Effect of *Clostridium perfringens* on the degradation of collagen in bones and cartilage: A Review

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Abstract
This narrative study seeks to investigate the interaction between *Clostridium perfringens* and ECM proteins present in bones and cartilage, which are important factors in the body's movement in individual division, causing the decomposition of dead tissues in the body into necessary materials for their nutrition, which reliably prevents innate and acquired immune responses, thus providing amino acids and other nutrients necessary for the bacteria to flourish and multiply. Recently, live and laboratory experiments have found that bacteria produce collagenase and stimulate the decomposition of collagen present in bone and cartilage tissues which may cause them to lose their natural structure and physiological functions. Finally, it has been shown that the importance of the research has reached the effect of the pathogen on the degradation of collagen in bones and cartilage.

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1. Introduction

Collagen plays a crucial role in the strength and elasticity of bones and cartilage. It maintains the structural framework and integrity of the tissue, thus contributing to bone density and cartilage ossification. Collagen type I is the major component of bone and is thus necessary for the maintenance, growth, and repair of the skeletal system. Type II collagen is essential for the structural and functional integrity of cartilage. Hence, the study of C. perfringens, which is implicated in various diseases and conditions, along with collagens, which are crucial in the structural and functional integrity of bone and cartilage, can provide a platform for studying how these matrix-degrading bacteria have a more profound impact on these tissues (Alcaide-Ruggiero *et al*.2021) [4] (Owczarzy *et al*.2020) [60] (Ouyang *et al*. 2023) [59]. In this review, the interplay of this microbe with these tissues has been explored and reported thoroughly in respective sections. This review explores the effect of C. perfringens on the degradation of the structural proteins of the body, namely collagen type I in bones and type II in cartilage. In the next section, the pathogenesis of C. perfringens has been reported in two main categories: the mechanisms leading to infections in general and invasive myonecrosis in particular (Mehdizadeh and A2021) [50] (Van *et al*. 2021, Van *et al*. 2020) [78, 77]. Next, the readers will come across the social, epidemiological, genetic, and other facets of these organisms in a defined manner (Krieger, 2024) [38]. Therefore, the objective of the present review is to provide insights into the pathogenicity of this particular microorganism with an embedded approach toward the degradation of the body's structural proteins, i.e., collagens type I in bones and type II in cartilage (Sharma *et al*., 2023) [70] (Qiu *et al*.2021) [64]. The paper traces the interaction between C. perfringens and the ECM proteins in bones and cartilage that are important effectors of body movement in the individual sections. It is reported that the principles behind their degradation are established and presented at length (Karsdal *et al*. 2021, Matsubayashi, 2022) [36, 49].

1.1. Background and Significance

Clostridium perfringens has long been of interest in the arenas of microbiology and pathology, particularly with a focus on its pathogenicity (Mora *et al*. 2020) [5]. Historically, ranches and farming communities have associated the pathogen with the onset of sudden deaths. Liquid accumulations in the joints have been observed in young animals, particularly fine wool sheep, but due to the initial rather benign clinical signs in affected animals, the pathogen has often been disregarded (Abo *et al*. 2024) [55]. In humans, due to pre-clinical neglect of early observations, the pathogen has been mostly used as a model microorganism in food microbiology and currently has no established medical claim, despite its being one of the leading Clostridium species involved in foodborne outbreaks. Once in the body, C. perfringens can induce severe tissue damage, including: a) gas formation in tissue, possibly causing crepitant swelling of rapid onset, mostly in well-supplied tissues; b) purple-black, possibly hemorrhagic discoloration; c) rapid liquefaction and progressive tissue degradation (Mora *et al*. 2020, Finnie & Uzal, 2022) [5, 24]. For instance, C. perfringens has been associated with: gas gangrene, a rapidly progressing, possibly fatal condition involving tissue destruction; necrotizing enteritis; and non-Clostridium difficile bone, skin, and soft tissue infections. Of significance is the renewed focus on hyper acute necrotic enteritis in commercial livestock (Mehdizadeh and A 2021, Mora *et al*. 2020) [50, 55]. Collagen is the principal organic matrix of the extracellular matrix of both bone and cartilage: accounts of 90–95% and 60–70% of intramuscular connective tissue in pork and beef, respectively. Collagen renders matrices tougher and elastic, able to withstand deformation from bone fractures; and the cartilage resists compression and shear in diarthrodial joints. Disruption of the collagen molecules in bone and cartilage renders them brittle due to reduced structural stability, a point of fracture for bone and surface erosion in bones and cartilage is the initiating event in osteoporotic fractures and osteoarthritis, respectively (Karamanos *et al*. 2021, Lin *et al*., 2020) [35, 43]. Degradation of the molecule re-populates the site with fragments unable to restore the original biomechanical function they might have originally served. Inadequate healing and repair can cause lasting harm (Wilkinson & Hardman, 2020, Mishra *et al*., 2022) [84, 51]. These structural and functional changes are associated with at-risk populations and diseases of public health concern. Collagen rearrangements are thought to contribute to the increased risk of vertebral fractures in Type 1 diabetes, and to the abnormalities in bone architecture in overweight children (Dragoun Kolibová, 2024, Liu *et al*., 2024) [22, 45]. Impaired chondrogenesis might also contribute to synovitis as a result of exposure of bone beneath the cartilage's surface in obese children, possibly representing an initiating event in osteoarthritis, thereby predisposing them to further bone damage and pain (d'Angelo *et al*., 2021, Sun *et al*. 2021) [19, 74]. There are no specific treatments to prevent fractures and/or decrease pain in bone disorders, or to prevent a joint from becoming arthritic. Therefore, to modulate collagen-related pathways as a treatment strategy, it is necessary to understand the requirements of degradative enzymes acting at least on the bone and cartilage since degradative enzymes associated with the mechanical environment within the chondrium may differ (LeBoff *et al*. 2022, Bertoldo *et al*. 2022) [40, 11].

2. *Clostridium perfringens*: Overview

One of the anaerobic bacteria capable of degrading the dead tissues of the body back to substances necessary for its nutrition is *Clostridium perfringens*. Although many microorganisms are capable of this activity, it has been shown that this microorganism plays a significant role in the process. *Clostridium perfringens* is among the lowest in the phylogenetic classification of bacteria and is assigned to the family Clostridiaceae. It lacks a cell membrane but has a thick layer of peptidoglycan. *Clostridium perfringens* can ferment sugars to produce an acid and a gas or an acid only, although some strains are described as acaligenesalbus (Mora *et al*. 2020, Hussain *et al*. 2024) [55, 31]. Various enzymes have been reported to be produced by C. perfringens, such as collagenase, endo-cellulase, gelatinase, and various other degradative enzymes that could degrade collagens are produced by some species of *Clostridium perfringens* and other Clostridium species as well; however, none of these enzymes has been immunologically characterized. *Clostridium perfringens* is a set of phenotypically similar bacterial types or strains with a broad virulence and toxic spectrum (Van *et al*. 2021, Van *et al*. 2020) [77, 78]. In pathogenicity tests, these can be tested against bubble-free and unencapsulated strains, which show the colony characteristic of the pathogenic, but are unencapsulated, meaning that the capsule has an antiphagocytic effect, and this phenotypic character, which may change due to mutation or other factors, leads to deletion from the outbreak. *Clostridium perfringens* strains have been categorized into five toxinotypes according to the toxins they produce (Forti *et al*. 2020, Abdel-Glil *et al*. 2021) [25, 1]. Each toxinotype produces a different toxin. These strains are designated as types A-E depending on the toxins they produce. Although the genus Clostridium produces morbid indicators with the role of spores, more than 20 toxins and more than 50 extracellular enzymes that are considered to be one of the enzymes whose pathogenesis has been clarified in at least 100 diseases and cause tissue damage and neurological paralysis have been clarified. Therefore, these bacteria, which appear in the differential diagnosis of a large number of human and veterinary medical diseases, create great economic losses along with public health (Mehdizadeh and A 2021, Mora *et al*. 2020) [50, 55].

2.1. Characteristics and Classification

Clostridium perfringens is a strictly anaerobic, endospore-forming, Gram-positive, straight or slightly curved bacillus with a flagellum. A few typical strains that were previously identified were round or irregular in shape and did not produce spores. Spores that appear sporadically have central structures, and the size of C. perfringens is 0.5–1.5 µm × 1.0–3.0 µm. C. perfringens grows under microaerobic or anaerobic conditions at 20–40 °C and pH 5.6–7. C. perfringens uses carbohydrates or amino acids as a carbon source and can ferment a variety of substances to produce energy. The physiological reaction is negative for various sugars and positive for a few sugars. When classified according to the characteristics of fermentation, C. perfringens is a Bacteroides group 1 organism. The produced endospore is oval, containing oval or spherical gas. (Andryukov *et al*. 2020, Weligala Pahalagedara, 2021, Bhatia, 2021, Hanim *et al*. 2021) [8, 83, 12, 29].

According to the activity of toxin production and pathogenicity for animals, C. perfringens was divided into five types. Type A is an organism not producing alpha-toxin and is less pathogenic. Other organisms that produce alpha-toxin include beta toxin, epsilon toxin, iota toxin, and other toxins known or unknown. Some strains may have no alpha-toxin plasmids and are named C. perfringens A. Infection with C. perfringens A, beta, epsilon, or iota is usually an acute outbreak of disease, and possible symptoms range from severe to mild. The alpha-toxin of type CPB rarely shows cytotoxic activity (Mehdizadeh and A 2021, Mora *et al.* 2020) [50, 55]. Most strains that produce beta and epsilon toxin belong to type CPB and produce iota toxin. Type C can be considered more pathogenic than type A; however, it also depends on other plasmid-encoded minor toxins that have not been identified. The most important factor determining the pathogenicity of the isolated strain is the alpha-toxin activity rather than a type resulting in the diagnosis and cure (Gangwar *et al.* 2022, Ali *et al.*, 2024) [26, 5]. The identification of C. perfringens is based on colonial morphology, the smell of cultural characteristics, the growth characteristics of classic strains, and the use of anaerobic procedures to isolate, culture, and possess lecithinase activities (Mohiuddin *et al.* 2020, Achakzai *et al.* 2020) [53, 3].

3. Collagen in Bones and Cartilage

Bones are composed of collagen, a composite material, mainly type I collagen fibrils, and hydroxyapatite crystals, which provide both toughness and elasticity, thus ensuring the mechanical strength of the bones. Bone serves as the locomotion scaffold but also has a role in mineral homeostasis (Veiga *et al.* 2022, Osuchukwu *et al.* 2021) [79, 58]. Cartilage is a form of connective tissue composed of a network of collagen and the major shaping molecule, aggrecan, and plays a critical role in bearing loads during locomotion. The main building materials of cartilage are type II collagen and highly hydrated proteoglycans (Alcaide-Ruggiero *et al.* 2021, Bloebaum *et al.*, 2021) [4, 14]. Collagen in bones is mostly type I, whereas in cartilage, it is type II. Cartilage serves as a major tissue in joints, transducing the compression applied on the articular surfaces into a hydraulic flow of liquid that communicates with the underlying bone, ensuring appropriate bone remodeling by the cells residing in bone (Alcaide-Ruggiero *et al.* 2021, Bielajew *et al.*, 2020) [4, 13].

The type of collagen present depends on the location in the bone. In cortical bone, it is mostly composed of type I collagen, whereas in trabecular bone, types II, III, V, and XI are also present. In cartilage, the collagen is composed of type II fibrillar molecules that aggregate to form long fibrils (Rico-Llanos *et al.* 2021, Li & Gong, 2021, Al-Qudsy *et al.* 2023) [67, 42, 6]. In healthy bones and cartilage, the integrity and hence the interaction of glycosaminoglycans, proteoglycans, and collagens is critical for the function of the tissues. Type I, type II, and type III collagens are the major collagens in bones and cartilage (Alcaide-Ruggiero *et al.* 2021, Rico-Llanos *et al.*2021) [4, 67]. The collagen molecule has a unique triple-helix structure, where three peptide α-chains twist intermolecularly into a right-handed triple helical conformation. It is critical that every third position in the Gly-X-Y tripeptide chain sequence is a glycine, essential for the triple helix formation. Collagen fibrils are the basic unit of fibrils, and further packing of the collagen microfibril network ensures bone mechanics. The packing and size of collagen fibrils are bone quality assays that indicate hard bone from the soft and are used for clinical purposes (Zhao *et al.* 2021, Kang *et al.* 2024, Qi *et al.* 2022) [88, 34, 63].

3.1. Structure and Function

Collagen fibers—the most abundant extracellular protein in living organisms essential for the support of load-bearing tissues such as bones and cartilage—possess several unique structural features that stem from their primary structure: a triple helix of repeating amino acid sequences, where proline and hydroxyproline residues are dominant at specific positions. (Lin *et al.* 2020, Skedros, 2024) [44] Collagen fibers exhibit a hierarchical organization, with bundles of single molecules, called fibrils that further aggregate to form larger, macroscopic fibers. In these tissues, compactly arranged and tightly packed collagen molecules confer mechanical properties such as tensile strength, ductility, and flexibility so that bones are bendable without fracturing and joints provide shock absorption upon impact (Sarrigiannidis *et al.* 2021, Bose *et al.*, 2022) [69, 15]. In addition to their roles as structural units, collagen has also been understood to provide a large surface area for cellular activity, serving the support of multiple cellular functions that influence, for example, their adhesion, migration, proliferation, and differentiation. Furthermore, they can interact with other components of temporal extracellular matrices necessary for tissue repair, maintenance, and remodeling during the convalescent period to complement more permanent reparative procedures (Mathew-Steiner *et al.*, 2021, Rezvani *et al.* 2021) [48, 66].

Tissues are in a state of turnover and continual matrix degradation and the resynthesis of new extracellular matrix confers functional properties distinct from one tissue to another, and in order for functions to be preserved and regenerated, a dynamic, ongoing process must occur. For example, in normal healthy humans, a significant amount of collagen is produced per day, while a larger amount of collagen can be degraded (Reilly & Lozano, 2021, Wang, 2021, Khatri *et al.*, 2021) [65, 80, 37]. The highly ordered and repetitive arrangement of collagen molecules, in combination with several molecules that precisely interact with collagen fibrils, influences how particular proteolytic enzymes may degrade collagen and causes some pathogens to recognize and interfere specifically with collagen. It has been proposed that highly repetitive sequences of certain extracellular matrix proteins, including collagen, may lead to incorrect or inappropriate folding of these domains and more accessible sites for protease cleavage (Zhang & Han, 2024, Qiu *et al.* 2021) [86, 64]. The formation of the collagen triple helix intensely stabilizes the folded structure but may also serve to expose less conventional areas of collagen and draw particular attention from harmful pathogens and proteolytic enzymes. The non-Gly-Pro-Hyp enriched domains of the triple helix in collagen of temperature-stable collagens certainly provide a high resistance against bacterial collagenase (Salvatore *et al.* 2021, Zhang *et al.*, 2022) [68, 87]. The pathogenic consequences of toxins from a variety of diseases in livestock and humans are hypothesized to either largely or exclusively be due to their enzymatic capability to rapidly and plentifully degrade barrier tissues, credibly preventing innate and acquired immune responses. This pathway of cellular matrix degradation may be desirable as it aids in the breakdown of tissues, thereby providing amino acids and other nutrients necessary for bacteria to flourish and multiply, and provides access to spaces lent and unbound by

immune system cells and anti-infection agents (Gao *et al*., 2020, Brown *et al*. 2021) [27, 16].

4. Mechanisms of Collagen Degradation

The major structural protein in the body is type I collagen, which contributes the principal tensile strength to the bones and also provides the structural integrity of cartilage tissue. Collagen exists in various proportions in different parts of the body, and the methods of degradation are assumed to be parallel to the method of construction dominated by individuals. In physiological situations, connective tissues catabolize and anabolize simultaneously. This degradation is shown to be essential for matrix remodeling for tissue repair or when the tissue is subjected to mechanical stress or other physiological and pathological conditions. (Rico-Llanos *et al*. 2021, Naomi *et al*., 2021, Amirrah *et al*. 2022, Bose *et al*., 2022) [67, 15, 56, 7].

Collagen degradation is possible through two ways: enzymatic and non-enzymatic. The enzymatic method of degradation of fibrillar collagens, e.g., collagen type I present in bone and type II present in cartilage, has gained much interest as several diseases are arising due to its changes, and the collagen-deficient state can result in several bone and cartilage conditions (Ledwoń *et al*., 2021, Sun, 2021) [41, 61]. The past decade has shown many advances in the path of degradation of several enzymes that are responsible for fibril collagen, hydrolysis-dependent and/or non-enzymatic factors. In an attempt to deal with technical issues associated with an enzymatic method focusing on new approaches, it is important to review the factors affecting collagen degradation in healthy and diseased bones and cartilages and the method of fibril degeneration (Żylińska *et al*. 2021, Moo *et al*. 2022) [90, 54]. The present review describes the involvement of several factors in the degradation of collagen in bone and cartilage and revises the method of collagen degradation in a well-defined ground protein. (Topol *et al*. 2021, Barreto *et al*. 2022) [76, 10].

4.1. Normal Degradation Processes

In normal physiological conditions, the complex molecule of collagen is degraded by the concerted activities of matrix metalloproteinases and other members of the so-called astacins family. This section is devoted to describing in detail the actual present knowledge concerning the involvement of these enzymes to discuss their possible role in the tissue remodeling processes related to normal tissue physiology (Costa *et al*. 2022, Dolmatov *et al*., 2021) [18, 21]. The control of collagen degradation represents an important issue and may be considered within the overall context of the degradation processes in different tissues. In normal physiological conditions, a controlled resorption of a series of tissue components represents a prerequisite for proper regeneration of the damaged tissues (Singh *et al*. 2023, Zhu *et al*. 2024) [71, 89]. They are a family of zinc-dependent endopeptidases that either degrade ECM components or cleave adhesion molecules of cells. Bone and cartilage are tissues of the connective tissue family that are permanently under stressful conditions not only because of the high mechanical stress but also because of their role in the locomotor system (Cherkas *et al*. 2023, Azimi, 2020) [17, 9]. Bone and cartilage are involved in a permanent remodeling process or a permanent collagen desquamation process to maintain the balance and renewal of collagen and proteoglycans. Both racemases do not only hydrolyze free lathyrine, but also lathyrine residues that are incorporated in collagenous proteins and in proteins that are related to these collagenous proteins, including collagens and elastin (Dudaric *et al*. 2023, Peng *et al*. 2021) [23, 61]. This degradation sequence is characteristic for MMPs that are involved in normal metabolism, since their action results in Gelactin with a typical Mr of 80 kDa that is composed of collagen I and one molecule of denatured collagen (Mixon *et al*. 2022) [52].

5. Impact of *Clostridium perfringens* on Collagen Degradation

Clostridium perfringens is a ubiquitous pathogen that causes multiple disorders in humans and livestock. Recently, *in vivo* and *in vitro* experiments have found that bacteria produce collagenase and induce the degradation of collagen that exists in bone and cartilage tissues, which can cause them to lose their normal structure and physiological functions. However, there are few reviews on this content (Ita *et al*. 2020, Mao *et al*., 2023) [32, 47]. This review focuses on *Clostridium perfringens*, one of the clostridial subtypes, and summarizes its effects on collagen degradation. The main mechanism of C. perfringens initiating collagenase to degrade collagen by releasing toxins is emphasized, and the latest developments in microorganisms and collagenase-related diseases are combined. The influencing factors affecting the mechanism of action also need further study (Mehdizadeh and A2021, Van *et al*. 2021) [50, 77].

Bacterial infections can lead to the degradation of collagen and accumulate some of the breakdown products, such as collagen peptides, which can then induce the loss of physiological function in bone and cartilage tissue. C. perfringens is a toxin-producing pathogen that has been demonstrated to synthesize and release collagenase and has been found to be negative in tissues, both *in vitro* and *in vivo* (Van *et al*. 2020, Mehdizadeh and A2021, Kumon *et al*., 2024) [78, 50, 39]. In patients, clinical doctors have also conducted a series of relevant studies, all of which suggest that the establishment of C. perfringens can degrade the integrity of the tissue. Few studies have been reviewed on this critical issue. In this review, a literature search identified *in vitro* and *in vivo* studies that demonstrated that C. perfringens induced the degradation of collagen in the tissue. *Clostridium perfringens* also interacts with other inducers of collagen degradation, so future studies are needed on the long-term collagen degradation mechanism (Van *et al*. 2020, Mehdizadeh and A2021) [78, 50].

5.1. *In vitro* Studies

This section reviews studies that target the interactions between *Clostridium perfringens* and collagen, the main constituent of bones and cartilage. These studies used various experimental approaches and conditions to approximate the *in vivo* environment of wound and bone tissues affected by clostridial myonecrosis to observe C. perfringens' capacity for collagenolysis. Key results from experiments are shown in a table (Tomczyk-Warunek *et al*. 2021, Qiu *et al*. 2021, Sharma *et al*., 2023) [75, 70, 64]. The research found that C. perfringens was involved in accelerating collagen degradation when these experiments were performed under diverse conditions, such as culturing different C. perfringens strains at different initial loads using collagen of different origins from various animal species, different tissues, and environmentally vital statuses. Knowledge from ex vivo and *in vitro* typing of the role of C. perfringens in the

etiopathogeny of clostridial myonecrosis, especially regarding the C. perfringens or pharmaceutical solution activity on collagen, is discussed in this section (Jarman *et al*. 2024, Wang *et al*. 2024) [33, 34].

In addition to summing up results and procedures, objectives, methods, and conclusions thereof, this paper also discusses the relevance of *in vitro* research on these topics that simulate *in vivo* research and use real statistics to help hypothesize and interpr*et al*l preclinical experiments, especially those ex vivo (Oosterlaken *et al*., 2021, Wang, 2021) [57, 80]. Nevertheless, no experiment or statistics were conducted that analyzed *in vivo* infections, and their results do not provide evidence or support for any other data observed and recorded (Wu *et al*., 2020, Welen *et al*. 2022) [85, 82]. The present paper focuses on examining data in a clinical, even *in vivo*, environment, which applies to human infections and not animal ones. It supposes that these results and understandings are relevant in the absence of publication on these themes (Lopez-Suarez *et al*., 2022, Prestat & Thierry, 2021) [46, 62]. This paper tags each clinical research with at least *in vitro* or even ex vivo research to continue in this direction (Green *et al*. 2020) [28].

6. Conclusion

The aim of this review was to summarize the effect of the pathogen on collagen degradation in bones and cartilage, since most of the chronological evidence points towards collagen being the main causative factor behind bone and joint softening in the above-mentioned diseases. The pathogen can also degrade collagen, which is present in healthy bones and joints; several points of contact of proteins and organelles of this pathogen on collagen and two pore-forming toxins secreted by its help in this process. There is a need for an in-depth knowledge of how the interactions between collagen and this pathogen (in the normal hosts) happen in the clinics, which will not only help in a comprehensive understanding of the process of bone and joint degradation, which can sometimes be reversed, but will probably also aid in the early diagnosis of these diseases.

Under normal physiological conditions, collagen is degraded with the help of matrix metalloproteinases and cathepsins; new collagen is ultimately made to replace the old one. Sometimes, the existing collagen in bones and joints can act as food for the pathogen, which can kick-start a pathway for the degradation of collagen; this degradation process is then perpetuated in a chain-like domino effect by various other bacterial virulence factors secreted by the pathogen. Apart from the SMase and Cpa, the pathogen secretes other highly specific virulence factors to other macromolecules present inside collagen fibrils as well as on collagen. Future research could probably be directed towards a cross-talk between, for example, dermal fibroblasts, osteoblasts, osteoclasts, and the pathogen for collagen degradation, in which the above bacteria produce and secrete various other metabolic factors, which are sometimes health promoting and are sometimes bone degrading. An interdisciplinary approach combining computer models, genetics, cellular and bacterial biology techniques, and microbiological culture methods could help to bring about new treatment possibilities for collagen- and bone-related diseases. Other bacterial and viral factors that play a role in collagen degradation could also be identified for the development of robust drugs against these collagen- and bone-degrading diseases. The results obtained in laboratory research should be dovetailed with the clinical data to pave the way for personalized medicine.

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