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Review

Focal Adhesion of Collagen-Based Bone Grafting Materials Enhances Bone Regeneration

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Abstract: Collagen, which has osteoconductive potential, has been widely used as scaffold material for bone repair and regeneration for more than 3 decades. Of late, collagen has been combined with other materials to produce collagen-based bone grafting materials with enhanced bone repair and regeneration capacities. However, varied results have been obtained with collagen-based grafting materials. To elucidate the mechanisms underlying the enhanced bone engineering capacity of these materials, we critically reviewed the current literature on the complex hierarchical structure and properties of native collagen molecules. This review emphasizes the scientific challenge of manufacturing collagen-based materials with suitable properties and shapes for specific biomedical applications, particularly bone repair and regeneration. This article sheds light on the interactions between collagen and cell receptor molecules to mediate biological pathways. In addition, this article clarifies the mechanisms of cell adhesion–mediated bone regeneration. The findings may guide future research on collagen-based biomaterials.

Keywords: collagen-based materials; cell adhesion; bone engineering; repair; regeneration

1. Introduction

Except in cases of bone defects caused by accidents, surgery, or injury, bone can repair itself by activating surrounding osteoprogenitor cells, leaving no residual scars. However, severe defects that are beyond bone's self-healing capacity can lead to bone nonunion, callus formation, and even permanent bone loss. Bone grafts are often used for repairing such defects.

Autologous bone grafting is the gold standard for the repair of severe bone defects. Autologous grafts facilitate the repair or regeneration of bone tissue through the differentiation and proliferation of adjacent or transplanted osteoblasts. These grafts are associated with a low risk of rejection, and because of their low antigenicity, the grafts can promote bone regeneration [1]. However, autologous bone grafting has some major disadvantages, such as poor osseointegration with artificial joints or tooth implants. Excessive bone constriction may occur when the defect is >9 cm or when the surrounding tissues do not provide sufficient blood supply due to scarring, infection, or irradiation [2]. Moreover, autologous grafting involves the sacrifice of healthy bone tissue at the donor site, resulting in additional morbidity [3]. Harvesting bone from appropriate donor sites requires a complex learning curve and may cause postoperative complications.

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In addition to autologous bone grafts, alternative grafts such as allogenous grafts, xenografts, and allografts are used for bone repair. These alternative grafts exhibit various physicochemical characteristics and varying reliability in surgical treatments [4,5]. Among all alternative grafts, collagen-based bone grafting materials are widely used in clinical settings. These materials are produced by combining collagen with other materials, thereby increasing bone repair and regeneration capacities.

Bone is a hard connective tissue with a hierarchical structure; it is composed of matrix, cells, and bioactive factors. The bone matrix is mainly composed of type I collagen and hydroxyapatite (HAP). Collagen fibers serve as a template for mineralization and play key roles in determining specific properties, such as compressive and shear behaviors, fracture mode, and toughness as well as reinforcing bone under external stress from applied loads [6,7].

Osteoblasts are anchorage-dependent cells. Therefore, the initial adhesion of osteoblasts to the biomaterial scaffold is pivotal for their migration, differentiation, and proliferation and subsequent bone formation. The adhesion of cells to the biomaterial surface is a complex process involving cell attachment, cell spreading, and cell–scaffold interaction; this process is called focal adhesion.

Collagen is the most abundant protein, constituting more than one-third (by weight) of the body's total protein content [8]. Type I collagen is the most common type of collagen found in the extracellular matrix (ECM), particularly in bone tissue [9]. The ECM plays vital roles in the morphogenesis and cellular metabolism of new tissues, conferring mechanical and biochemical properties [10]. The collagen matrix is essential for bone tissue engineering [11] because of its abundance, biocompatibility, high porosity, ability to combine with other materials, easy processing, hydrophilicity, low antigenicity, and absorbability [12,13]. It can promote cell adhesion, differentiation, and proliferation.

Recently, scientists have designed innovative collagen-based biomaterial scaffolds, leveraging the extensive evidence on collagen organization, structure, and properties. The use of these materials has considerably enhanced bone engineering. The design of resorbable collagen-based medical scaffolds requires knowledge of the anatomy and biological function of tissues or organs as well as an understanding of the role of collagen's physicochemical properties and structure in tissue or organ regeneration. Different collagen-based scaffolds have been developed for different bone engineering applications. These scaffolds promote biological responses, such as cell signaling, and serve as artificial biomimetic extracellular matrices that guide bone tissue regeneration. The initial step of bone regeneration involves the adhesion of cells to the collagen-based scaffold; the subsequent steps are material–tissue interaction, cell differentiation, cell proliferation, and new bone formation [14,15].

Cell adhesion is initially activated by the interaction of the collagen matrix with integrin. The mechanisms through which integrin signaling activates cell adhesion are discussed in the following sections.

2. Mechanisms of Collagen Binding and Cell Adhesion

Integrin, a cell surface receptor, plays an essential role in regulating cell signaling, migration, survival, and adhesion to various ECMs, including collagen, fibronectin (FN), and laminin (LN) [16–18]. The arginine–glycine–aspartic acid (Arg–Gly–Asp [RGD]) peptide of collagen constitutes a specific site for its recognition by integrin. Integrin is a key regulator of cell–cell and cell–extracellular microenvironment communication.

Various ECM-binding integrins that facilitate the adhesion of osteoblasts on biomaterial surfaces have been identified. Six integrins play major roles in cell adhesion: $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha V\beta 1$, $\alpha 5\beta 1$, and $\alpha 11\beta 1$ (Geographic abstract A) [16,19,20].

Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that is activated upon integrin binding to the ECM at the site of focal adhesion [21]. Integrin-mediated adhesion at tyrosine 397 leads to the autophosphorylation of FAK, creating a binding site for the proto-oncogene tyrosine-protein kinase (Src) homology 2 (SH2) domain of Src; the FAK–Src complex, in turn, phosphorylates other tyrosine residues in FAK, thus maximizing its kinase activity and creating additional protein-binding

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sites [22]. This active FAK–Src complex activates Ras-related C3 botulinum toxin substrate 1 (Rac1) by recruiting and phosphorylating the p130 Cas scaffold protein (p130 Crk-associated substrate, known as breast cancer anti-estrogen resistance 1 [Bcar1]) [23]. The phosphorylated p130 Cas recruits Dock 180 and engulfment and motility 1 (ELMO1; a *Ced-12* ortholog) by binding to v-crk (sarcoma virus CT10 oncogene homolog crk; proto-oncogene, adaptor protein). The Dock 180-ELMO1 complex functions as an unconventional GEF for Rac1 and promotes the formation of membrane protrusions [24,25]. The activation of Rac1 and cell division cycle 42 (Cdc42) is also inhibited. This suppresses actomyosin contractility and enhances actin-mediated protrusion. The activity of Rac1 and Cdc decreases, whereas that of RhoA gradually increases, which promotes the formation of stress fibers and the maturation of focal adhesion (Geographic abstract C) [26,27].

The FAK–Src complex also phosphorylates paxillin-kinase linker (the GEF for Cdc and Rac1, which is also known as G-protein-coupled receptor kinase-interacting protein 2 [GIT2] and Pak-interacting exchange factor-beta [βPIX, also known as cloned out of library-1 {Cool-1} and Rho guanine nucleotide exchange factor 7 {Gef7}]). βPIX recruits and activates Rac1 through direct interactions with focal adhesions and membrane protrusions [28]. Notably, paxillin-kinase linker and βPIX are phosphorylated by Src, which further modulates their activity in response to integrin-mediated adhesion [29,30]. Thus, integrin signaling through Src family kinases (SFKs) can regulate the localization and activity of GEFs to control the formation of membrane protrusions.

The FAK–Src complex activates several pathways; subsequently, activated Rac and Cdc42 GTPases facilitate the formation of a membrane protrusion at the site of integrin binding in the early stages of cell spreading. Simultaneously, this complex, together with syndecans, mediates the suppression of actomyosin contractility by reducing the activity of RhoA. In the later stages of cell spreading, integrins promote the activity of several GEFs, leading to a shift in the balance between RhoA and Rac1 activity in favor of RhoA activity, thereby enhancing RhoA-mediated actomyosin contractility. The integrin $\alpha 5\beta 1$ is particularly efficient in promoting the second phase of cell spreading, which may involve the force-induced activation of SFKs.

The enhanced activity of FAK promotes RhoA activity, thereby promoting cellular contractility through ROCK. Crosstalk between RhoA–ROCK and the extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) pathway induces the phosphorylation of p44/42 MAPK (ERK1/ERK2) through MAPK kinase (MEK), which regulates the activity of the osteogenic transcription factor runt-related transcription factor 2 (RUNX2). RUNX2 controls the expression of osteogenic genes such as osteocalcin (OCN), alkaline phosphatase (ALP), and bone sialoprotein, ultimately driving differentiation toward mature osteoblasts (Geographic abstract D) [31].

3. Collagen-Based Materials

Collagen-based materials promote the differentiation of human-induced pluripotent stem cells into osteoblasts [32]. The collagen matrix combined with other ECM or materials also enhances cell differentiation and proliferation. Other ECMs or materials include LN [33], FN [34,35], chitosan (CS) [36], HAP [37,38], calcium phosphate cement (TCP), fibrin, and fibrinogen [39]. Collagen-based materials are also applied as coating on the implant to enhance osteoblast adhesion to the implant surface and osseointegration between bone and the implant [40–44]. The techniques and mechanisms are described in the following sections.

3.1. Orientation of Collagen Fibers

The orientation of collagen-coated poly-lactide-co-glycolide (PLGA)/poly-caprolactone (PCL) fibers was reported to enhance bone regeneration through cell adhesion. HanBang Chen et al. demonstrated that adipose-derived stem cells exhibited the highest expression of adhesion-related genes, such as those encoding integrin β1, cadherin 11, Fn-1, LN, and N-cadherin [45]. Electrospinning technology was used to fabricate collagen/HAP fibers to improve bone regeneration. Collagen/HAP fibers support cell adhesion and bone regeneration. Yuanyuan Zhou et al. produced

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collagen/HAP composite fibers through electrospinning, and after 6 days of culture, the composite fibers exhibited higher viability and ALP activity than did myeloma cells [46].

3.2. LN

Selective cell retention has been widely used as a bone tissue engineering technique. LN is a main component of the ECM and the basement membrane. LN plays a key role in mediating cell–matrix adhesion, leading to cell proliferation and differentiation [32,33]. A collagen-binding domain (CBD) containing the core functional amino acid sequences of LN-4 (CBD-LN peptide) was introduced on the functional surface of a collagen-based decalcified bone matrix scaffold. The decalcified bone matrix/CBD-LN scaffold maintained osteoblast proliferation and induced osteogenic differentiation through early cell adhesion mediated by upregulated integrin β1 expression [33]. In tissue engineering, bioactive molecules have been introduced into three-dimensional porous scaffolds to mimic the in vivo microenvironment.

3.3. FN

FN is an ECM glycoprotein with a size ranging from 230 to 270 kDa. The dimer is formed by α and β subunits. FN includes types I, II, and II domains. The type III domain is the most sensitive to unfolding. Several receptors can bind to FN—for example, α2β1, α3β1, αvβ1, and α5β1—and improve focal adhesion, cell proliferation, and cell migration [47]. FN combined with type I collagen can promote focal adhesion, as evidenced by elevated filopodium formation, increased cell circularity, and accelerated spreading in mesenchymal stromal cell line [34]. Coating FN combined with OCN on the collagen matrices enhanced the adhesion of osteoblast-like cells (MC3T3-E1) and mRNA levels of osteogenic markers, such as RUNX2, ALP, and collagen type I in these cells [35].

3.4. Ceramic and Combined Materials

A hybrid scaffold composed of granular HAP and collagen was designed to mimic the microenvironment for the adhesion, viability, and osteoinduction of human bone marrow-derived mesenchymal stem cells (BMSCs) [48].

Moreau et al. reported that collagen incorporated into calcium phosphate bone cement increased the attachment and osteogenesis of osteoblasts [49]. Gutierrez et al. [50] proved the adhesion activity of HAP. The nano apatite–collagen composite appeared more similar to natural bone in terms of biomimetics than was nano apatite cement without collagen [49]. Functionally graded CO_3 apatite–collagen containing magnesium (FGMg-Ap-collagen) was also reported to enhance osteoblast adhesion to apatite and to promote bone formation [51]. CS combined with collagen/HAP in mesenchymal stem cells (MSCs) [52] and rats [53] and collagen/β-TCP in the human osteoblast cell line MG63 [54] increased cell adhesion and proliferation, resulting in osteogenesis.

Yu et al. reported that intrafibrillar mineralized Col-HA-based scaffolds, constructed with either cellular or lamellar microstructures, exhibited enhanced bone regeneration capacity in a mouse model. Moreover, Fe/Mn incorporation promoted the osteogenic potential of the lamellar scaffolds, facilitating the in vitro osteogenic differentiation of BMSCs and the in vivo bone regeneration in the presence of fresh bone marrow cells [55].

3.5. CS

CS, a nontoxic natural polymer, is primarily composed of −1,4-linked *N*-acetyl-D-glucosamine and D-glucosamine units. CS exhibits high solubility in dilute acidic solution with pH < 6.5 [56]. This polymer forms covalent bonds with FN, improving cell adhesion [57]. CS binding to collagen/HAP or CS/collagen/β-TCP leads to the formation of a three-dimensional structure that enhances cell adhesion and proliferation, resulting in osteogenesis [53,54]. Osteoblast adhesion increases with increasing β-TCP and CS contents [54,58].

3.6. Fibrin and Fibrinogen

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Fibrin is widely used for enhancing the focal adhesion of collagen [59]. Santos et al. reported that the combination of collagen sponge and fibrin glue exerted hemostatic effects and ensured more favorable bone formation than did only collagen sponge [59,60]. B.-S. Kim et al. found that type I collagen-derived atelocollagen/fibrin composite gel with the addition of an optimal concentration of fibrinogen supported human MSC growth in vitro and bone formation in vivo [61].

3.7. Cytokines and Chemokines

Cytokines or chemokines, such as bone morphogenetic proteins (BMPs), chemotactic cytokine ligand (CXCL)12, and CXCL13, can enhance cell adhesion to the scaffold. Osteoblast adhesion to collagen or collagen-based materials leads to increased cell proliferation. BMP4 was immobilized in a CBD and bound to the collagen–polyglycolic hybrid scaffold; the BMP-immobilized hybrid scaffold supported the adhesion and proliferation of MSCs [62].

Claude Laflamme et al. revealed that a mixture of BMP-2/BMP-7 homodimers enhanced osteoblast adhesion and growth following culture on a collagen scaffold. Osteoblast adhesion and proliferation increased 4 days after culture on the collagen scaffold. BMP-2/BMP-7 promoted bone regeneration through different mechanisms involving interleukin-6 and matrix metalloproteinase inhibitors [63].

Sylvia Weeks et al. reported that the incorporation of the chemokines CXCL12 and CXCL13 into a poly-L-lactic acid–collagen-based scaffold increased cell adhesion. Combining CXCL12 with an FN- and collagen-coated scaffold increased MSC adhesion to the scaffold through α4 and α5β1 binding [64]. This scaffold promoted the differentiation of MSCs into osteoblasts, resulting in bone formation.

3.8. Small Molecules

Grafting collagen with other material to fabricate a scaffold is an effective strategy for enhancing cell proliferation and ALP expression [65]. Collagen can improve cell adhesion because of the presence of its Pro-α3(V) chain in bone. Through the N-terminal peptide of this chain, collagen adheres to osteosarcoma cells [66]. OCN, a small bone ECM protein, accelerated bone formation in a rat model when added to HAP/collagen composites. Histological findings indicate that OCN activates both osteoblasts and osteoclasts during early bone formation [37].

3.9. Implants

Collagen is widely used to coat biomedical implants. Geißler and Hempel et al. have reported that the collagen type I coating of Ti6Al4V promoted the initial adhesion of osteoblasts in the presence of fetal calf serum. Moreover, 60% to 90% of all osteoblasts adhered to collagen type I–coated surfaces, whereas 30% of the initial cell number remained adhered to uncoated surfaces. Collagen type I, which includes the RGD peptide, effectively promotes cell adhesion by interacting with $\alpha 1\beta 1$ and $\alpha 2\beta 1$ [40]. The type I collagen–coated Ti–6Al–4V alloy facilitated osseointegration and bone-to-implant contact [41].

Titanium-based implants exhibit osseointegration and are widely used in dental and orthopedic care. However, cell growth and differentiation capacity on the surface of titanium-based implants are limited. To address these limitations, various functionalization strategies for titanium surfaces have been developed—for example, bioactivated coating. One of the most used peptides to functionalize biomaterials is a cell adhesion peptide containing the RGD sequence, which is found in collagen, FN, and bone sialoprotein. RGD peptide–enriched materials interact with integrin on the cell surface and can enhance the proliferation of osteoblasts and bone marrow-derived stem cells [42].

In dental care, zirconia is used to manufacture implants. To enhance implant compatibility, nano-HAP is bound to the surface first and then type I collagen is immobilized on the surface. Compared with cases with no coating or with nano-HAP-only coating, cases with nano-HAP-collagen coating exhibited increased osteoblast attachment and spreading on the surface; higher osteoblast differentiation was confirmed by higher ALP activity and mineralization [43]. HAP and extensive collagen coating of an implant surface can facilitate cell attachment because of the presence

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of increased hydroxyl groups on the surface, which results in the formation of a low contact angle and the activation of carboxylic groups, which are beneficial for osteoblast adhesion and proliferation [44]. A list of articles reporting the modification of cell adhesion by collagen-based bone grafting materials for enhancing bone regeneration is provided in Table 1.

Table 1. Articles on collagen-based grafting materials.

Characterization	Technology	Results	Article	Author	Publisher
Orientation Alignment of collagen-based scaffold Bone-mimetic oriented (type I) collagen scaffolds	Using extrusion to obtain collagen and then fabricating the scaffold	Human induced pluripotent stem cell-derived osteoblasts exhibited favorable responses to the collagen scaffolds, as confirmed by the actin structure	Superior alignment of human iPSC-osteoblasts associated with focal adhesion formation stimulated by oriented collagen scaffold	Ryosuke Ozasa et al. [32]	*International Journal of Molecular Sciences* (June, 2021)
Orientation Collagen-based scaffold and PLGA, PCL through electrospinning PLGA/PCL/type I collagen electrospun scaffolds	The eletrospun scaffold made of polymer contained type I collagen	Upregulated expression of adhesion-related genes (β1, Cadherin 11, and Fn-1), with ADSC adhesion	Enhanced osteogenesis of ADSCs by the synergistic effect of aligned fibers containing collagen I	HanBang Chen et al. [45]	*ACS Applied Materials & Interferences* (Oct, 2016)
Orientation Collagen-based scaffold and HAP through electrospinning Electrospinning of collagen/HAP fibrous composite	HAP mixed with type I collagen	Cells exhibited increased viability on the collagen/HAP composite nanofibers	Greener synthesis of electrospun collagen/ hydroxyapatite composite fibers with an excellent microstructure for bone tissue engineering	Yuanyuan Zhou et al. [46]	*International Journal of Nanomedicine* (Apr, 2015)
Orientation Poly(lactide-co-glycolide)/CS scaffolds with collagen	Immersed scaffold in a solution containing type I collagen	Cell adhesion efficiency increased by approximately 1.2 fold; promotion of stem cell differentiation into osteoblasts	Effect of surface-modified collagen on the adhesion, biocompatibility and differentiation of bone marrow stromal cells in poly(lactide-co-glycolide)/CS scaffolds	Yung-Chih Kuo et al. [13]	*Colloids and Surfaces B: Biointerfaces* (Oct, 2010)
Laminin Collagen-based scaffold and laminin Collagen-based decalcified bone matrix scaffold modified with laminin α4	Collagen-binding domain (CBD) containing laminin alpha 4 on the scaffold	Promotion of early cell adhesion	Laminin alpha 4 promotes bone regeneration by facilitating cell adhesion and vascularization	Yong Tang et al. [33]	*Acta Biomaterialia* (Mar, 2021)

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Fibronectin Fibrillar complexes based on collagen type I and fibronectin	Fibronectin solution was added into collagen solution; then, KH_2PO_4 was added to form fibril shapes	MSCWJ-1 cells were elongated and had increased area on the composite fibril, which were confirmed by the actin cytoskeleton	The structural interactions of molecular and fibrillar collagen type I with fibronectin and its role in the regulation of mesenchymal stem cell morphology and functional activity	Yuliya Nashchekina et al. [34]	*International Journal of Molecular Sciences* (Oct, 2022)
Fibronectin Fusion protein, human OCN (hOCN) with $FN_{III9-10}$ combines with collagen	rhOCN/$FN_{III9-10}$ was crosslinked with collagen to form the matrix	rhOCN/$FN_{III9-10}$-functionalized collagen matrix increased not only the adhesion but also the differentiation of MC3T3-E1 cells	Osteocalcin/fibronectin-functionalized collagen matrices for bone tissue engineering	Kim S. et al. [35]	*Journal of Biomedical Materials Research Part A*, (Oct, 2015)
Ceramic and combined materials Collagen-based scaffold with silicon and HAP Silicon, collagen, and HAP	Silicon, collagen, and HAP	After 7 days, osteoblasts exhibited similar interaction with the scaffold and bovine bone	Analysis of in vitro osteoblast culture on scaffolds for future bone regeneration purposes in dentistry	Sandra J. Gutie´rrez-Prieto et al. [50]	*Advances in Pharmacological Sciences* (2019)
Ceramic and combined materials Collagen-based scaffold and the mixture of tetracalcium phosphate and dicalcium phosphate Anhydrous Calcium phosphate bone cement (CPC) with type I bovine collagen	CPC powder mixed with collagen powder	Two-fold increase in osteoblast attachment	Self-setting collagen-calcium phosphate bone cement: Mechanical and cellular properties	Jennifer L. Moreau et al. [49]	*Journal of Biomedical Materials Research Part A* (July, 2008)
Ceramic and combined materials Collagen-based scaffold and $FGMgCO_3Ap$ $FGMgCO_3Ap$ and atelocollagen composite pellet	$FGMgCO_3Ap$ mixed with atelocollagen	Osteoblast-like cells adhered more effectively to the composite than to the Ti plate	Action of $GMgCO_3Ap$-collagen composite in promoting bone formation	Y. Yamasaki et al. [51]	*Biomaterials* (May, 2023)
Ceramic and combined materials Collagen-based scaffold with CS and HAP Collagen/CS sponges (composed of collagen, CS, and HAP)	Homogenization of the collagen gel, CS gel, and HAP	Collagen coating and RGD coating exhibited good compatibility	Use of collagen/CS sponges mineralized with hydroxyapatite for the repair of cranial defects in rats	M.A.S. Munhoz et al. [53]	*Injury* (Sep, 2018)

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Ceramic and combined materials Collagen-based scaffold with HA Collagen–HAP scaffold combined with Fe^{2+} or Mn^{2+} ions	The scaffolds made of poly(acrylic acid) and type I collagen then substituted by Fe^{2+} or Mn^{2+} were shaped as a disc piece, whose diameter and thickness were 5.5 and 1 mm, respectively	MC3T3 cells exhibited viability and attachment when collagen was used; the parameters improved when Mn^{2+} and Fe^{2+} were added, as confirmed by the formation of pseudopodia	Intrafibrillar mineralized collagen-hydroxyapatite-based scaffolds for bone regeneration	Le Yu et al. [55]	*ACS Applied Material & Interfaces* (Dec, 2020)
Ceramic and combined materials Collagen-based scaffold with HAP Collagen-hemostat and granular HAP scaffold	Scaffold was prepared by mixing granular HAP and collagen hemostat and then dried overnight	After 21 days, human bone marrow-derived mesenchymal stem cells exhibited higher growth on the scaffold and exhibited high viability and cytoskeleton structure as the cell attachment	Enhanced osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by a hybrid HAP/collagen scaffold	Elisa Mazzoni et al. [67]	*Frontiers in Cell and Develop Bio* (Jan, 2021)
CS Collagen-based scaffold with β-TCP and CS Collagen, β-tricalcium phosphate, and CS matrix	Different ratios of CS and β-TCP formed with collagen	Composite made of β-TCP/collagen led to enhanced cell adhesion and mechanical properties	Bioactivity and mechanical properties of collagen composite membranes reinforced by CS and β- TCP	Sang-Bae Lee et al. [54]	*Society For Biomaterials* (Apr., 2012)
Fibrin and fibrinogen Collagen-based fibrin Fibrin–collagen sponges	A fibrin–collagen sponge was immersed in fibronectin–gelatin solution to generate fibrin	Favorable cell attachment and increased ALP activity	Improvements of osteoblast adhesion, proliferation, and differentiation in vitro via fibrin network formation in collagen sponge scaffold	Beom-Su Kim et al. [59]	*J Biomedical Materials Research Part A* (July, 2013)
Fibrin and fibrinogen Collagen-based scaffold with fibrin glue–modified collagen sponge	Fibrin glue composed of human fibrinogen, aprotinin, and thrombin	The sponge promoted new bone formation in a rat model of calvarial bone defect	Effect of collagen sponge and fibrin glue on bone repair	Thiago de Santana SANTOS et al. [60]	*J Appl Oral Sci.* (Sep, 2015)
Cytokine and chemokine Collagen-based scaffold and PLGA with BMP-4 Bone morphogenetic protein-4 immobilized in a collagen-PLGA hybrid scaffold	PLGA was crosslinked to type I collagen and then immersed in BMP-4	Mesenchymal stem cell adhered to the scaffold and exhibited uniform distribution on the scaffold with BMP-4	Spatial immobilization of bone morphogenetic protein-4 in a collagen-PLGA hybrid scaffold for enhanced osteoinductivity	Hongxu Lu et al. [62]	*Biomaterials* (June, 2012)

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Cytokine and chemokine Collagen-based scaffold with BMP-2 and with BMP-7 CollaTape scaffolds	CollaTape (taken from bovine deep flexor [Achilles] tendon)	The collagen scaffold with BMP-2/BMP-7 promoted osteoblast adhesion	Effect of BMP-2 and BMP-7 homodimers and a mixture of BMP-2/BMP-7 homodimers on osteoblast adhesion and growth following culture on a collagen scaffold	Claude Laflamme et al. [63]	*Biomedical Materials* (Feb, 2008)
Cytokine and chemokine Collagen-based scaffold with PLLA and chemokines Type IV collagen and some chemokines were coated on the scaffold made of PLLA	PLLA-coated coverslips were incubated with fibronectin, type IV collagen, or heparin with chemokines (CXCL12 and CXCL13)	Combined CXCL12 and collagen enhanced cell adhesion compared with the outcomes noted with collagen alone	The effects of chemokine, adhesion and extracellular matrix molecules on binding of mesenchymal stromal cells to poly(L-lactic acid)	SYLVIA WEEKS et al. [64]	*Cytotherapy* (May, 2012)
Small molecule Collagen-based scaffold and HAP with osteocalcin HAP/collagen composites help in the secretion of osteocalcin in the scaffold or electrospinning	Nanocrystalline HAP implants contained 2.5% type I collagen/graphene oxide, HAP combined with collagen	The expression of adhesion proteins (osteopontin, bone sialoprotein, and CD44) was increased; electrospinning-coated alloys increased cell adhesion and viability	Osteocalcin enhances bone remodeling around hydroxyapatite/collagen composites; Novel hydroxyapatite/graphene oxide/collagen bioactive composite coating on Ti16Nb alloys by electrodeposition	Stefan Rammelt et al. & Yılmaz, E. et al. [37,38]	*Journal of Biomedical Materials Research Part A (March, 2005); Materials Science and Engineering: C* (2019)
Small molecule Collagen-based scaffold and PLGA, HAP with BMP-4 Nano-HAP–poly(D,L-lactide-co-glycolide)–collagen biomaterial	Multistep polymerization and fabrication process	Increased cell proliferation and ALP expression	Mechanical properties and osteogenic potential of Hydroxyapatite-PLGA-collagen biomaterial for bone regeneration	Didarul B. Bhuiyan et al. [65]	*Journal of Biomaterials science* (May, 2016)
Implant Collagen was coated on alloy Collagen type I coating of Ti6Al4V	Ti6Al4V alloy coated with type I collagen	The alloy coated with type I collagen enabled osteoblasts to attach better and faster; they were recognized by integrins α1β1 and α2β1	Collagen type I coating of Ti6Al4V promotes adhesion of osteoblasts	Geißler et al. [40]	*J Biomed Mater Res* (2000).
Implant Collagen was coated on alloy Ti–6Al–4V alloy combined with collagen	Ti–6Al–4V alloy coated with type I collagen	The alloy coated with type I collagen led to the high levels of new bone formation	An alternative ex vivo method to evaluate the osseointegration of Ti–6Al–4V alloy also combined with collagen	Francesca Veronesi et al. [41]	*Biomedical Materials* (Feb, 2021)

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Implant Collagen crosslinked to alloy Collagen crosslink on titanium (Ti6Al4V) surfaces	Different crosslinkers (EDC/NHS, riboflavin, and lysyl oxidase) were used for coupling the collagen with alloy	Cells exhibited favorable attachment to the material surface modified by crosslinkers, which was confirmed through immunofluorescence	Transglutaminase enables highly hydrolytically and proteolytically stable crosslinking of collagen on titanium surfaces and promotes osteogenic differentiation of human mesenchymal stem cells	Alena L. Palkowitz et al. [42]	*Society For Biomaterials* (Dec, 2023)
Implant Collagen was immobilized on alloy Zirconia dental implants coated with collagen	Coating the surface with nano-HAP and then immobilizing type I collagen on it	Compared with cases with no coating and with nano-HAP-only coating, those with nano-HAP-collagen coating exhibited increased osteoblast attachment, spreading, mineralization, and differentiation	Bioactive surface of zirconia implant Prepared by nano-hydroxyapatite and type I collagen	Hun Kim et al. [43]	*Coatings* (Sep, 2022)
Implant Collagen was immobilized on alloy Stainless steel (SS316L) immobilized with HAP, collagen on stainless steel (SS316L) with polydopamine	HAP and collagen immobilized on polydopamine and then grated on the implant surface	Presence of hydroxyl groups on the surface, resulting in a low contact angle and carboxylic group activation, may be beneficial for osteoblast adhesion and proliferation	Synthesis and characterization of collagen–hydroxyapatite immobilized on polydopamine grafted stainless steel	Zafirah Tapsir et al. [44]	*Surface and Coatings Technology* (Jan, 2016)

Abbreviations: CS, chitosan; HAP, hydroxyapatite.

4. Conclusion

Over the last decades, extensive research has been conducted on collagen-based materials to improve their biological and mechanical properties for supporting efficient bone regeneration. Advanced strategies for regulating collagen's structure and properties by modifying materials and refining processing techniques can yield highly biomimetic substrates, which can advance tissue engineering.

Collagen-based materials enhance FAK activity and activate the ERK–MAPK pathway. This stimulates the phosphorylation of ERK1/ERK2 through MEK, regulating the activity of osteogenic transcription factor RUNX2. RUNX2 controls the expression of osteogenic genes such as OCN, ALP, and bone sialoprotein, ultimately driving differentiation toward mature osteoblasts.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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References

1. Bharadwaz, A. and A.C. Jayasuriya, Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. Materials Science and Engineering: C, 2020. **110**: p. 110698.
2. Ndukwe, K.C., et al., Reconstruction of mandibular defects using nonvascularized autogenous bone graft in nigerians. Nigerian Journal of Surgery, 2014. **20**(2): p. 87-91.
3. Zimmermann, G. and A. Moghaddam, *Allograft bone matrix versus synthetic bone graft substitutes.* Injury, 2011. **42**: p. S16-S21.
4. Graziani, G., et al., A Comprehensive microstructural and compositional characterization of allogenic and xenogenic bone: Application to bone grafts and nanostructured biomimetic coatings. Coatings, 2020. **10**(6): p. 522.
5. Trajkovski, B., et al., Hydrophilicity, viscoelastic, and physicochemical properties variations in dental bone grafting substitutes. Materials, 2018. **11**(2): p. 215.
6. Weiner, S. and H.D. Wagner, *THE MATERIAL BONE: Structure-Mechanical Function Relations.* Annual Review of Materials Research, 1998. **28**(Volume 28, 1998): p. 271-298.
7. Thula, T.T., et al., In vitro mineralization of dense collagen substrates: A biomimetic approach toward the development of bone-graft materials. Acta Biomaterialia, 2011. **7**(8): p. 3158-3169.
8. Li, P. and G. Wu, Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino acids, 2018. **50**: p. 29-38.
9. Lin, X., et al., The bone extracellular matrix in bone formation and regeneration. Frontiers in pharmacology, 2020. **11**: p. 757.
10. Ferreira, A.M., et al., *Collagen for bone tissue regeneration.* Acta biomaterialia, 2012. **8**(9): p. 3191-3200.
11. Li, Y., et al., *Collagen-based biomaterials for bone tissue engineering.* Materials & Design, 2021. **210**: p. 110049.
12. Leisten, I., et al., 3D co-culture of hematopoietic stem and progenitor cells and mesenchymal stem cells in collagen scaffolds as a model of the hematopoietic niche. Biomaterials, 2012. **33**(6): p. 1736-1747.
13. Kuo, Y.-C. and C.-F. Yeh, Effect of surface-modified collagen on the adhesion, biocompatibility and differentiation of bone marrow stromal cells in poly (lactide-co-glycolide)/chitosan scaffolds. Colloids and Surfaces B: Biointerfaces, 2011. **82**(2): p. 624-631.
14. Kafi, M.A., et al., Adhesion, proliferation and differentiation of human mesenchymal stem cell on chitosan/collagen composite scaffold. Journal of Materials Science: Materials in Medicine, 2019. **30**: p. 1-12.
15. Teixeira, B.N., et al., Evaluation of bone marrow stem cell response to PLA scaffolds manufactured by 3D printing and coated with polydopamine and type I collagen. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2019. **107**(1): p. 37-49.

 doi:10.20944/preprints202505.2026.v1

16. Popov, C., et al., Integrins $\alpha 2\beta 1$ and $\alpha 11\beta 1$ regulate the survival of mesenchymal stem cells on collagen I. Cell death & disease, 2011. **2**(7): p. e186-e186.
17. Mao, L., et al., The role of integrin family in bone metabolism and tumor bone metastasis. Cell Death Discov, 2023. **9**(1): p. 119.
18. CASTOLDI, M., et al., Osteoblastic cells from rat long bone II: adhesion to substrata and integrin expression in primary and propagated cultures. Cell biology international, 1997. **21**(1): p. 7-16.
19. Kapp, T.G., et al., A comprehensive evaluation of the activity and selectivity profile of ligands for RGD-binding integrins. Scientific reports, 2017. **7**(1): p. 1-13.
20. Lu, J., et al., Basement membrane regulates fibronectin organization using sliding focal adhesions driven by a contractile winch. Developmental Cell, 2020. **52**(5): p. 631-646. e4.
21. Salasznyk, R.M., et al., Activation of FAK is necessary for the osteogenic differentiation of human mesenchymal stem cells on laminin-5. Journal of Cellular Biochemistry, 2007. **100**(2): p. 499-514.
22. Mitra, S.K. and D.D. Schlaepfer, *Integrin-regulated FAK–Src signaling in normal and cancer cells.* Current opinion in cell biology, 2006. **18**(5): p. 516-523.
23. Chodniewicz, D. and R.L. Klemke, *Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold.* Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2004. **1692**(2-3): p. 63-76.
24. Lafer, G. and A. Brugner, The effect of nutrient content on quality and storage losses of'Braeburn'apples. 2002.
25. Kiyokawa, E., et al., *Activation of Rac1 by a Crk SH3-binding protein, DOCK180.* Genes & development, 1998. **12**(21): p. 3331-3336.
26. Huveneers, S. and E.H. Danen, *Adhesion signaling–crosstalk between integrins, Src and Rho.* Journal of cell science, 2009. **122**(8): p. 1059-1069.
27. Wan, Q., et al., *Rac1 and Cdc42 GTPases regulate shear stress-driven β-catenin signaling in osteoblasts.* Biochemical and Biophysical Research Communications, 2013. **433**(4): p. 502-507.
28. Ten Klooster, J.P., et al., *Targeting and activation of Rac1 are mediated by the exchange factor β-Pix.* The Journal of cell biology, 2006. **172**(5): p. 759-769.
29. Brown, M.C., et al., Src and FAK kinases cooperate to phosphorylate paxillin kinase linker, stimulate its focal adhesion localization, and regulate cell spreading and protrusiveness. Molecular biology of the cell, 2005. **16**(9): p. 4316-4328.
30. Feng, Q., et al., Cool-1 functions as an essential regulatory node for EGFreceptor-and Src-mediated cell growth. Nature cell biology, 2006. **8**(9): p. 945-956.
31. Khatiwala, C.B., et al., ECM compliance regulates osteogenesis by influencing MAPK signaling downstream of RhoA and ROCK. Journal of bone and mineral research, 2009. **24**(5): p. 886-898.
32. Ozasa, R., et al., Superior alignment of human iPSC-osteoblasts associated with focal adhesion formation stimulated by oriented collagen scaffold. International Journal of Molecular Sciences, 2021. **22**(12): p. 6232.
33. Tang, Y., et al., Laminin alpha 4 promotes bone regeneration by facilitating cell adhesion and vascularization. Acta Biomaterialia, 2021. **126**: p. 183-198.
34. Nashchekina, Y., et al., The structural interactions of molecular and fibrillar collagen type I with fibronectin and its role in the regulation of mesenchymal stem cell morphology and functional activity. International Journal of Molecular Sciences, 2022. **23**(20): p. 12577.
35. Kim, S., et al., *Osteocalcin/fibronectin-functionalized collagen matrices for bone tissue engineering.* Journal of Biomedical Materials Research Part A, 2015. **103**(6): p. 2133-2140.
36. Przekora, A., et al., Hybrid chitosan/β-1, 3-glucan matrix of bone scaffold enhances osteoblast adhesion, spreading and proliferation via promotion of serum protein adsorption. Biomedical Materials, 2016. **11**(4): p. 045001.
37. Rammelt, S., et al., *Osteocalcin enhances bone remodeling around hydroxyapatite/collagen composites.* Journal of Biomedical Materials Research Part A, 2005. **73A**(3): p. 284-294.
38. Yılmaz, E., et al., Novel hydroxyapatite/graphene oxide/collagen bioactive composite coating on Ti16Nb alloys by electrodeposition. Materials Science and Engineering: C, 2019. **101**: p. 292-305.

 doi:10.20944/preprints202505.2026.v1

39. Wei, M., et al., Enhanced mechanical properties and anti-washout of calcium phosphate cement/montmorillonite composite bone-cement for bone-repair applications. Ceramics International, 2022. **48**(23): p. 35185-35197.
40. Geissler, U., et al., *Collagen type I-coating of Ti6Al4V promotes adhesion of osteoblasts.* Journal of biomedical materials research, 2000. **51**(4): p. 752-760.
41. Veronesi, F., et al., An alternative ex vivo method to evaluate the osseointegration of Ti–6Al–4V alloy also combined with collagen. Biomedical Materials, 2021. **16**(2): p. 025007.
42. Palkowitz, A.L., et al., Transglutaminase enables highly hydrolytically and proteolytically stable crosslinking of collagen on titanium surfaces and promotes osteogenic differentiation of human mesenchymal stem cells. Journal of Biomedical Materials Research Part A, 2024. **112**(6): p. 812-824.
43. Kim, H., et al., Bioactive surface of zirconia implant prepared by nano-hydroxyapatite and type I collagen. Coatings, 2022. **12**(9): p. 1335.
44. Tapsir, Z. and S. Saidin, Synthesis and characterization of collagen–hydroxyapatite immobilized on polydopamine grafted stainless steel. Surface and Coatings Technology, 2016. **285**: p. 11-16.
45. Chen, H., et al., Enhanced osteogenesis of ADSCs by the synergistic effect of aligned fibers containing collagen I. ACS Applied Materials & Interfaces, 2016. **8**(43): p. 29289-29297.
46. Zhou, Y., et al., Greener synthesis of electrospun collagen/hydroxyapatite composite fibers with an excellent microstructure for bone tissue engineering. International journal of nanomedicine, 2015: p. 3203-3215.
47. Parisi, L., et al., A glance on the role of fibronectin in controlling cell response at biomaterial interface. Japanese Dental Science Review, 2020. **56**(1): p. 50-55.
48. Mazzoni, E., et al., Enhanced osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by a hybrid hydroxylapatite/collagen scaffold. Frontiers in Cell and Developmental Biology, 2021. **8**: p. 610570.
49. Moreau, J.L., M.D. Weir, and H.H. Xu, *Self-setting collagen-calcium phosphate bone cement: Mechanical and cellular properties.* Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2009. **91**(2): p. 605-613.
50. Gutiérrez-Prieto, S.J., et al., *Analysis of in vitro osteoblast culture on scaffolds for future bone regeneration purposes in dentistry.* Advances in Pharmacological and Pharmaceutical Sciences, 2019. **2019**(1): p. 5420752.
51. Yamasaki, Y., et al., Action of FGMgCO3Ap-collagen composite in promoting bone formation. Biomaterials, 2003. **24**(27): p. 4913-4920.
52. Ou, K.-L., et al., Effects of the nanostructure and nanoporosity on bioactive nanohydroxyapatite/reconstituted collagen by electrodeposition. Journal of Biomedical Materials Research Part A, 2010. **92A**(3): p. 906-912.
53. Munhoz, M., et al., Use of collagen/chitosan sponges mineralized with hydroxyapatite for the repair of cranial defects in rats. Injury, 2018. **49**(12): p. 2154-2160.
54. Lee, S.B., et al., Bioactivity and mechanical properties of collagen composite membranes reinforced by chitosan and β-tricalcium phosphate. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2012. **100**(7): p. 1935-1942.
55. Yu, L., et al., Intrafibrillar mineralized collagen–hydroxyapatite-based scaffolds for bone regeneration. ACS applied materials & interfaces, 2020. **12**(16): p. 18235-18249.
56. Yi, H., et al., *Biofabrication with Chitosan.* Biomacromolecules, 2005. **6**(6): p. 2881-2894.
57. Custódio, C., et al., *Immobilization of fibronectin in chitosan substrates improves cell adhesion and proliferation.* Journal of Tissue Engineering and Regenerative Medicine, 2010. **4**(4): p. 316-323.
58. Moutzouri, A.G. and G.M. Athanassiou, *Insights into the alteration of osteoblast mechanical properties upon adhesion on chitosan.* BioMed Research International, 2014. **2014**(1): p. 740726.
59. Kim, B.S., J.S. Kim, and J. Lee, Improvements of osteoblast adhesion, proliferation, and differentiation in vitro via fibrin network formation in collagen sponge scaffold. Journal of Biomedical Materials Research Part A, 2013. **101**(9): p. 2661-2666.

 doi:10.20944/preprints202505.2026.v1

60. Santos, T.d.S., et al., *Effect of collagen sponge and fibrin glue on bone repair.* Journal of Applied Oral Science, 2015. **23**: p. 623-628.
61. Kim, B.S., et al., The effects of fibrinogen concentration on fibrin/atelocollagen composite gel: an in vitro and in vivo study in rabbit calvarial bone defect. Clinical Oral Implants Research, 2015. **26**(11): p. 1302-1308.
62. Lu, H., et al., Spatial immobilization of bone morphogenetic protein-4 in a collagen-PLGA hybrid scaffold for enhanced osteoinductivity. Biomaterials, 2012. **33**(26): p. 6140-6146.
63. Laflamme, C. and M. Rouabhia, Effect of BMP-2 and BMP-7 homodimers and a mixture of BMP-2/BMP-7 homodimers on osteoblast adhesion and growth following culture on a collagen scaffold. Biomedical Materials, 2008. **3**(1): p. 015008.
64. Weeks, S., et al., The effects of chemokine, adhesion and extracellular matrix molecules on binding of mesenchymal stromal cells to poly (l-lactic acid). Cytotherapy, 2012. **14**(9): p. 1080-1088.
65. Bhuiyan, D.B., et al., Mechanical properties and osteogenic potential of hydroxyapatite-PLGA-collagen biomaterial for bone regeneration. Journal of Biomaterials Science, Polymer Edition, 2016. **27**(11): p. 1139-1154.
66. Yamaguchi, K., et al., Pro-α3 (V) collagen chain is expressed in bone and its basic N-terminal peptide adheres to osteosarcoma cells. Matrix biology, 2005. **24**(4): p. 283-294.
67. Mazzoni, E., et al., Enhanced Osteogenic Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells by a Hybrid Hydroxylapatite/Collagen Scaffold. Frontiers in Cell and Developmental Biology, 2021. **8**.