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Review Article

THE NATURAL AND COMMERCIAL SOURCES OF HYDROXYAPATITE/COLLAGEN COMPOSITES FOR BIOMEDICAL APPLICATIONS: A REVIEW STUDY

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ABSTRACT

Bone is considered the core unit that forms the human body's skeleton, consisting primarily of hydroxyapatite (HA) and collagen (Col). The composites of hydroxyapatite/collagen had been prepared through different fabricated techniques and were used in many bone defects as biomaterials for bone tissue engineering. The incorporation of HA and collagen is possible due to the biocompatibility of collagen and the high mechanical properties of the HA. HA/Col composites have been used in many medical and biological fields. Current study have been discussed the synthesis and characterization techniques of HA/Col composites; the study have been included to study the cytotoxicity and cell attachment of the composites, along with their applications, as well as barriers that still remain to their successful development for clinical application.

Keywords: Hydroxyapatite, Collagen, HA/Col composites, Biomedical applications

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INTRODUCTION

Bone defects may occur for several reasons, such as injuries, illnesses, surgical interventions, and accidents, some of which may heal on their own. However, bone defects greater than 1/3 an inch (≈ 8 mm) cannot be healed on their own [1]. Therefore, bone substitutes were used to fill up and enhance bone defects to allow for the rapid healing process [2]. These substitutes provide structural and mechanical support to enhance bone tissue formation or fill gaps to facilitate the healing of bone tissue. Bone substitutes have been widely used in plastic surgery, oral, maxillofacial, dental, and orthopedic surgery, making it one of the most implanted tissues in the medical field [3].

The structure of natural bone is a composite material comprised of organic and inorganic elements [4]. The organic materials are mainly Col fibers containing tropocollagen, which make up most of the organic constituent of bone, and provide strength to the bone [5]. The inorganic materials are mainly calcium (Ca) and phosphorus (P) in the form of HA [6, 7]. However, both of HA and Col are formed the structure of the bone, naturally.

The incorporation of HA and Col is possible due to the biocompatibility of Col and high mechanical properties of the HA, and it is widely utilized as the biomaterial to enhance the healing of bone defect, as well as a replacement material for bone defects. Nonetheless, despite its advantages, several issues of HA/Col composite concern the public. Tampieri *et al.* [3], reported the successful development of HA/Col composite that showed excellent bioactivity properties, which lead to its use as a bone filler for the defect bones. The composite of HA/Col had been prepared through different fabricated techniques and were used in many bone defects as biomaterials for bone tissue engineering. The research were focusing on the years from 2010 into 2021, but there is some old references because some of them as a books and important to add.

Preparation methods of HA/Col composites

The HA/Col composites have been fabricated and widely studied for bone engineering purposes. As have been mentioned, pure Col has weak mechanical properties; however, the use of col is limited and to enhance the mechanical properties of Col, the researchers have combined it with HA using different methods.

Significant aim to synthesis HA/Col composites to produce materials that have identical properties of the natural composites. There are many techniques have been reported to produce HA/Col composites such as freezing-drying [8], 3D printing [9], situ precipitation [10], co-precipitation [11], electrospinning [12], and dehydrothermal [13], fig. 1 shows the most synthesis techniques which have been used to prepared composites of HA/Col. The previous studied have been focused on these two materials due to their mechanical properties and biocompatibility. The researchers were reported to modify the methods of the preparation for the composites of HA/Col. The study of Ficai *et al.*, [14], has been reported to prepared composites of HA/Col by using self-assembled; briefly, 80:20 (HA/Col) were mixed together, the pH of the mixture has been adjusted at 9 via using NaOH as a calibration solution and the temperature kept during the synthesis process of the composites at ~37 °C. The study was reported to new way to calculate the SD for the fibres of the 2D composites. Walsh *et al.*, 2019, have been reported to synthesize composites of HA/Col via using lyophilisation method, the ratio of composites was 70:30 (HA: Col).1.8 g of Col has been dissolved in 0.05M acetic acid. Col mixture was centrifuged for 90 min 15,000rpm. 3.6g of HA dissolved in 0.05M acetic acid, then added slowly to the Col mixture.

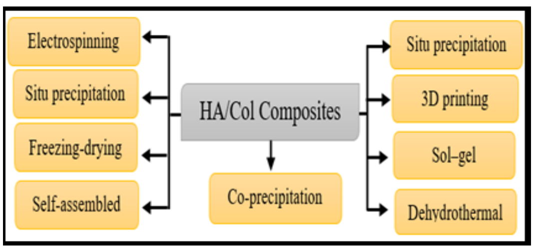

Fig. 1: Synthesis techniques of HA/Col composites (Source: author)

The HA/Col composites were synthesis by using the self-assembling method, the method includes two steps. The first step include to treated Col with the solution of $Ca(OH)_2$, then stirred for 24 h. The second step the solution of H_3PO_4 was added to the mixture. The base of $Ca(OH)_2$ have been added to adjusted the pH [17, 18]. The freeze-drying method has been used to preparation HA/Col. The study of Siswanto *et al.*, [19], has been reported to synthesis the composites from natural sources. HA (marine) has been dissolved in phosphoric acid and the Col (bone chicken) have also been dissolved in acetic acid. The solution of HA and Col have been mixed together and stirring them to make a homogeneous solution. The mixture of HA-Col were frozen under −80 °C, for 6 h.

Recently the applications of HA/Col as a Nano sizes have been gotten bulk of attention in materials engineering due to the properties of Nano-materials which could be used in many applications. The ability of the composites can be investigated via analysis of their chemical, physical and biological properties during using many measurements.

Table 1: Synthesis methods of HA/Col composites

No	Method	Composites	Application	Ref
1	Freezing-drying	Chitosan/Col/HA	Cartilage tissue engineering	[8]
2	3D printing	HA/Col	Osteochondral regeneration	[9]
3	3D printing	Zinc Silicate/Nano HA/Col	Bone regeneration	[10]
4	Situ precipitation	HA/Col	Bone tissue engineering	[11]
5	Co-precipitation method	HA/Col-magnetite	Bone cancer treatment	[12]
6	Electrospinning	HA/Col	Bone regeneration engineering	[13]
7	Electric field orientation	HA/Col	Cortical bone defect	[14]
8	Dehydrothermal	HA/Col	Bone repair	[16]
9	Self-assembled	HA/Col	Bone regeneration engineering	[15]
10	Sol–gel	HA/Col	Injectable bone substitute	[16]
11	Lyophilisation	HA/Col	Bone defect	[17]

The composites of HA/Col incorporate with materials

The HA/Col composites had been prepared through different fabricated techniques and were used in many bone defects as biomaterials for bone tissue engineering. The composite have been incorporated by different sizes (Nano-size and micro-size) with many other materials such as calcium phosphate (CaP) [20], poly(L-lactide) (PLLA) [21], Poly(vinyl alcohol) (PVA) [22], chitosan [23], and metals such as iron (Fe) [24], to develop the properties of the composites, which will possess high compatibility and bio-degradable properties for bone repair.

The composites of HA/Col/natural polymers

The composites of HA/Col have to be incorporated with many natural polymer to improve the properties. The previous studies have been reported to prepared the composites of HA/Col with natural polymers such as gelatine [25], cellulose [26], chitosan [27], chitin [28] and starch [29] as it shown in the table 2. Kaviani *et al.*, [22], was reported to incorporate HA/Col with chitosan by using freezing method. The application of the composites was for cartilage tissue engineering. This study included a cheaper way and environmentally friendly for the preparation of the composite.

The properties of the HA/Col have been improved by incorporated with gelatine. The unidirectional freeze-casting method has been used to synthesize the composites. The results of the availability study against human bone-derived osteoblast, which have been compared with the control cell, showed improved in proliferation, differentiation and adhesion [26, 30]. Study of He *et al.*, [27], reported to prepared HA/Col/cellulose by using in-situ precipitation method for bone tissue engineering. The results showed improved in the swelling ratio of the composites. The mechanical properties and biodegradation have been investigated; the compression strength was increased to 20-40 MPa, which is almost nearby the compression for the natural bone.

Table 2: The HA/Col composites incorporated with the natural polymers

Natural polymer	Techniques	Ref
Gelatine	Unidirectional freeze-casting	[26]
Cellulose	In-situ precipitation	[27]
	Simple mixing method	[28]
Chitosan	The freeze-gelation process	[22]
	Simple mixing method	[31,32]
Chitin	The freeze-thawing process	[33]
Starch	Simple mixing method	[29]

The composites of HA/Col/synthetic polymers

The composites of HA/Col/synthetic polymers were synthesis and has shown unique properties that enable it to be used in many fields. Many synthetic polymers have been incorporated with the composites of HA/Col, such as Poly (L-lactide) (PLLA) [34], Polylactide-co-glycolide (PLGA) [35], Polyvinyl alcohol (PVA) [36], Poly (methyl methacrylate) (PMMA) [28] and Polycaprolactone [37], as shows in table 3. The previous studies were reported to study the ability of the composites of HA/Col for bone tissue engineering through determined the chemical, physical and biological properties of the composites.

Zhou *et al.*, [35], incorporated PLLA with the HA/Col composites by using electrospinning method. The composites were characterized for their biocompatibility against mouse osteoblasts MC3T3-E1. The results have been shown to enhance the spreading, proliferation and adhesion for the cell. The biodegradation study was determined during 80 d, and the composites showed high stable composition and didn't change in the morphology of the composites. Ariesanti *et al.*, [38], were reported to prepared HA/Col/PVA by using a simple mixing method. The cytotoxicity of the composite of HA/Col/PVA showed high cell availability in the MTT assay.

Table 3: The HA/Col composites incorporated with the synthetic polymers

Synthetic polymer	Techniques	Ref
Poly (L-lactide) (PLLA)	Optimized sol-gel method.	[34]
Polylactide-co-glycolide (PLGA)	Electrospinning	[35]
Polyvinyl alcohol (PVA)	Stimulate method	[36]
Polycaprolactone	freeze-dried method	[37]
Poly(methyl methacrylate) (PMMA)	Simple mixing method	[28]

The composites of HA/Col/metals

The parameters of metallic materials have significant properties, which have been shown to have properties higher than ceramics, which gives it a priority to be used in the field of tissue engineering. The biocompatibility of the metallic is lower than ceramics, which leads to allergic reactions in the blood clots [39]. The metallic nanomaterials have unique properties such as anti-microbial activity, high ratio of surface area, and biological, mechanical and physical properties [40, 41]. The composites of HA/Col were incorporated with metals and metals oxide for example, gold (Au) [42], silver (Ag) [43, 44], magnetite (Fe_3O_4) [45], graphene oxide (GO) [46] and Iron oxide (Fe_2O_3) [11] as it shown in table 4. Characterization of the composites has been shown high ability to be used to inhibition the growth of bacteria [47, 48], in addition, to use in the regeneration of bone tissue engineering [49, 50]. The HA/Col composites don't have ability to inhibition the growth of bacteria; however, recent studies have been focused to incorporated composites of the HA/Col with nanomaterials to use as an antibacterial [51, 52]. The compatibility of the composites HA/Col with metals was investigated and show high compatibility *in vitro* study [54].

Ciobanu *et al.*, [45], reported to synthesis HA/Col with Ti for antibacterial activity. The composite was determined its ability to use as an antibacterial against *staphylococcus aureus* (S. aureus) and *Escherichia coli* (E. coli), the coated composites showed significant function against both of these bacterial. HA/Col composite was incorporated with Au nanoparticles by using the microwave-assisted green method and investigated for the biocompatibility study against MG-63 cells. The results show high availability of the MG-63 cell after 24 h, and interaction was observed clearly in the SEM image [42]. Song *et al.*, [9], reported to synthesis of composites of HA/Col with zinc silicate by using the hydrothermal method for bone regeneration. Biocompatibility study have been determined for the ZS/HA/Col composites against bone marrow stromal cells (BMSCs) and the availability of the BMSCs after 24 h was very high. Accoutring to previous studies the composites of HA/Col with the metals have been shown high biocompatibility which can be used safely in the implants.

The composites of HA/Col/Drugs

The present studies have been taken to trend to the drug-delivery system. The systems of the drug delivery has the ability to use in the bone pathologies such as osteosarcoma, osteomyelitis, and osteoporosis. The composites of HA/Col were incorporated with wide range of drugs such as paclitaxel [23], cisplatin [55], vancomycin [56], tetracycline [57] and alendronate [58]. The most studies have been focused to incorporate the composites with the drugs to design new antibiotic for inhibition of bacteria growth [59]. The other applications of using drugs in the drug delivery system are anti-cancer [23], biocompatibility [60], and anti-osteoporosis [61].

Table 4: The HA/Col composites incorporated with metals and metal oxides

No	Metals and metal oxide	Methods	Applications	Ref
1	Gold (Au)	Microwave-assisted	Tissue engineering and drug delivery	[47]
2	Silver (Ag)	Simulated body fluid (SBF)	Orthopedic	[43]
3	AgNPs	Co-precipitation	Antimicrobial	[44]
4	Titanium (Ti)	Biomimetic method	Bone implants	[42]
5	Zinc (Zn)	Freeze-dryer	Bone regeneration	[9]
6	Magnetite (Fe_3O_4)	Co-precipitation	Bone cancer treatment	[11]
7	Graphene oxide (GO)	Electrodeposition	Antibacterial effect	[53]
8	Iron oxide (Fe_2O_3)	Co-precipitation	Bone fractures	[23]
9	Titanium (Ti)	Electrochemical deposition	Biocompatibility	[55]

Table 5: The HA/Col composites incorporated with drugs

No	Drugs	Methods	Applications	Ref
1	Paclitaxel	Hydrothermal	Anticancer	[23]
2	Cisplatin	Bone Cancer	In situ treatment	[55]
3	Vancomycin	Electrospinning	Antimicrobial Activity	[56]
4	Vancomycin gentamicin	Electrospinning	Antimicrobial Activity	[57]
5	Vancomycin	Freeze-dryer	Biocompatibility	[58]
6	Vancomycin	3D printing	Enhance Osseo integration and antimicrobial activity	[62]
7	Tetracycline	Mineralization	Antimicrobial Activity	[60]
8	Gentamicin	Hydrothermal	Antibacterial	[61]
9	Alendronate	Freeze-dryer	Bone regeneration as a anti-osteoporosis	[62]
10	Alendronate	Freeze-drying	Bone regeneration	[63]

Many studies have been reported to incorporate HA/Col with wide range of antibiotics. The vancomycin has been used widely with composites. The study of suchý *et al.*, [60], reported to loaded of vancomycin with the HA/Col via using the electrospinning method for antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, the results showed a significant effect against both of the bacterial compared with the HA/Col composites which did not show any inhibition for the growth of the bacteria. The vancomycin/HA/Col composites were modified by incorporated with gentamicin to enhance the ability of the composites against both of the bacteria [65].

These composites have been investigated for the biocompatibility study through tested *in vivo* against wide range of the cells such as human osteoblast-like cell line (SAOS-2 cells) [56, 57], stromal cells (MSCs) [58], MG-63 cells [59], and MC3T3-E1 osteoblastic cells [62]. The composites show high availability for the cells after 24 h; however, the composites are very safely to be used in bone tissue engineering.

The characterization techniques of HA/Col composites

The prepared HA/Col composites were characterized for their chemical, physical and biological properties. The characterized techniques for the implant composites include two important things biocompatibility and morphology of the composites. Previous studied focused on the *in vivo* study [63]. The biocompatibility test include two parts; firstly, the cytotoxicity study, which give the viability of the cultured cells with the composites after cultured for kwon time. The cytotoxicity studied of HA/Col composites was determined by different techniques such as MTT assay [64], alamar blue assay [66], and flow cytometry analysis [49].

The hydroxyapatite and collagen don't have any toxic itself; moreover, the composites of HA/Col aren't toxic [45], but, there will be cause for concern when combined with other potentially toxic substances, which will lead to the toxicity of the final product. Previous studies have dealt with the study of the toxicity of composites when combined with other materials, as shows in table

5. The study of Popa *et al.*, [49], reported to combined Zn with the composites of HA//Col, the results have been shown viability for the HeLa cell lines more that 95%. The composites of HA/Col were modified for the osteoblast via incorporated with chitosan. The results did not show any toxic against MC3T3-E1 cells for the 7days.

In the conclusion according to the previous studies, the composites of the HA/Col did not show any toxicity against human cells line; moreover, the composites have been combined with many materials, and the results did not show any toxicity; however, the composites are safe and can be used in the bone tissue engineering.

The cell attachment study of the HA/Col composites was demonstrated in wide range of the previous studies. The composites showed promising attached with the vivo cells. The HA/Col/chitosan was prepared for the restoration of maxillofacial mandible bone. Cell attachment was investigated against mesenchymal stem cells; the results have been shown more than 90% of cells were attached to the composites after 24h of the cultured [67]. The study of Cao *et al.*, [68], reported to prepared HA/Col for the application of bone graft. Study of cell attachment was determined during cultured with murine L929 cells, 75% of the cells attached with the composites after 24h. Table 6 shows summaries for the previous studies which have been mentioned to the study of cell attachment for the composites of HA/Col.

In the conclusion of this part, the composites of HA/Col have been cultured with the human cell line for the known duration time, and the results were demonstrated to high biocompatibility with the human cells line. According to the previous study, the composites of HA/Col is suitable to use in bone tissue engineering due to its high biocompatibility.

The second part of the characterization techniques are chemical, physical and biological scan for the composites. Many techniques have been used to confirm this part, such as SEM, EDX, XRD, XPS, FTIR, AFM, antibacterial, anticancer and swelling ratio, etc., as shows in fig. 2. The chemical composition of the HA/Col composites has been confirmed by using XRD, EDX, XPS, and FTIR.

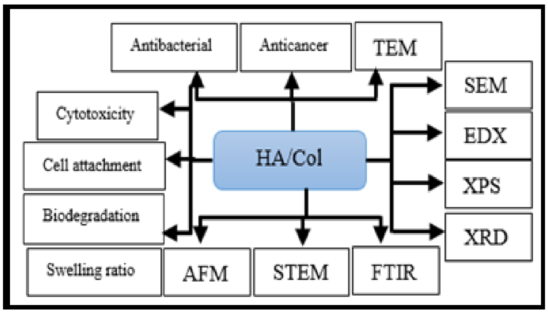

Fig. 2: Characterization techniques of HA/Col composites (Source: author)

The characterization techniques of HA/Col composites aimed to identify the chemical composition, surface morphology, mechanical properties, and biological ability for the prepared composites. The chemical composition was conformed by using XRD [69, 70], FTIR [39], EDS [71] and XPS. The XRD was used to determine crystal, size of the particle and shape. The XPS and EDS aimed to confirm the elements that possible to be present. The FTIR analysis has been used to investigate the chemical groups which is constituted the structure of the composites [74, 75], as shows in table 7.

The morphology of the HA/Col composites was determined by using TEM, AFM and SEM. These instruments were used to identify the homogeneous of the surface sizes and shapes of the particles. The biological properties of the composites were determined by using many assays such as antibacterial, anticancer, degradation, swelling ratio and cell attachment. The results of the previous studies showed good ability for the composites to be used as osteoconductivity and biocompatibility [69, 72]; table 7 shows more details about them.

Table 6: Cytotoxicity and cell attachment of HA/Col composites

No	Composites	Test	Cells type	Results	Application	Ref.
1	HA/Col/Zn	Flow cytometry analysis	HeLa cell lines	More than 95%.	Bone regeneration	[49]
2	Nano-HA/Col	Cytotoxicity (MTT)	Fibroblast L929 cell line	85-100 %	Cytocompatibility	[66]
3	HA/Col	Cell attachment, *In vitro* cytotoxicity	Murine L929 cells	75% of the cells attached with the composites.	Bone graft	[67]
4	Nano-HA/Col	Cell Counting Kit-8 (CCK8), flow cytometry, Cell adhesion	MC3T3-E1	80-100 % cell viability after 3 d.	Osteogenesis	[68]
5	HA/Col/chitosan	Cytotoxicity tests, attachment cells	Mesenchymal stem cells	The concentration less than 1 mg doesn't has any toxic. More than 90% of cells were attached to the composites.	Restoration of maxillofacial mandible bone	[69]
6	Nano-HA/Col/chitosan	Cytotoxicity (CCK-8 assay)	MC3T3-E1	The MC3T3-E1 cells were grow normally during the 7 d that gave advantage to be non-toxic materials.	Biocompatibility and Osteoblast	[70]
7	HA/Col/calcium phosphate (CaP)	MTT	hMSCs	The MTT assay for the composites didn't show any toxic. However, the present of CaP did not effect on the final products.	Tissue engineering	[71]
8	HA/col/pectin	MTT assay	MEF-WT cells	The viability of the MEF-WT cells after 7 d were from 83-93%, the results have been shown there is not any toxic for the prepared composites	Bone replacement	[72]
9	HA/Col	Cell attachment	MG-63 cells	The attachment study for the HA/Col composites was investigated for duration time from 1-7 d, the results have been shown good attached to the cell in the SEM image.	Bone tissue engineering	[73]
10	HA/Col	Cell attachment	Mesenchymal stem cells	SEM image shown excellent attachment for the cells with the components of the composite	Bone regeneration	[74]

Table 7: Characterization techniques of HA/Col composites

No	Composites	Chemical composition	Morphology	Biological/Medical/Application	Ref.
1	HA/Col	EDS and XRD were used to determine the chemical composition of the composites. The EDS shows to present of an element, Ca, P, O, Cu, and Al. The ratio of Ca/P was 1.5. XRD analysis conform to present all standard peaks of functional groups for HA and Col.	Study of the morphology has been done by using FESEM, the SEM image showed a homogeneous surface with a rod-like shape for the composites.	Bone tissue engineering	[75]
2	HA/Col	XRD and FTIR were used to investigate the chemical composition of HA/Col composites. XRD was conformed to present of functional groups of HA and col. The FTIR shows major peaks at 3000 cm^{-1} belong to Amide I group. The Po_3 and OH were conformed to present as well.	This study did not mentioned for the morphology tests.	MTT assay have been used to determine the cytotoxicity of the prepared composites; results showed 100% percentage of the cell to present after 24h cultured. Osteoconductivity And Biocompatibility have been confirmed.	[42]
3	HA/Col	XRD and FTIR were used to conform all functional groups of HA and Col. The results showed to present for all major groups that was correspond to HA and Col.	SEM has used to determine the morphology. The SEM image showed very well distributing for HA in the matrix of Col. The shape of composites have been confirmed as a plate-like shape.	In the biology part the author mentioned to study of swelling and degradation ratios. The results show swilling ratios were from 250% to 650%, and the stability of the composites after 7 d was 80%, while it's degraded to 50% after 28 d.	[76]
4	HA/Col	XRD showed crystal structure for the composite according to sharp peaks of XRD spectrum. FTIR showed all functional groups of HA and Col.	TEM and SEM were used to conform the morphology of the composites. Results shows nano size from 200-400 nm with the a homogeneous surface	Degradation study of the prepared composites showed slow degraded.	[77]
5	HA/Col	FTIR, EDS, and XRD were used to analysis the chemical structure of the composites. The results have been shown the chemical groups of the HA and Col in the FTIR, while the XRD showed the major peaks corresponding for both of them.	The SEM image showed spherical shape for the HA particles and very well distribution in the matrix of Col.	The cell attachment with MC3T3-E1 cells, the SEM images showed very well attached to the cell with composites.	[78]
6	HA/Col/chitosan/carbon	XRD, FTIR, and EDS aimed to analysis the chemical structure of the composites.	SEM image showed good pore size with high porosity (98 ± 0.15 to 95.7 ± 0.1%).	The MTT assay used to analysis the cytotoxicity of prepared composites. The results showed non-toxic for the composites.	[79]

The applications of HA/Col composites

The micro and Nano HA/col scaffold developed for the applications of bone tissue engineering due to the pure Col showed unwanted foreign body reactions [79, 80, 81]. HA has been developed from tri-calcium phosphate (TCP) cement by modifying the particle size of the starting cement powder, which is then precipitated in the solution of Col. The emulsification technique has been used to fabricate the micro-carriers of the ceramic slurry. The material has shown a synergistic effect that leads to enhanced differentiation and proliferation of cells. The scaffold of HA/Col that has been developed to be used is coated with titanium (Ti), then implants to improve the osseointegration [82]. The group that was coated had been created (Ti–6Al–4V) using plasma technique spraying with HA, and was dropped into the collagen solution. The Co/HA scaffold has been implanted in the muscles of rabbits and has shown high improved osteogenesis [83, 84].

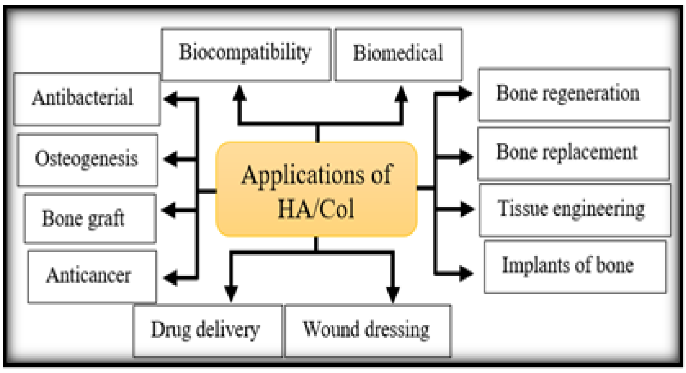

Fig. 3: Applications of HA/Col composites (Source: author)

Fig. 3 shows examples for the applications which have been mentioned in the previous studies. Among them, there were many applications that have been mentioned, such as bone regeneration [49], bone graft [67], osteogenesis [70] and biocompatibility [58], etc. in conclusion, the HA/Col composites possesses unique properties that enable it to be used in wide fields such as medical, biological and industrial fields.

Sources of HA/Col composites

The composites of HA/Col were fabricated for the defects of bone, and Col was obtained from different sources such as porcine dermal [85], bovine serosa [86], calf hide [87], rat tails [89], bovine skin, horse tendon [88, 90], while the most of HA was from natural [65] and synthetic [91] sources.

Table 8: Shows the sources of Col and HA from different composite formulation

The scaffolds	HA sources	Collagen sources	Form of scaffolds	Ref
HA/Col nanocomposite	Synthetics	porcine dermal	Paste	[92]
HA/Col	Synthetics	bovine serosa	Solid	[93]
HA/Col	Synthetics	bovine femur	Spongy	[94]
HA/Col	Synthetics	calf hide	Fiber	[95]
HA/Col	Synthetics	bovine skin	Solid	[96]
HA/Col	Synthetics	calf skin	Solid	[97]
HA/Col	Synthetics	mineralized blend	cylindrical shape	[98]
HA/Col	Synthetics	bovine tendon	Solid	[99]
HA/Col	Synthetics	Bovine	Solid	[100]
Col/HA/pectin	Synthetics	Rabbit skin	cylindrical	[101]
Col/HA Nano composites	Synthetics	calf skin	Fiber	[102]
Col/HA	Synthetics	rat tails	Hydrogel	[103]
Col/HA	Synthetics	bovine tendon	lyophilized	[105]
Col/HA	Synthetics	rat tail tendons	Solid	[106]
Col/HA	Synthetics	rat tail tendons	Solid	[107]
HA/Col/Calcium phosphate	Synthetics	bovine skin	Solid	[108]
HA/Col/polycaprolactone	Synthetics	Bovine	Fibers	[109]
HA/Col/Fe	Synthetics	horse tendon	Solid	[110]
Han/Col	Nano-powder	tail tendons rats	lyophilized	[111]
HA/Col	Micro-powder	Rat	Gel	[112]
HA/Col	Synthetics	tail tendon	Solid	[113]
Col/HA/Cisplatin	$Ca(NO_3)_2.4H_2O$, and $NH_4H_2PO_4$	calf hide	Solid	[114]
Col/HA	$Ca(NO_3)_2.4H_2O$, and $NH_4H_2PO_4$	calf hide	Solid	[115]
Col/HA	Synthetics	bovine tendon	Solid	[116]
Col/HA/PLCL	Sigma–Aldrich	Sigma–Aldrich	Solid	[117]
PVA-Col-HA	Berkeley, CA, USA	rat tail	Fibers	[118]
Col/HA Nanocomposite	$Ca(NO_3)_2.4H_2O$, and $NH_4H_2PO_4$	Porcine	Solid	[119]
Col/HA	Synthetics	calf skin	lyophilized	[120]
Col/HA/PVA	Synthetics	rat-tail	Hydrogel	[121]
Col/HA/hyaluronic acid	$Ca(NO_3)_2.4H_2O$, and $NH_4H_2PO_4$	bovine Achilles tendon	Solid	[122]
Col/HA	Synthetics	Calf Hides	Solid	[123]
Col/HA/Chitosan	Synthetics	bovine tendon	Solid	[124]
silica/Col/HA	Synthetics	Bovine	hydrogels	[125]
Col/HA/PLCL	Synthetics	Purchase from Fisher Scientific	Solid	[126]
Col/nHA/PVA	Purchase from aap Implantate AG, Germany	Rat tail	Fibers	[127]
`Col\HA\ PLLA	purchased from PURAC	bovine tendon	Solid	[128]
Col/HA nano/Chitosan composite	Synthetics	bovine dermis	Fibers	[129]
Col/HA	Plasma Biota Limited	Fetal calf skin	Solid	[130]
Col/HA	Synthesized	calf hide	Solid	[131]
Col/HA	Sigma–Aldrich	tail tendons of rats	Spongy	[132]
Col/HA	Synthesized	calf hides	Solid	[133]
Col/HA	purchased from Merck	bull skin	Gel	[34]

CONCLUSION

HA/Col composites were investigated as a functional biomaterial, which was used in many medical applications; the composites that were mentioned in this section were fabricated from various sources. The previous studies demonstrated for many sources for the HA and Col; the wide source for hydroxyapatite was synthetic, while the col was isolated from an animal source. In conclusion, Accordingly, previous studies have been documented to prepare composites of hydroxyapatite/collagen (HA/Col) from synthetic and natural sources. The majority of these studies were using hydroxyapatite from synthetic sources of chemicals that may cause toxicity when applied in the future. Col was used from natural sources such as porcine dermal, bovine skin, rat tails and bovine tendon. However, the recent problem of animal diseases such as Hyaline Membrane Disease (HMD) and mad cow disease made animal-based HA not a good alternative of HA. A lot of HA/Col composites were used in tissue engineering applications due to the higher properties that can be obtained from them. Despite the exceptional and harmonic properties of these substances and the way they are synthesized as compounds for the medical and biological fields of many prosthetic processes to enhance the growth and cohesion of the bones, many operations have failed due to bacterial infection.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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