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Review on the fish collagen-based scaffolds in wound healing and tissue engineering

Gomathy Meganathan [1], Balamuralikrishnan Balasubramanian [2], Arun Meyyazhagan [1], John Paul [3], Aditi Chaudhary [1], Manikantan Pappuswamy [1,*]

[1]Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru-560029, India
[2]Department of Food Science and Biotechnology, College of Life Sciences, Sejong University, Seoul. South Korea
[3]Department of Zoology, St. Joseph's University, 36 Lalbagh Road, Bengaluru, Karnataka, India

ABSTRACT: Collagen from marine organisms is an emerging source in tissue engineering, an alternative to bovine and porcine collagen. Despite the positive results on wound healing from fish collagen scaffolds, the comprehension and advancements in its properties are limited. Given this context, this study aimed to carry out a systematic review to examine the effects of collagen scaffolds on different models of experimental skin wounds, the advantages and its limitations. The search was conducted according to the orientations of Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) and the MeSH (Medical Subject Healings) terms used were "Fish Collagen" AND "Wound Healing" AND "Scaffold". 36 articles in total were sorted out from the databases of Google Scholar and PubMed. After the analysis, the current review covers 10 articles from the beginning of 2017 to March 2023. The results are mainly focused on the different methods of collagen extraction, preparation of scaffolds and its treatment on the animal model along with its effects. To infer, this current review states that, despite the positive effects of collagen on tissue repair and regeneration, no product is available for medical purposes. Thus, this review also demonstrates the huge potential for collagen in tissue engineering.

1. INTRODUCTION

Collagen belongs to the class of fibrous protein and is the most abundant polymer in animals constituting about 25-30% of protein mass in humans (Cruz et al., 2021). Collagen is classified into 28 subtypes till date and there are 5 most common types present in humans, amongst which, the most prevalent type of collagen is present in skin, cartilage and tendons is Type I (Blum, 2011). Cartilage is made up of Type II collagen, Reticulate fiber is made up of Type III collagen, Extracellular matrix (ECM) has Type IV collagen, Cell surface, hair and placenta are formed of Type V collagen (Sun et al., 2021). Type 1 is the significant and abundant type of collagen due to its ubiquitous presence in connective tissue fibers (Hulmes, 2002). The chief role of this particular collagen is to provide mechanical and organizational support to the ECM (Vogel, 2001). In the case of diabetes and aging, changes in tissue stiffness are due to the non-enzymatic cross-linking of collagen and with respect to arthritis, the fibrillar collagen confirmation is misidentified as non-self by matrix metalloproteinases mediated proteolysis of cartilage thereby leading to tissue damage (Rosloniec et al., 2001).

Collagen is made up of two α1 chain and α2 chain forming a triple helix structure and the most abundant motif in the sequence of amino is Glycine, Proline and X (where X can be any amino acid other than Glycine, hydroxyproline and Proline) (Shenoy et al., 2022). In the triple helix stand, 18 amino acids are present in one turn also the left-handed helix is formed by the molecule's three α-chains (Hofmann et al., 1978). The helical structure is created when the three chains that are distanced from one another by one residue in a right-handed manner around a central axis is supercoiled (Fraser et al., 1979). Although collagen contains hydroxyproline significantly at higher concentrations than the other proteins, this amino acid is not unique to collagen alone and is therefore employed as a particular index of collagen synthesis and concentration (Shoulders & Raines, 2009). As shown in Figure 1 (a and b), there is at least one triple-helical region in every molecule of collagen and confirmation of the same makes the helical structure extremely proteolytic resistant because, the peptide bonding with adjacent amino acids are hidden inside the molecule (Brodsky et al., 2008).

* Corresponding author.
E-mail address: manikantan.p@christuniversity.in (Manikantan Pappuswamy)

Collagens are essential for the healthy development and growth of any individual animal and humans as well. Mutations in the various collagen genes are in charge of numerous clinical diseases pertinent to the skeleton, skin, and joints (Campos et al., 2023). Collagens are crucial to the healing of wounds, i.e., platelets are activated by fibrous collagen leading to a hemostatic clog formation, granulation of tissue and control of cell migration after injury to various types of tissues (Mathew-Steiner et al., 2021). Collagen can operate as survival factors and often stop the adhering cells from dying. Other than normal growth and support to body tissues, collagen also is an inhibitor of angiogenesis. Proteolytic fragments of Type XVIII are Endostatin (Marneros & Olsen, 2005), Restin is a proteolytic fragment of type XV collagen (Mutolo et al., 2012) proteolytic fragment of Type IV Collagen such as Arrestin, Canstatin, tumstatin have been found in the extracellular matrix, which are potent endogenous inhibitors of tumorigenesis (Arakaki et al., 2021).

The extracellular matrix of the human skin tissue comprises 3 main constituents like glycosaminoglycan, proteoglycans, and fibrous protein. Of the fibrous proteins, collagen is a building block for skin, bone and cartilage (Frantz et al., 2010). As positive feedback to injury, collagen invites platelet activation and aggregation of WBC (white blood cells) thus leading to fibrin clot in the site of injury. Collagen degradation releases peptide factors responsible for the proliferation of fibroblast and growth factors responsible for re-epithelialization and angiogenesis (Mathew-Steiner et al., 2021). The type and the amount of organization in the collagen determines the elasticity and strength of the recovered wounded tissue. Accumulation of type III collagen at the wounded area marks the initial stages of wound healing, which is further modified into type I collagen (Singh et al., 2023). Lysyl oxidase enzyme enhances the initial accumulation of collagen during the granulation tissue formation, induces covalent bond linking for the final conversion and modification of collagen (Reilly & Lozano, 2021).

Collagen peptides are formed in the ribosome alongside the endoplasmic reticulum, after mRNA is translated, the formation of α1 and α2 chains begins. These peptide strands are called pre-procollagen and have the capacity of signaling amino acid sequences on their terminal ends (Dunsmore, 2006). The pre-peptides are further moved to the Rough Endoplasmic reticulum and these signaling peptides are broken down in the endoplasmic reticulum leading to the formation of pro-alpha chains. Amino acids such as lysine and proline are hydroxylated in the lumen, and certain hydroxylysine are then further glycosylated. Procollagen is synthesized in the endoplasmic reticulum with 2 strands of α1 chain and 1 strand of α2 chain.

Animals are buildup of macroscopic fibres and networks found in bone, tissue and basement membranes using a single collagen triple helical structure known as tropocollagen, which assembles in a stratified manner. Collagen is widely distributed, making the triple helix structure of recurring N-H(Gly)O=C(Xaa) with hydrogen connections, which are abundant in the animal kingdom i.e., amide-amide hydrogen bond (Jenkins et al., 2005). Based on their supramolecular organization and structure, collagens are split up into fibril-forming collagens, fibril-associated collagens, network-forming collagens, anchoring fibrils, basement membrane collagens, transmembrane collagens and other types based on the particular activity (Gelse et al., 2003). The tropocollagen of type I collagen undergoes random coiling confirmation as the tropocollagen is unstable at body temperature (Leikina et al., 2002). Furthermore, collagen must be assembled into strong macromolecular structures in order to support the torsional stress in any given dimension (Buehler, 2006).

Recent information has made it possible to regulate the structure of the collagen cartilage fibrils to an instant resolution (4 nm). The structure demonstrates the heterotypic microfibril structure of collagen fibrils in cartilage, which means that the surface of the fibril has ten evenly spaced microfibrils, while the fibril core has four equally spaced microfibrils (Holmes & Kadler, 2006). Type I collagen fibres in tendons can be up to 1 cm and 500 nm in length and diameter, respectively. The diameter and length of a single triple helical collagen structure are both about 300 nm. Clearly, the natural collagen fibril's structural dimensions need fibrillogenesis on an exceptional scale. The fact that collagen fibrils are D periodic (D = 67 nm) is their most distinguishing trait. A tropocollagen monomer's accurate length is 4.46D, resulting in 0.54D and 0.46D gaps and overlaps respectively, which causes the banded structure seen in images of collagen fibrils through transmission electron microscopy. The collagen fibril and microfibril structural models must take into account this regular overlap region and an array of gaps (Shoulders & Raines, 2009).

Hydroxylase enzyme is involved in the final modification of collagen from pro-collagen, while in case of disorders like scurvy, deficiency of Vitamin C occurs. This vitamin C acts as a cofactor for the synthesis of Hydroxylase enzyme.

The kind, quantity, and organization of collagen, which alters as the wound heals, determine the tensile strength of the recovered skin. Wound healing involves four major steps: Homeostasis, Inflammation, Proliferation and Maturation/Remodeling (as shown in Figure 2). During the initial phase of wound healing (as mentioned in Figure 2), type III collagen is synthesized, later replaced by type I collagen, one of the prominent skin collagen types (Gould, 2016). The lysyl oxidase enzyme enhances the first random deposition of collagen and promotes covalent cross-linking during the development of granulation tissue (Mathew-Steiner et al., 2021). If the wound heals without any interruptions, collagen remodeling occurs for several months, and the restored tissue gains tensile strength that is around 80–85% of that of the unrestored tissue (Fitridge & Thompson, 2011).

During healing, the major producer of collagen is fibroblast (Sorushanova et al., 2019). The biosynthesis processes of collagen that produce fibrils have been the subject of most investigation and involve a number of complex phases in the spatial and temporal coordination of many biochemical

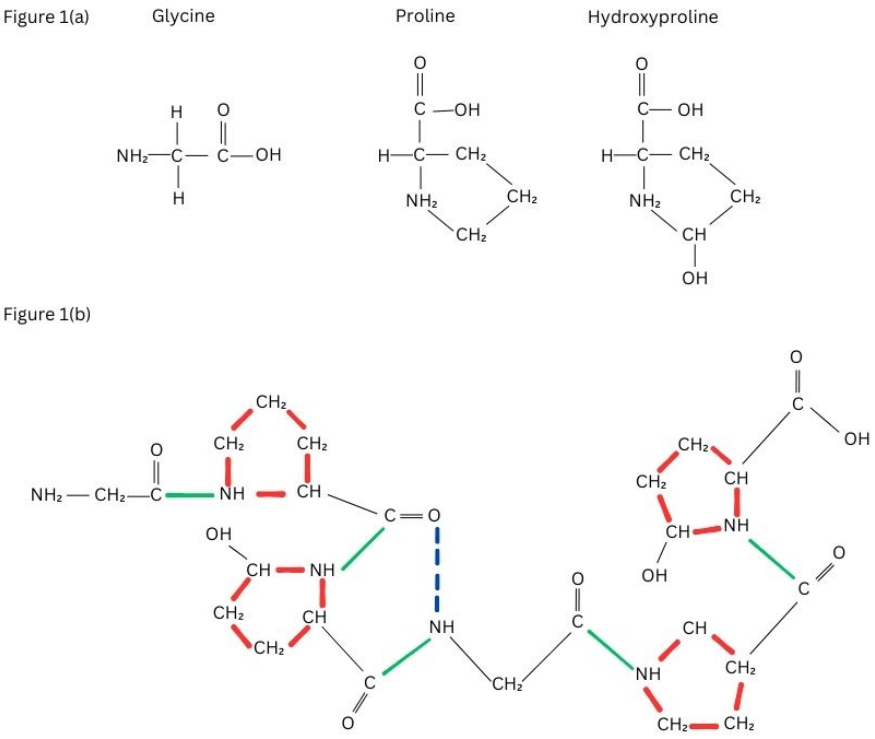

Figure 1. Figure 1a. The common motif of collagen– Glycine, Proline, Hydroxyproline. Figure 1b. Triple helix structure of collagen. The side chains of proline and Hydroxyproline (shown in red) are on the outside of the molecules and the bonds (shown in green) are the peptide bonds between the amino acids present inside the triple helix. The hydrogen bonds shown in blue dotted lines stabilize the triple helix.

activities (X. Liu et al., 2019). After transcription, the endoplasmic reticulum modifies the nascent/pre-pro-collagen through post-translational modification, removing signal peptides from the N-terminus to create pro-collagen. The triple helical collagen structure is created as a result of the glycosylation and hydroxylation of amino acid (Owczarzy et al., 2020). The triple helical pro-collagen framework is further supported by chaperone proteins and packaged for processing and maturation of the pro-collagen takes place in the Golgi apparatus.

After being put together into secretory vesicles, the pro-collagen is subsequently extruded into the extracellular space, where it undergoes enzymatic modification to become tropocollagen (Revell et al., 2021). The cross-linking process includes disulfide bonds, cross-links (mature) are produced through the lysyl oxidase pathway. Advanced glycation and transglutaminase cross-links, end product-mediated cross-links, define the mechanical property of collagen, i.e., elasticity and reversible deformation. A multi-layered hierarchical structure is created by minute differences in cross-linking that depend on the type of collagen and the uniqueness of the tissue (X. Liu et al., 2019).

Since the 1990's, after the emergence of the term tissue engineering, in order to contribute to structural stability and a favorable domain for cellular regeneration, scaffolds based on biomaterial have been a study of interest, functionally "imitating" the original tissue (Zhong et al., 2010). The selection of an appropriate polymer is an important step in the preparation of scaffolds. Collagen, Hyaluronic acid, chitosan, gelatin and fibrin, and silk are a few examples of biopolymers that have been applied as scaffolds. Scaffolds are made by synthetic or natural biomaterials in various forms such as nanofibers, sponges, 3D structures hydrogen mats, foams, nanogels and membranes (Jafari et al., 2017). Wound healing scaffolds are created using a variety of techniques and a few of them are extrusion, molding, freeze-drying and electrospinning. Availability for use, lower cost, flexibility, and scalability, the electrospinning process continues to be among the most popular of these (Ramalingam et al., 2019).

The following essential components should be present in a scaffold used for wound healing applications: suitable mechanical and physical properties, proliferation, and differentiation of high physiological backdrop to enable cell–to-cell adhesion (Negut et al., 2020). The scaffold should ideally be both biocompatible and biodegradable, rate of degradation that coincides with the time required for the wound to heal. A moist environment must be maintained by the scaffold in order to offer vital cues for cellular adhesion, migration, and growth to trigger angiogenesis, to accelerate the development of granulation tissue followed by re-epithelialization (Talikowska et al., 2019).

Porcine-based and bovine-based collagen application has been forbidden due to religious constraints, dietary regimes and specific needs. Additionally, foot-and-mouth disease, transmissible spongiform encephalopathy and bovine spongiform encephalopathy (Kumagai et al., 2019) that occurred over the

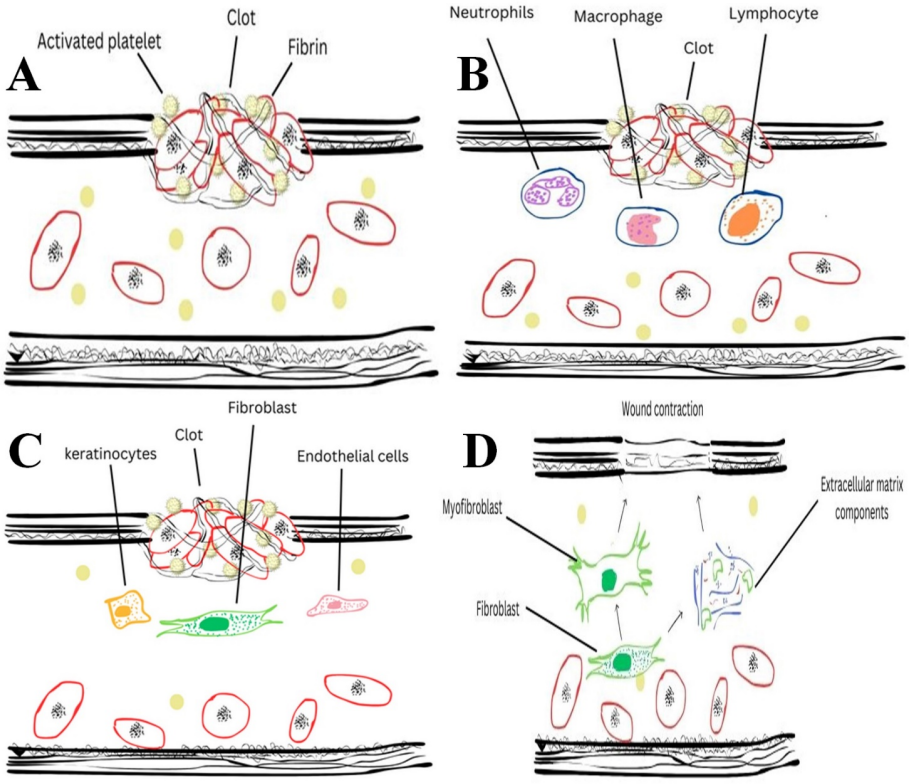

Figure 2. Indicates the stages of wound healing. A), Homeostasis, B), Inflammatory Phase, C) Proliferative Phase, (D) Remodeling Phase.

past few years around the world, primarily in Asia and the United Kingdom, a wider segment of the population began to express concern about the use of products derived from cows (Coppola et al., 2020). Because they may serve as a means of spreading these diseases, the usage of items generated from cattle is greatly restricted (regulations CE n. 999/2001 and UE n. 142/2011). Therefore, there is a need for an alternative source of collagen.

In this light, the last 20 years there has been a significant increase in interest in marine sources (such as fish, Mollusca and sponges) as secure, prominent and alternative sources for the production of collagen. Marine collagen is free of religious objections and exhibits a naturally decreased threat of communicable infections. Additionally, the fishing industry generates an array of waste or byproducts (such as skin, heads, fins, bones, intestines, and scales) per year that make up roughly 85% of the average catch weight and cause serious damage to the environment (Pal & Suresh, 2016). Fish byproducts are therefore valuable for collagen production, making fish collagen both profitable and cost-effective while still being environmentally beneficial (D. Liu et al., 2015).

Despite the constructive outcome of fish-based collagen, there is still only a limited understanding of the use of fish collagen into wound healing. The idea of this review article is, to sum up the recent use of fish collagen in preparation of wound healing scaffolds. The extraction of collagen, preparation of scaffold and linkages in preparation of scaffolds, merits and demerits of use of fish collagen are dealt with in detail. In this context, the purpose of the review was to perform a systematic literature survey to explore the effect of fish-based collagen tissue wound healing in experimental models with reference to in-vivo studies.

2. REVIEW PROTOCOL

Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) guideline was followed to perform the review analysis. The review was performed during the month of April and May 2023 using Google Scholar and PubMed databases. The literature review was performed according to the directions of PRISMA. The MeSH terms were defined as “Fish Collagen” AND “Wound Healing” AND “Scaffold”. Further, other synonyms and advanced searches were used for detailed understanding.

3. ELIGIBILITY CRITERIA

3.1. Inclusion Criteria

1. In-vivo experiment
2. In-vivo models like skin injury and burns treated with collagen scaffolds
3. Manuscripts published and written in English during 2017 and later

3.2. Exclusion Criteria

1. In-vitro studies, reviews, clinical trials, and case reports Collagen other than fish source

2. Lack of description of the skin wound treatment and preparation of scaffolds
3. Animal models associated with systematic diseases like diabetes or osteoporosis

3.3. Data extraction

The important variables examined here are scaffold preparation and wound healing. Moreover, other variables like biomaterial type, animal sex, weight, age, wound size, wound type, implantation time, extraction protocol of collagen, protocol of scaffold preparation, cross-linking method, in-vivo analysis performed and the overall results were analyzed.

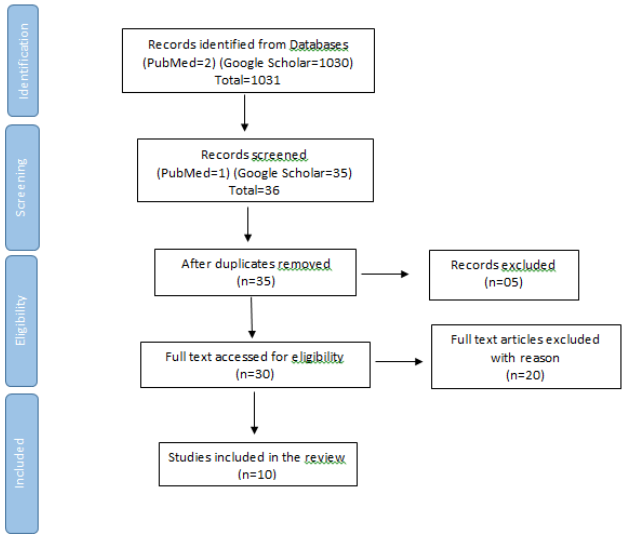

Figure 3. Indicates the PRISMA flow diagram forthe systematic review.

4. RESULTS AND DISCUSSION

The PRISMA flow diagram represents the search scheme of the current review (Figure 3). A total of 36 research papers were retrieved from the Google Scholar and PubMed databases. The duplicated articles were removed (n=1) Thirty-five full research articles were evaluated for the inclusion criteria out of which, 25 articles were removed based on exclusion criteria. Eventually, 10 research articles were included and analyzed in the current review.

DTSCS- Dialyzed tilapia skin collagen sponges; STSCS-self-assembled tilapia skin collagensponges;EDC/NHS-N-hydroxysuccinimide,1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; FSC- Fish scale derived collagen

From Table 1, it is evident that collagen was used as the biomaterial and certain studies have extracted collagen from a specified fish scale while few other studies have not specified the species of the fish collagen. Of the 10 studies included in this review, 4 studies have extracted collagen from tilapia (Sun et al., 2021; Wang et al., 2021; Zhou et al., 2017), 1 study extracted collagen from Panaeolus olivaceus (Chandika et al., 2021), 1 study extracted collagen from Cyprinus carpio (Hartinger et al., 2020) 1 study extracted collagen from Ctenopharyngodon idellus (Shi et al., 2020), 1 study extracted collagen from Scyliorhinus canicula (Lahmar et al., 2022) 2 studies have not specified the fish species because both these studies obtained fish collagen from laboratories.

depicts the variables assessed in the included studies, species/strain, age, weight, skin wound size, animal sex, implantation period and protocol of treatment. The animal models used for these are ICR mice (Chandika et al., 2021), Wistar rats (Hartinger et al., 2020) Sprague- Dawley rats (Hu et al., 2021; Sun et al., 2021; Wang et al., 2021; Zhou et al., 2017) New Zealand White rabbits (Shi et al., 2020), BALB/c albino rats (Lahmar et al., 2022) Mice (Li et al., 2018). Five Studies used male animals (Chandika et al., 2021; Hu et al., 2021; Sun et al., 2021; Zhou et al., 2017) remaining five studies have not specified the sex of the animal model. The age of the animal varied from 6-10 weeks (Chandika et al., 2021; Hu et al., 2021; Lahmar et al., 2022; Wang et al., 2021; Zhou et al., 2017). However, the remaining five studies have not reported the age of the animal model. The weight of the animal varied mostly between 200-380g in the nine studies reported except for one study where the weight of the mice reported was 30g (Chandika et al., 2021). The skin wounds were of 3 different types, five studies performed excision wounds (Chandika et al., 2021; Hu et al., 2021; Lahmar et al., 2022; Zhou et al., 2017), three studies performed Incision wounds (Hartinger et al., 2020; Li et al., 2018; Wang et al., 2021) and two studies performed on burn wounds (Shi et al., 2020; Sun et al., 2021). The wound size varied from 0.5 to 2 cm.

Most of the studies used one-time treatment and there was no change of dressing or scaffold. The scaffolds were inserted and sutured (Hartinger et al., 2020; Hou et al., 2020; Li et al., 2018; Shi et al., 2020; Sun et al., 2021; Wang et al., 2021). One study changed the dressing at regular intervals of two days (Lahmar et al., 2022). The remaining three studies used sponges for treatment (Hu et al., 2021; Sun et al., 2021; Zhou et al., 2017). Table 2 shows the details about the extraction protocol of collagen, membrane manufacturing technique, physical and morphological characteristics of scaffolds of the prepared scaffolds and sponges. Of all the extraction protocols used, extraction using acetic acid is the most common technique (Chandika et al., 2021; Hartinger et al., 2020; Lahmar et al., 2022; Li et al., 2018; Shi et al., 2020; Wang et al., 2021). Some of the studies were based on the obtained fish collagen commercially (Hou et al., 2020; Hu et al., 2021; Sun et al., 2021; Zhou et al., 2017). Five studies used N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride as crosslinking agents (Hartinger et al., 2020; Hou et al., 2020; Hu et al., 2021; Sun et al., 2021). Two studies used glutaraldehyde as a cross-linking agent (Shi et al., 2020; Zhou et al., 2017). Three studies have not specified about the cross-linking agents used (Lahmar et al., 2022; Li et al., 2018; Wang et al., 2021).

Table 1
List of studies using collagen from marine sources, the types of animal model used, the type of woundcreated and protocol of wound dressing performed on animal models

Author	Strain or Strain	Animal sex	Mean age	weight	Biomaterial's origin	skin wound type	Implantation period	Skin wound size	Protocol of treatment
Chandika et al. (2021)	ICR mice	Male	8 week old	30g	Collagen from P. olivaceus	Excision wounds	21 days	1.5 x 1.5 cm^2	The Fish collagen and Porcine collagen bilayer scaffold was applied to the wound so that the L2 layer touched the wound area, and it was then sutured.
Hartinger et al. (2020)	Wistar rats	NOT SPECI-FIED	Adult	380g	Type 1 collagen from C.s carpio	Incision wound	8 days	NOT SPECI-FIED	The formulation was inserted after wound creation using a catheter
Wang et al. (2021)	Sprague–Dawley rats	NOT SPECI-FIED	6-7 weeks	200-250g	Purified Tilapia skin, Dialyzed tilapia skin collagen sponges (DTSCS) and self-assembled tilapia skin collagen sponges (STSCS)	Incision wound	14 days	1cmx1cm	After being sterilized by irradiation, DTSCS and STSCS samples were affixed using surgical sutures to the wound. The other two wounds were patched with gauze and bovine collagen sponge .
Sun et al. (2021)	Sprague-Dawley (SD) rats	Male	NOT SPECIFIED	200-220g	Type I collagen from Nile tilapia skin	Scald wound	28 days	2 cm	Dressing of wounds were done with radiation-sterilized bi-layered composite dressing samples (Inner layer: noncrosslinked collagen sponge or EDC/NHS cross-linked collagen sponge; outer layer: medical spun-laced nonwoven covered with 3% chitosan solution with 30% glycerin)

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Table 1 continued

(Hou et al., 2020)	Sprague-Dawley (SD) rats	Male	8-10 weeks	180-220g	Species Not specified, marine collagen	Full thickness excision wound	14 days	2cm x 1cm	The wound area was treated with scaffold. After implantation, an air-permeable covering was placed over the dorsal area to stop the scaffolds from slipping out of the wound.
Shi et al. (2020)	New Zealand White rabbits	NOT SPECI-FIED	NOT SPECIFIED	2.5 kg	Collagen from C. idellus	Burn wounds	28 days	2x2cm	The wounds on rabbit were covered with five FSC scaffolds that had been soaked in saline. All scaffolds were securely secured to the rabbit's body using an elastic adhesive bandage and a commercially available breathable non-woven application.
Hu et al. (2021)	Sprague-Dawley	male	7-8 weeks	NOT SPECIFIED	Fish Collagen, species not specified	Full thickness excision wound	4 weeks	6mm	Electrospun scaffolds that were randomly aligned, and latticed were cut to a circle (8 mm in diameter) and positioned below the wound.To keep the wound region safe, Tegaderm film were applied over the wound. In order to lessen the contraction of the dorsal muscle, annular silicone splints were next sewn to the film and the skin beneath the wound.

Continued on next page

Table 1 continued

Lahmar et al. (2022)	BALB/c albino rats	NOT SPECIFIED	NOT SPECIFED	NOT SPECIFIED	S. canicula fish skin collagen,	Excision wound	15 days	6mm	Different sponges containing collagen extract were used to cover the wounds on the mouse groups that had been treated. In order to remove undesirable cells from the wound bed, sponges were changed every 2nd day for 15 days.
Li et al. (2018)	Mice	NOT SPECIFIED	NOT SPECIFED	NOT SPECIFIED	Tilapia collagen microfibrous matrix scaffold	Incision wounds	30 days	0.5cm	One non-absorbable nylon suture stitch was used to affix the implant to the inner dorsal wall. The mice were then routinely provided with enough food and water, their entire bodies were cleaned, and the wounds were checked regularly.
Zhou et al. (2017)	Sprague-Dawley (SD) rats	Male	6-8 weeks	200-250g	Tilapia skin collagen	Full thickness excision wound	15 days	1.8cm	Collagen/bioactive glass nanofibers was used as wound dressing for the test group. The control group received no materials at all. The dressings were fastened to the wounds with the use of adhesive Tegaderm (3M) polyurethane films.

Table 2
Presents the collagen extraction protocol, the crosslinkers used, and scaffold preparation technique.

Author	Collagen extraction protocol	Crosslinking	Membrane Manufacturing protocol	Physical and morphological characteristics
Chandika et al. (2021)	The washed skin was soaked in 0.1M NaOH for 2 days. After thoroughly washing in ice-cold distilled water. Acetone was used to defeat the skin, and distilled water was used to thoroughly clean it. The skin was initially immersed in citric acid for 48 hours before being moved to 0.5M acetic acid. Futher it was centrifuged at 18000 rpm for 1.5h, the suspened collagen was colled and salted with NaCl. The precipitate was collected after 12h and centrifuged at 18000rpm for 30 min. The pellets were resuspended in acetic acid at 4°C for 5 days. The pellets were freeze dried at -80°C	N-hydroxysuccinimide (NHS) (200 nM) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride (EDC)(200mM) 2 hours in pure ethanol at room temperature, followed by 200 nM of 10% (w/v) COS in pure ethanol.	Collagen 10% (w/v) and PCL 10% (w/v) solutions were produced after being dissolved in 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP). Different mass ratios of collagen and PCL were then combined (90:10, 50:50, and 10:90). With various working conditions, electrospinning was carried out using a syringe pump, 24-gauge needle, stainless-steel spinning collector, and high voltage power source are included. Then, by altering the mass ratios of the two layers. The thickness ratio of the 1:4 (L1:L2) bilayer fiber meshes was used to manufacture them.	Fish collagen bilayer nanofibrous three-dimensional scaffolds was morphologically homogeneous and had compositionally similar architecture to that of Extracelluar matrix
Hartinger et al. (2020)	Preliminary extraction of collagen was done using phosphate and citric buffer. Collagen extraction was done by soaking for 24hrs in 0.5M acetic acid and further centrifuged. 0.1M acetic acid was used for dialyzing the liquid portion in the dialysis tubing. Further it was stored at -15°C and lyophilized at -105°C.	Cross-linking with ethanol solution comprising NHS (N-hydroxysuccinimide) and EDC(N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) at a molar ratio of 4:1 increased the stability of the sandwich and homogeneous collagen sponge.	The process used to create an aqueous collagen dispersion involved swelling collagen in deionized water for three hours at ten degrees, homogenising it with a disintegrator for ten minutes, letting it sit for twenty minutes at room temperature, then homogenising it again for five minutes. Collagen dispersion concentration in the core was 5 wt%, whereas it was only 1 wt% in the surrounding layers. The resulting 1 wt% dispersion was applied to the core in separate containers, impregnated, and allowed to sit at room temperature for 3 hours before being frozen at -80°C for 6 hours and lyophilized. The resulting 5 weight percent dispersion was then put into containers, frozen at -80 degrees Celsius for six hours, and lyophilized.	The intersection of the hard core's lower porosity and the excessively porous periphery was seen in the SEM image. The interface between the layers with different porosities shows no signs of delamination.

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Table 2 continued

Wang et al. (2021)	To create Dialysed tilapia skin collagen sponges (DTSCS), in acetic acid that had been dialyzed to a pH of about 7, purified tilapia skin collagen was dissolved. In order to create collagen microfibers, collagen molecules self-assembled, with longer, thicker creating microfibers between the microfibers. Collagen's acidic solution was combined with Phosphate buffer solution, and the pH was raised to neutral. After standing for an hour, a collagen gel that had self-assembled itself was produced. To make self-assembled tilapia skin collagen sponges (STSCS), collagen microfibers were well stirred, collected in a sieve, resuspended, and then freeze-dried.	No cross-linking agents were used	To create DTSCS, in acetic acid, which was dialyzed to a pH of nearly 7, purified tilapia skin collagen was dissolved.Self-assembly was employed in the preparation of STSCS. Acidic collagen solution was combined with PBS, and the pH was raised to 7. After standing for an hour, a collagen gel that had self-assembled itself was produced. To make STSCS, collagen microfibers were well stirred, was filtered using a sieve, resuspended in water, and then freeze-dried.	Both species of sponge showed unique reticular structures and porosities. Cell growth and biomedical applications seemed to be more suited to the structure and pore size of STSCS.

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Table 2 continued

Sun et al. (2021)	Commercially available type I collagen from Nile tilapia fish was produced in laboratories.	N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide/N-Hydroxy succinimide (EDC/NHS) cross-linked collagen sponges	Bi-layered composite wound dressings used both commercial Beiling collagen sponges or EDC/NHS cross-linked sponges were used for the inner layer. Release paper, acrylic resin glue with a strong viscosity, and medical spun-laced nonwoven cut into 5x5 cm sizes with microcellular structure and good air permeability made up the substrate for the outer layer. With or without 30% (solute mass) of glycerin, medical-grade chitosan was produced in concentrations of 1%, 2%, 3%, 4%, and 5%, and carboxymethyl chitosan in concentrations of 1%, 2%, 3%, 4%, and 5%, respectively. The medical spun-laced nonwoven that served as the top layer of wound dressings was either individually coated with a triangle coating rod in just one direction or it was soaked for 10 minutes in a beaker of the chitosan or carboxymethyl chitosan solutions mentioned above, followed by drying in an electric-heated, constant-temperature oven.	The coating preparation process produced a more homogeneous surface than soaking. To further investigate chitosan's colour and consistency, 1%, 2%, and 3% of chitosan solutions with or without glycerin in the form of coating and 1% of chitosan solution with glycerin in the form of soaking might be used.
Hou et al. (2020)	Obtained marine collagen commercially	ColNAC composite with polyamide (col NAC -EDC/NHS solution)	To make a 14% collagen solution. 0.05 mol/l acetic acid was used to dissolve the collagen. The collagen solution was placed in a petri dish with a diameter of 60 mm and a height of 2-3 mm. The petri dish was filled to a similar height with the collagen solution before the processed PA nanofibers (3 cm * 3 cm square) were placed on top. After being lyophilized at 40 kPa and 20 °C, the PA-Col scaffolds were recovered and placed in Col -EDC/NHS solution for crosslinking.	Showed a much higher tensile strength.

Continued on next page

Table 2 continued

Shi et al. (2020)	The scales were cut up into little pieces; to decalcify it was immersed for 24 hours in 1 M acetic acid. Acid-soluble collagen from grass carp was obtained after filtration and centrifugation at 8000 rpm for 5 minutes. For 24 hours at room temperature, the filtrate was combined with 0.01 M acetic acid solutions containing 5% pepsin. Pepsin-soluble collagen was obtained after filtration and centrifugation at 8000 rpm for five minutes. Solutions of pepsin, acid-extracted collagen, and an equivalent volume of a 1M sodium chloride solution were mixed. After 4 hours, the mixture was centrifuged at 8000 rpm for 5 minutes to produce collagen gel. The collagen gel was added to 0.5 M acetic acid solutions and stirred until dissolved. The solution was dialyzed for 24 hours against 0.1 M acetic acid and then for 24 hours against ultrapure water to produce purified collagen gel. The collagen gel was freeze-dried so that it would last and be ready for future experiments.	sponge was crosslinked in 0.25% glutaraldehyde solution	For the purpose of producing a stable solution with 1.5% concentration, PSC was added to 1mM HCl and agitated at 1000X for one hour. The liquid was promptly decaned into the prepared mould, and then placed in an 80°F refrigerator to immediately freeze-dry. The silicone sheet with several 20mm 20mm 5mm cuboids were used as moulds. The dried sponge was repeatedly cleaned with ultrapure water after being crosslinked in 0.25% glutaraldehyde solution for 24 hours. The sponge was freeze-dried to create the PSC scaffolds. Similar to how PSC scaffolds were prepared, FSC scaffolds also were prepared.	The porosity was high 98±0.5%. Had a rough outer surface.

Continued on next page

Table 2 continued

Hu et al. (2021)	Fish collagen was commercially obtained and not extracted from a particular fish species	To cross-link Fish collagen (FC), the membranes were soaked in a solution of 50 mM 1-ethyl-3-(3-dimehylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide in 10 mM ethanol for 24 hours at 4°C.	Scaffolds were fabricated using electrospinning method.PLGA (20% by weight) and FC (2% by weight) To dissolve the solutions, 1,1,1,3,3,3-hexafluoro-2-propanol solvent was added to a plastic syringe with a flat-tipped 21-gauge (G) needle (inner diameter, 0.5 mm). A high voltage of 7 kV and a distance of 16 cm separated the needle and the collector. The electrostatically charged fibre for the random group was launched towards the grounded collector in a strong electric field. An electroconductive wire net resembling a chess board was utilised as the collector for latticed group. The collecting drum's rotating speed was set for the aligned group at 2800 rpm. For 24 hours at 4°C, the membranes were submerged in a solution of 50 mM 1-ethyl-3-(3-dimehylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide and ethanol of 10mM to cross-link FC.	Showed a higher tensile strength.
Lahmar et al. (2022)	The skin of fish was treated with 0.5M NaOH for 24 h and cleaned with distilled water and cut into small pieces weighing about 5 g. The sliced skin was combined for 6 hours at 4°C with 0.1 M NaOH. The skin was treated and suspended in 0.5M acetic acid for 48 hours after being further cleansed with distilled water. To precipitate the collagen, the resulting solution was centrifuged at 5000 rpm for 1h at 4°C. The pellets formed after centrifuging the precipitates for 30 minutes at 4000 rpm were then dissolved in 0.5 M acetic acid for 24 hours before being freeze-dried.	NOT SPECIFIED	A plant extract with a concentration of 1 mg/mL was combined with 20% collagen, further blended for 15 minutes to create a homogeneous solution, dialyzed in deionized water, and then lyophilized, streilized by CO 60, and heated to 4 °C.	The extracted collagen showed the property of type 1 collagen with 2 alpha chains and 1 beta chain

Continued on next page

Table 2 continued

Li et al. (2018)	ASC- Extracted using 0.5 M acetic acid in a ratio of 1:50 (Sample/solution) for 48 hours. Further centrifuged at 1000rpm for 30 mins and stored at 4°C for further use. PSC-The pre-treated skin was continuously stirred for 48 hours while being dissolved in 0.5 M acetic acid with 0.1% (w/v) pepsin. Centrifuging the mixture at 10,000 g for 30 min. at 4 °C. After 48 hours using 0.5M acetic acid containing 0.1% (w/v) pepsin, and the extract was centrifuged. By using the gradient salting out technique, the pooled extracts were salted out to provide a NaCl concentration of 0.9 mol/L. To inactivate the pepsin, the supernatant was then dialyzed (50KD, 31mm) against 0.02mol/L Na2HPO4 for 12 hours, with solution changes made every 2 hours. The ASC was followed throughout the remainder of the extraction and sterilisation procedure.	NOT SPECIFIED	The PSC sample that had been lyophilized was dissolved in 0.1 M acetic acid by stirring at 4 C until the concentration reached 6.0 mg/mL. To begin the assembly procedure, collagen solution was combined with the same volume of Na-phosphate buffer (pH 6.8, 90 mM), which contained 210 mM NaCl, at 4 C in an ice bath. The resultant mixtures were then homogenized for five minutes and incubated at 37 C for six hours. After that, the pH was changed to 7.0–7.6. In the end, the matrices were lyophilized.	ASC and PSC both have amide A bands at 3340.11 cm^{-1} and 3424.96 cm^{-1}, respectively. The findings showed that ASC's NH groups participated in hydrogen bonds to a greater extent than PSC's. The amide B's 2920–2944 cm^{-1} -CH_2 symmetric stretching vibration absorption band. ASC and PSC both had amide B bands at 2927.41 cm^{-1}. The wavenumbers of the collagen's amide I, amide II, and amide III bands are all closely related to its structure.

Continued on next page

Table 2 continued

Zhou et al. (2017)	Collagen was commercially obtained from laboratory	The nanofibers were exposed to the vapor of glutaraldehyde for 24 hours before being placed in a vacuum drying oven.	Tilapia collagen was dissolved in a hexafluoropropanol solution to yield an 8% collagen solution, which was then combined with the Bioactive glass precursor solution at a volume ratio of 10:1 to make a composite solution of collagen and Bioactive glass. Using a syringe holding the polymer solution, the electro spun Collagen/Bioactive glass nanofibers were further grown at high voltage (16–18 kV). The needle could potentially be placed up to 10-15 cm from the aluminum foil collector when electrospinning at rate of 1.0 mL/h. After being crosslinked by glutaraldehyde vapor for 24 hours, the nanofibers were kept in a vacuum drying oven.	FTIR spectroscopy and the X-ray diffraction spectra of the Collagen and Bioactive glass nanofibers were not changed significantly after crosslinking. Additionally, it was found that the collagen/bioactive composite nanofibers' tensile strength could be greatly increased by the addition of an appropriate amount of bioactive glass.

PCL- Polycaprolactone;DTSCS- Dialyzed tilapia skin collagen sponges; STSCS- self-assembled tilapia skin collagen sponges; Col- Collagen; PSC- Pepsin soluble collagen; FSC- Fish scale derived collagen; PLGA- Poly (lactic-co-glycolic acid); FC- Fish collagen; ASC- Acid soluble collagen; Col/NAC: collagen (Col) with N-acetylcysteine

Freeze drying technique was the most common type of technique performed in the preparation of collagen-based scaffolds or sponges $. Four studies used the electro-spinning technique for the preparation of scaffold (Chandika et al., 2021; Hu et al., 2021; Sun et al., 2021; Zhou et al., 2017).

Pore size is one of the important parameters in scaffold preparation as these pores act as a niche for the developing cells and the higher the pore size greater the growth of the developing cells, similarly this can be achieved by the addition of collagen at a higher ratio. From the above studies, it is also clearly evident that collagen alone cannot be used for the preparation of scaffolds due to its lower melting point and tensile strength. Hence, most of the studies involve the synthetic polymer and crosslinking agents to stabilize the structure. Other parameters which determine the quality of scaffolds are biodegradability, tensile strength and water absorption capacity. Table 3 represents the details of the scaffold's pore size and of which one study didn't examine the mechanical or structural properties of the prepared scaffold (Lahmar et al., 2022). One study showed the highest pore size (Shi et al., 2020). Table 4 represents the wound healing rate in animal models and also its histological analysis. Of all the studies, one study showed a complete wound healing on day 21 (Wang et al., 2021) while all other studies showed a longer time duration (up to 30 days) for a complete wound healing. The cellular behavior in the case of wound healing is directly affected by the scaffold architecture as ECM influences specific Integrin-ligand interaction between the cells and its surrounding environment (Murphy et al., 2010). All subsequent actions inside the scaffold such as proliferation, migration, and differentiation are mediated by the initial cell attachment (Anselme, 2000). Because the cells are able to recognize small ECM changes that may have an impact on their behavior, pore size can have an impact on a number of biological scaffolding parameters, including cell attachment, infiltration, and vascularization (Boyan et al., 1996; Harley et al., 2008). As a result, a balance must be maintained between the proper pore size for cell movement and a specific surface area for cell adhesion (Karageorgiou & Kaplan, 2005).

4.1. Pore Size

By modifying the molecular weight of the employed polymer molecular-weight distribution and architecture in the solution, the properties of the polymer solution like viscosity, conductivity and surface tension (Grenier et al., 2019; Haugh et al., 2010) the pore size can be adjusted. Process parameters can also be altered in the electrospinning technique for obtaining the desired pore size are the electric potential, the flow rate of the polymer through the needle, the distance between the needle's tips and the shape of the collector, temperature, humidity and air velocity (Rnjak-Kovacina & Weiss, 2011). Freeze drying method technique is one of the most commonly used method for the preparation of scaffolds. The scientific findings demonstrate that the freezing temperature was lowered, leading to the reduction in the pore size of the scaffold. Additionally, it was discovered that the addition of an annealing step while freeze-drying caused a notable increase (40%) in pore size (Haugh et al., 2010). The range of 100 to 400 μm is the perfect line for scaffold pore size. Under static seeding circumstances, pores greater than 500 μm are too large as to allow the cell and scaffold to come in contact; as a result, cells pass through the scaffolds without adhering to them (J.H. Nam et al., 2020).

4.2. Biodegradability

When grafted into living organisms, scaffolds should eventually degrade chemically or enzymatically because they only serve as a non-permanent platform for the growing cells or tissues. Biodegradability is how quickly the scaffolding materials are being degraded (Chocholata et al., 2019). The rate at which the scaffold degrades should ideally match the rate at which new bone grows or tissues regenerate. Scaffold will be eventually replaced by new tissue through a process known as "creeping substitution" once they have effectively been designed and merged with the host bone (Molina et al., 2021). The leftovers non-toxic part of the scaffold will either be recycled as metabolites in additional biochemical processes or depart the living system without endangering any other tissue or surrounding organs (Krishani et al., 2023).

4.3. Bioactivity and Biocompatibility

The Bioactivity of scaffolds implies the capacity of scaffold to communicate with the created tissue's biological components and the environment (Nitti et al., 2021). It must also be extremely biocompatible for cell growth and adhesion showing minimum immune response in order to avoid severe inflammatory responses that could slow healing and cause the transplanted scaffold to be rejected. There are proven studies in Null mice that wound healing is independent of inflammatory cells and this inflammatory phase can be skipped (Martin et al., 2003). Bioactive scaffolds are created to prevent processes like scarring and to promote the best migration of cells or differentiation, regeneration of tissue or neo-tissue development, and their integration with the host. Traditional passive biomaterials, in comparison, often pose little to no environmental interactions (Roseti et al., 2017).

4.4. Mechanical Properties

Materials used for the scaffolds must possess intrinsic mechanical properties resembling those of the local tissues or bones at the anatomical site of implantation (Chan & Leong, 2008) (Chan & Leong, 2008). It offers mechanical support, structural stability, and helps to reduce the risk of stress shielding, implant-related osteopenia, and refracture that follows. The scaffold must also be sufficiently rigid to allow for surgical access for transplantation. Elastic modulus, fracture toughness, tensile strength, fatigue, and elongation percentage are a few examples of the mechanical qualities (Gurumurthy & Janorkar, 2021). The traditional methods for describing a scaffold's mechanical characteristics include compressive and tensile testing. The important obtained values are toughness

Table 3
Presents the basic parameters of scaffolds quality

Author	Mechanical analysis	Water uptake capacity
Chandika et al. (2021)	The Porosity and fibrous morphology were completely dependent on the proportion of collagen added. Also crosslinking solution help in present of collagen avowed diappearance of thin fibres as these fibres were interconnected with the neighboring fibres	Within 30 minutes of incubation, all nanofibrous scaffolds showed a great capacity to absorb water > 250% and reached equilibrium
Hartinger et al. (2020)	Sandwich-structured sponges could support heavier loads and were substantially stiffer than homogeneous collagen sponges.	NOT SPECIFIED
Wang et al. (2021)	The STSCS showed a highly porous structure of 50-150μm which is highly suitable for cell proliferation and adhesion while DTSCS showed less porosity of 15-40μm.porosity of STSCS and DTSCS Was 97.38% and 91.25% respectively.	The absorption rate of water was 20 times more the weight of the collagen sponge.
Sun et al. (2021)	The wound dressing material showed uniformity morphologically. The synergistic effects of the inner layer's three-dimensional microporous spongy structure with better absorption of wound exudates, better promotion of cells adhesion and proliferation after crosslinking, and the outer layer's medical spun-laced nonwoven with the obstruction of ambient bacteria and antibacterial property of chitosan may be responsible for the wound healing effectiveness of EDC/NHS cross-linked collagen-based composite dressing.	NOT SPECIFIED
Hou et al. (2020)	The PA-Col and PA-Col/NAC scaffolds had a lower Young's modulus than the PA scaffold, showing that these scaffolds were significantly softer and therefore better suited for use as a dressing for wounds.	PA-Col group showed a water absorption capacity of 450% which was much higher compared to other test groups
(Shi et al., 2020) Shi et al. (2020)	FSC showed a pore size of about 100- 200μm. On the SDS page, it was found that the FSC displays typical regions connected to the chain (about 100 kDa) and the chain (around 200 kDa).	FSCscaffold reached 47.8, FSC was able to withstand wate r for 5 hours.
Hu et al. (2021)	Tensile strength was 15.2Mpa in the aligned group	Water contact was least in the aligned group and howed higher warer absorption rate.
Lahmar et al. (2022)	NOT SPECIFIED	NOT SPECIFIED
Li et al. (2018)	The results showed that the isolated collagen from tilapia skin included type I collagen, a heterotrimer made up of two identical 1 chain and one 2 chains in the molecular form of α1(I)]2α2(I). The scaffold was also highly porus	NOT SPECIFIED
Zhou et al. (2017)	The scaffold showed a good pre size and had a diameter of 32.68°±2.54°	The contact angle of the composite nanofibers was 32.68°2.54° and they had good thermal stability and hydrophilicity.

DTSCS-Dialyzed tilapia skin collagen sponges; STSCS- self-assembled tilapia skin collagen sponges; PA-Col- poly amide nanofiber collagen; Col/NAC- collagen (Col) with N-acetylcysteine

Table 4
Overall results of wound healing on different animal models

Author	Macroscopic evaluation	Histological evaluation
Chandika et al. (2021)	The rate of wound healing in fish collagen-based scaffold reached up to 84.5 % on day 14 and 90.2 % on day 21.	The fish collagen group's wound healing was the most noticeable on day 21, the stratum corneum, stratum granulosum, and stratum spinosum of the epidermis are readily discernible, and epithelialization is almost complete.
Hartinger et al. (2020)	Mild inflammation was seen after 30 h.	The moderate connective tissue reaction, which includes the formation of granulation tissue and modest inflammation, is a common stage of the healing process.
Wang et al. (2021)	After 21 days of treatment, the wound closure rate in every group was very near to 100%. The STSCS was more effective than DTSCS due to its mechanical properties	Histological examination The wounds' capillary density significantly increased 21 days after injury, and the red granulation tissue eventually transformed into connective tissue as the epidermis thickened.
Sun et al. (2021)	On day 28 following scald, only the EDC/NHS cross-linked collagen-based composite dressing group saw full re-epithelization.	The stratum corneum was reconstructed and evident wound reepithelization took place on days 18 and 21 after scald, however the epidermis has not fully healed. Additionally, each group displayed thick dermal mesenchyme, spindle-shaped fibroblasts, reduced granulation tissue, and an increase in fibrous tissue.
Hou et al. (2020)	PA-Col/NAC scaffold treated group showed 80% healing rate on day 14	PA and PA-Col At 14 days, collagen appears to be misaligned, despite PA-Col/NAC showing substantial collagen bundle deposition and robust inflammatory cell infiltration, both of which are uniformly and regularly organized. This incident demonstrated that compared to the PA and PA-Col groups, the PA-Col/NAC group healed wounds more quickly.
Shi et al. (2020)	On 28th day the FSC treated group showed complete wound healing	No secondary damage was seen on the tissue while there was complete re-epithelization on day 28.
Hu et al. (2021)	Re-epithelialization was complete in all groups on day 14 with the exception of the lattice group, and all groups displayed mature stratified epithelia. On day 28 there was visible growth of hair follicle	Results show that there was neo vascularization and re-epithelization.
Lahmar et al. (2022)	Complete wound closure was seen on day 15	The granular layer was one cell thick and well-formed. The thin layer of keratin is composed of additional keratin. Low cellular collagen lines the dermis beneath, the fibres are horizontally orientated, and neither inflammation nor obvious vascularity are present.
Li et al. (2018)	At 20 days after scaffold implantation, a macroscopic examination of implanted site indicated no signs of inflammatory tissue reactions to the implantation of collagen tissue.	Histological research showed that over the period of implantation under study, connective tissue matrix grew within the implant pore spaces of the microfibre collagen matrix scaffolds.
Zhou et al. (2017)	Showed 90% would healing on day 14	Angiogenesis was prolierated by secretion of vascular endothelial growth factor

DTSCS- Dialyzed tilapia skin collagen sponges; STSCS- self-assembled tilapia skin collagen sponges; EDC/NHS- N-hydroxysuccinimide,1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; FSC- Fish scale derived collagen;PA-Col/NAC - poly amide nanofiber collagen with N-acetylcysteine

Table 5
Presents the different techniques used for the fabrication of scaffolds along with their advantages and disadvantages

Technique	Advantages	Disadvantages
Freeze Drying (Fereshteh, 2018)	No involvement of heat	Longer time, high energy, expensive
Electrospinning (Kishan & Cosgriff-Hernandez, 2017)	Good porosity, perfect architecture	Lack of cellular infiltration
Bioprinting method (Huang et al., 2017)	Affordable and higher efficiency	Depends on the cells
Thermal induced phase separation methods (Y.S. Nam & Park, 1999)	Controllable, Interconnected pores	Can be used only for thermoplastic
Stereolithography (Melchels et al., 2010)	High resolution, smooth surface and processing is fast	Toxic resin, Expensive and high temperature
Fused deposition model (Kamboj et al., 2021; Krishani et al., 2023)	No requirement of solvent	High temperature and filament required
Solvent casting and Particle leaching (Thadavirul et al., 2014)	High porosity, lower cost for production	Irregular shaped pores, poor inter connectivity
Gas foaming (Harris et al., 1998)	Low temperature, avoids usage of cytotoxic solvent	Closed pores, long procedure

Young's modulus, and compressive/ tensile strength. The literature states that the skin's range of Young's modulus is 0.42 MPa to 0.85 MPa.

There are various techniques known from the previous literatures that can be used for the fabrication of scaffolds, Table 5 represents some of the techniques used in the fabrication of scaffolds along with their advantages and disadvantages. From the current literature review, it is clearly evident that the most common technique involved in the fabrication of scaffolds are Freeze drying and electrospinning. The structure and characteristics of the resulting collagen scaffold can be accurately controlled by freeze drying and by carefully controlling the growth of the ice crystals. This is accomplished by adjusting a cycle of freezing rather than drying components (Brougham et al., 2017). This hasn't always been fully understood over the years, the focus has been on managing the sample's efficient sublimation rate and understanding the drying phases, where the vacuum is introduced. According to current knowledge, the focus point of any scaffolds, where users attempt to alter the ice crystal structure and porosity of a specific sample should be attained at the first freezing stage.

4.5. Freeze Drying

Other names for the freeze-drying procedure include lyophilization and ice templating. The three steps in this method are sublimation, solidification, and dissolution (Ho et al., 2004). First, the solvent is used to dissolve the chosen polymer. The resulting mixture is then poured into a mould and placed in the freezer to harden or freeze. The process can then be finished by mechanical refrigeration, dry ice in aqueous methanol, or liquid nitrogen, allowing it to cool either naturally or chemically. Keeping the temperature under control is important at this stage to prevent the growth of larger crystals that could subsequently damage the scaffold's characteristics (Capuana et al., 2021). Thirdly, the frozen component is subjected to the sublimation process to eliminate water and other solvent molecules. This method is ideal for creating scaffolds with numerous pores, which promote vascularization and aid cell growth and cell differentiation (Fereshteh, 2018). Combining the lyophilization process with salt leaching, gel casting, gas foaming and liquid dispensing methods will improve the properties of the scaffold (Kardan-Halvaei et al., 2023).

4.6. Electrospinning Technique

It is a straightforward process where high-voltage electricity is passed through solutions to create fibres (Jun et al., 2018). The interaction between electrostatic repulsion and the surface tension of the charged liquid that experiences significant decreases in voltage is the key idea behind this technology. A Power supply unit, a metallic needle, a syringe pump and a grounded collector are the machine's four main components. This method is frequently used to create nano-fibrous scaffolds (Zulkifli et al., 2023). The syringe pump's capillary tube receives the liquid injection. The electric field generated by a high-voltage power source exerts muscle strength that raises the liquid's surface tension when it emerges from the metallic needle's nozzle (Zhang et al., 2015). Additionally, electrostatic repulsion continuously whips the liquid jet, which is then collected as fibers in the grounded collector. Good porosity, aligned fibres and patterned architecture are characteristics of electrospun scaffolds that support cellular response and enhance cell regeneration (Hong et al., 2019). The electrospinning technique has limitations too i.e., it is difficult to precisely manage fiber creation, homogenous cell dispersion, and lack of cellular infiltration (Muthukrishnan, 2022).

5. CONCLUSION

From the overall review performed, it is seen that 70% of the research articles published have worked on bone repair using fish collagen-based scaffolds. And there are no direct fish collagen-based products available as a scaffold for direct treatment in patients. Irrespective of so much research work carried out over the past three decades, there is no successful output emphasizing scaffolds with fish-based biomaterials. On the other hand, fish-based products that are already available in the market and other biomaterials like mucus and oil from fish sources are known to

have a high wound healing rate. Therefore, there is a need right away to combine scaffolds with fish mucus or oil in scaffolds which would furthermore enhance the wound healing efficiency when merged together. This could be a novel way out in finding a solution for people suffering from diabetes and burn wounds. Hence, there is an urgent need for good quality research to be carried out in this particular area to boost up the market and industries to come out with a quality product in the field of wound healing processes.

ABBREVIATIONS

PRISMA: Preferred Reporting Items for Systematic review and Meta-Analysis

MeSH: Medical Subject Healings

ECM: Extracellular Matrix

SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation

WBC: white blood cells

CONFLICTS OF INTEREST

Given his role as Associate Editor, Balamuralikrishnan Balasubramanian has not been involved and has no access to information regarding the peer review of this article. Full responsibility for the editorial process for this article was delegated to Assocate Editor Si Mi. The authors hereby declare that they have no conflict of interest and have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ORCID

Gomathy Meganathan	0000-0003-3051-6801
Balamuralikrishnan Balasubramanian	0000-0001-6938-1495
Arun Meyyazhagan	0000-0002-1934-3906
John Paul	0000-0002-0998-1736
Aditi Chaudhary	0009-0003-0433-5799
Manikantan Pappuswamy	0000-0002-6047-3702

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