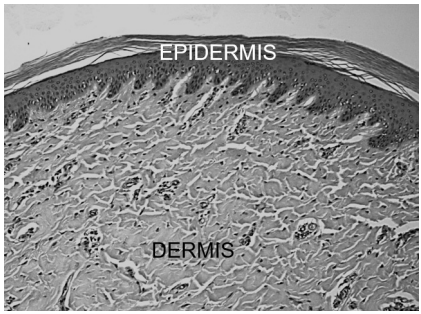


**Figure 2.1.** Histological image of epidermis and dermis layers of porcine skin stained with haematoxylin and eosin (2.5X magnification). Image produced and kindly donated by Dr Stuart John Brown (RAFT Institute, Mount Vernon Hospital, Northwood, UK).

In contrast to the epidermis, dermis is a highly vascularised tissue that contains appendages like hair follicles or sweat glands that are connected to the epidermis but penetrate deep into the dermal layer. The main cell type found in the dermis is fibroblasts, which are responsible for producing the structural components of the dermal ECM. The main component of the dermal ECM is collagen type I, which in conjunction with elastomeric elastin fibres, fibronectin, and proteoglycans (dermatan sulfate and hyaluronan), give skin its necessary elasticity, flexibility, and tensile strength. Apart from collagen I, which constitutes 80-85% of the dermal ECM, collagen III is also present (8-11%) (Menon 2002; Schultz *et al*. 2005). During normal wound healing after suffering an injury, a temporary fibrin matrix is initially formed. Fibroblasts are attracted into and attach to this fibrin matrix before depositing an ECM that is rich in collagen III (40% in wounded tissue). During the later remodelling phase of the wound healing process, which can last up to a year or even longer after injury, the disorganised collagen III bundles become organised and crosslinked to collagen I, resulting in a fully matured scar with stable