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EFFICACY OF COLLAGEN-ONLY SCAFFOLDS COMPARED TO POLYMER-ASSOCIATED COLLAGEN AND NANOMATERIALS IN SKIN WOUND REPAIR – A REVIEW

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Abstract

Wound healing remains a clinical problem, with cases of atrophic, hypertrophic, or keloid scars. Three-dimensional scaffolds have been used to restore skin function, facilitating cell migration, adhesion, and proliferation. Collagen is the most common, presenting low antigenicity, decreased inflammation, and replacement by autologous tissue. It is used as sheets/films, sponges, membranes, sprays, and hydrogels of various origins. This integrative literature review aimed to evaluate the application of unassociated collagen scaffolds for skin wound healing and compare them to collagen associations with nanomaterials and polymers. Properties such as applications in humans and other unconventional models cause burns, partial and full-thickness wounds, and others. Scaffold, biomaterials, collagen, wound, injury, repair, and healing were among the descriptors. We found 3,098 articles published between 1995 and 2022 (Mendeley platform), including clinical/experimental trials. After exclusion, 26 studies were identified and analyzed. Autologous and heterologous collagens are the most used in the clinic and favor wound closure by improving re-epithelialization and reducing inflammation but may present challenges in aesthetic acceptance and loss of repair function in the wound site. Furthermore, collagen integration with other nanomaterials improved wound repair and experimental models.

Keywords: Biogel. Collagen. Dressings. Matrix. Membranes. Sponges. Wound healing.

1. Introduction

Wound healing remains a pressing clinical and social problem. Skin injuries trigger several biological events for wound repair. The interaction of inflammatory events and macromolecules composing the ECM (extracellular matrix), such as collagen, elastin, and glycoprotein (e.g., fibronectin, proteoglycans, and laminins), must occur for tissue repair. Wound repair presents four phases in a temporal sequence: hemostasis, inflammation, proliferation (cellular infiltration, angiogenesis, and re-epithelialization), and maturation/remodeling (Larouche et al. 2018) (Figure 1). A scar is formed during skin wound repair, replacing the original tissue with collagen fibers deposited in an aligned manner (Figure 1-e-f). Inadequate collagen deposition can cause atrophic, hypertrophic, or keloid scars. There have been numerous proposals to improve the healing process aiming at restoring skin function, such as scaffolds (Gandhimathi et al. 2014).

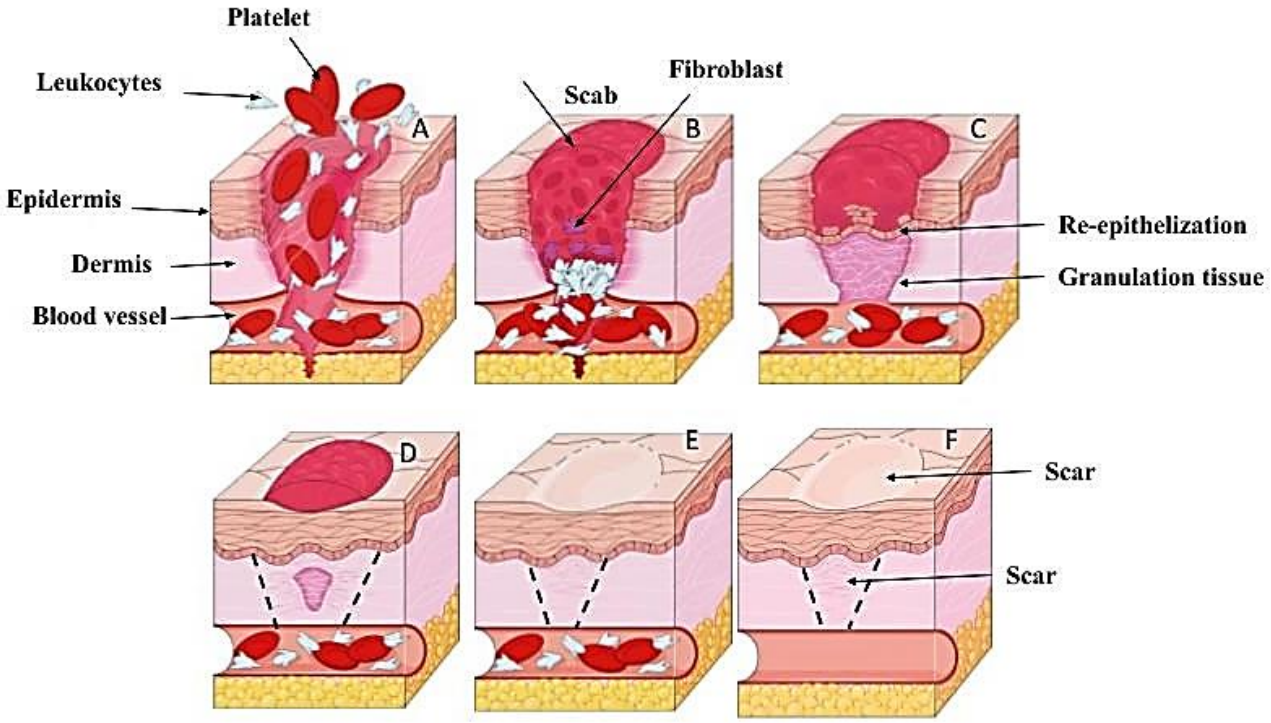

Figure 1. Four stages of skin wound repair: A- hemostasis; B- inflammation; C- granulation tissue (new tissue formation); D- remodeling. A: Hemostasis occurs soon after the injury, forming a fibrin plug; B: The inflammatory phase lasts around 72 hours after the injury, with leukocyte kinetics showing a peak of neutrophils up to 24 hours, macrophages up to 72 hours, and lymphocytes up to 96 hours after the injury. The open wound may present abundant bacteria and platelets. All skin appendages are torn and located at the edge of the injury, such as hair follicles and sebaceous glands. A crust forms on the wound surface, below which epidermis re-epithelialization begins. C: New tissue formation (granulation tissue) occurs approximately two to ten days after the injury, showing decreased inflammatory cells and increased fibroblasts that will deposit the new extracellular matrix. Re-epithelialization occurs. A skin wound occurs about seven days after the injury. Most cells from the earlier repair stage migrate from the injury. Scab forms on the wound surface and epithelial cell migration occurs under the eschar. D: After concluding re-epithelialization, the crust decreases until falling off, and the scar starts contracting. The re-epithelialized wound is slightly larger than the surrounding surface. E-F: Remodeling phase - The healed region does not contain normal skin appendages. A skin wound appears approximately one (E) and 12 (F) months after repair. Fibroblasts migrating into the wound and contracting it deposit disorganized collagen. The images were from the SMART (Servier Medical ART) public domain website.

Scaffolds have a three-dimensional structure that mimics the natural extracellular matrix and facilitates cell migration, adhesion, and proliferation (Kang et al. 2019). Collagen has been used as a scaffold, presenting low antigenicity, decreased inflammatory responses in the wound bed (Wang et al. 2016; Kang et al. 2019), complete body resorption, and replacement by autologous tissue (Rahmanian-Schwarz et al. 2014).

Due to its significance, the pharmaceutical industry has been using collagen as a dressing in different forms, such as sheets/films, sponges, membranes, sprays, and hydrogels (Ruszczak and Schwartz 1999). Collagen can have different origins: bovine, porcine, and equine, among others (Purba et al. 2014; Landsman et al. 2016), and it has been used differently for skin wound repair. Therefore, this literature review evaluated collagen use without associations with other materials to explain its effectiveness in skin wound repair and compared it to collagen associations with polymers and nanomaterials.

2. Material and Methods

It was a non-systematic literature review that searched the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) for studies that applied collagen scaffolds without adding other

materials. The PICO strategy (an acronym for Patient, Intervention, Comparison, and Outcome) allowed determining the guiding question, descriptors, and their variants. The question was: "How does the use of unassociated collagen scaffolds affect wound healing in experimental models compared to methods used in the clinic?" The used articles presented animal and human experimental models, interventions with unassociated collagen scaffolds, comparisons with methods used in the clinic, and the expected outcome of wound healing improvement from patient-specified parameters.

Exclusion criteria were scaffolds associated with other substances, wounds in regions other than the skin, review articles, and case studies. The included investigations showed unassociated collagen scaffolds, all collagen variations, different injuries, experimental model variations, and experimental studies.

Therefore, the following descriptors were used for database searching: 1) Scaffold: scaffold, scaffolding, truss, orlop, scaffolds, biocomposite, hydrogel, matrix, matrices, membrane, biomaterials, bio-gel, spray, sponge; 2) Healing: repair, regeneration, healing, recovery, priest, healing, wound repair, scar; 3) Wound: wound, raw, cut, wound, defects, injury, pain, Injuries; 4) Skin: skin, dermal, fur, fur, piece of fur, bark, dermis, leather, felt skin; 5) Collagen: collagen, bovine collagen, human collagen, porcine collagen, fish collagen, hydrolyzed collagen.

Then, another advanced search was performed on the PubMed platform to add descriptors and remove others that best described the topic of this study: 1) Scaffold: scaffold, hydrogel, matrix, membrane, biomaterials, sponge; 2) Collagen: collagen and hydrolyzed collagen; 3) Wound: wound defects, wound, wounds; 4) Healing: healing, repairing, regeneration healing, wound healing, wound repair, scar.

Inclusion criteria were clinical or experimental trials that applied collagen scaffolds for skin wound healing in various animal models. The exclusion criteria were collagen scaffolds associated with other substances or materials. The articles were included in the study after two independent reviewers, who applied the inclusion and exclusion criteria, read the titles and abstracts. The remaining studies were retrieved from the reference list of included articles in case they were relevant to the research object (Figure 2).

3. Results

First, applying the descriptors in the advanced PubMed search yielded 147 articles published from 1980 to January 2021. No studies appeared in the LILACS platform (https://lilacs.bvsalud.org/), and there were 105 articles from 1974 to January 2021 in the MEDLINE database (Figure 2).

Next, 1,780 articles published between 1995 and July 2022 were identified. Overall, 3,098 studies were found and joined in the Mendeley platform, in which their abstracts were analyzed based on the exclusion and inclusion criteria. The analysis provided 23 articles that were defined and examined as the study object and the basis for the present discussion (Figure 2). Seventeen studies used several experimental animal models (Table 1), and six included humans (Table 2).

These articles aimed to analyze and group the experiments according to collagen type, scaffold type, animal model, wound type, and days of treatment. The evaluated studies found scar size reduction, skin cell proliferation, re-epithelialization ability, and decreased wound contraction. Moreover, evaluations of the mechanical forces on the scaffold were listed, such as good adhesion, immunogenicity, softness, and biodegradation. The review added the topics of collagen scaffold associations with natural polymers and nanomaterials as a form of discussion and comparison to other methods applied in the clinic and experimental research. The other articles used in this phase were retrieved from the reference list of the identified studies in case they were relevant to the research object and had acknowledged authors in the subject (Figure 2).

4. Discussion

Collagen

Collagen is the main protein in the extracellular matrix of the skin. It is found in different self-organized superstructures, such as fibrils and macrofibrils, or organized in networks (Landsman et al. 2016).

These structures are formed by α chains and joined by hydrogen bonds, which later intertwine and acquire the triple-helix conformation that may be homotrimeric or heterotrimeric. Each α chain consists of a repeated sequence of amino acids [Gly – X – Y] n, where X is occupied by proline and Y by hydroxyproline. The triple helix can be continuous or segmented with non-collagen components. Today, 29 collagen types differ according to their respective α chains (Fraser et al. 1979; Perumal et al. 2008; Sorushanova et al. 2019).

The superstructure provides collagen interaction with other macromolecules and extracellular matrix components, which provide biological and structural properties to collagen, such as the ability to work in cell adhesion, differentiation, and proliferation, and the mechanical resistance and maintenance of the structure of cells and connective tissues (Khoshnoodi et al. 2006). Thus, collagen has been used in different therapeutic applications for tissue repair (Chan et al. 2016; Ghodbane and Dunn 2016).

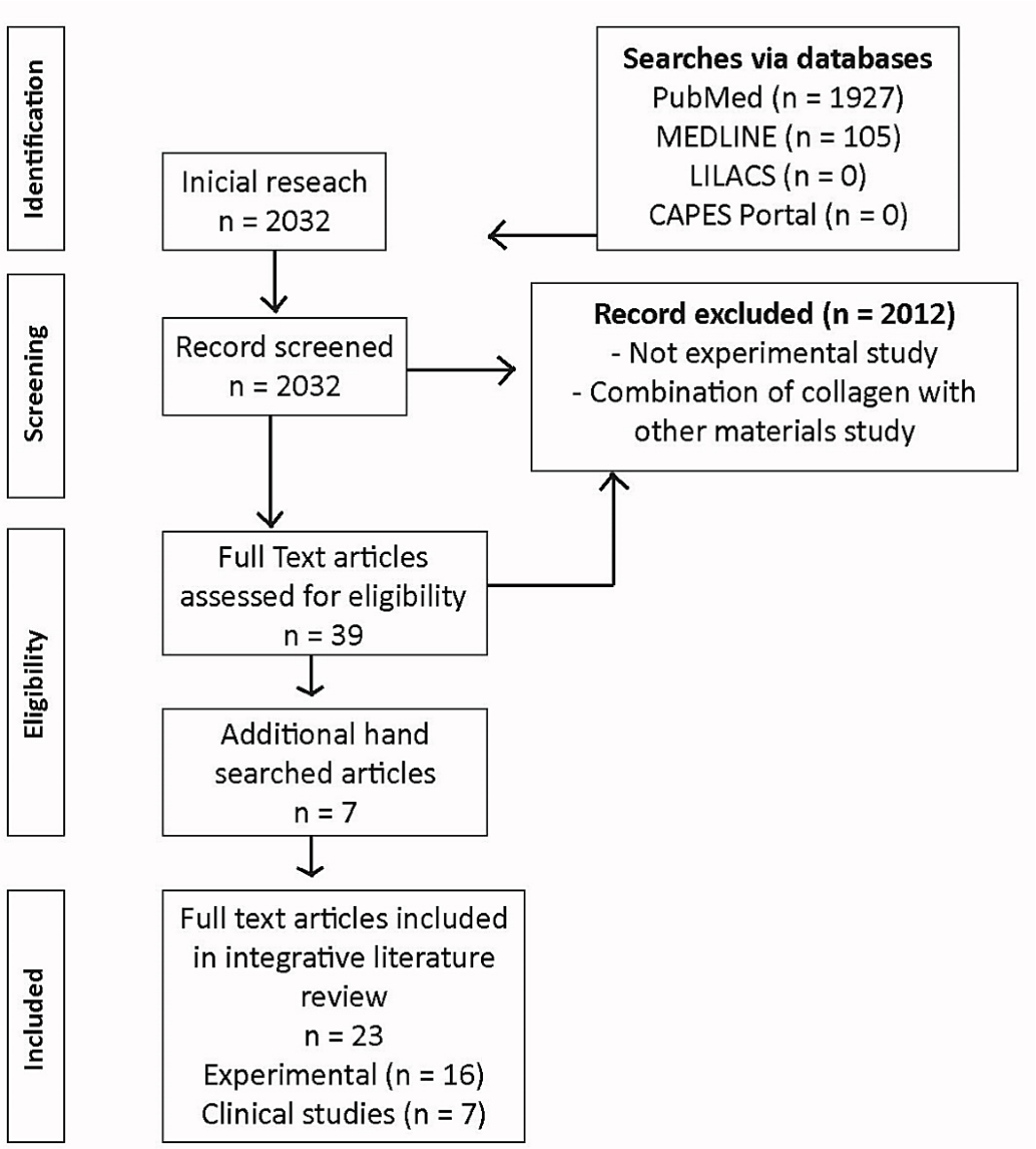

Figure 2. Flowchart of the research and screening process.

Collagen scaffolds for skin wound healing

Several promising technologies can heal or regenerate tissues such as the skin. Conventional substitutes of epidermal, dermal, and dermo-epidermal origin, such as autografts, allografts, and xenografts, are the gold standard in the clinic (Gautam et al. 2014). However, tissue engineering developed a strategy of using three-dimensional scaffolds, which provide an environment for cell adhesion, migration, and dissemination, allowing cell proliferation and extracellular matrix (ECM) synthesis (Veleirinho et al. 2012).

Scaffolds have been considered the best material to restore, maintain, and improve tissue function. They should preferably mimic the skin, promote wound healing, be permeable to moisture and oxygen, be

biocompatible, shield wounds from infection and irritation, allow exudate removal, and promote repair with good esthetics (Kumbar et al. 2008). Scaffolds can usually be produced from synthetic or absorbable polymeric materials, naturally occurring, biodegradable, or non-degradable. However, collagen scaffolds can also associate with other biomolecules or compounds, such as elastin, glycosaminoglycan, chitosan, and others, providing different forms of production and application (Ellis and Yannas 1996; Buijtenhuijs et al. 2004; Wu et al. 2007). Sponges, dressings, and membranes are the most common collagen-only scaffolds reported in studies or marketed (Collins et al. 1976; Takeda et al. 1983; Gao et al. 1992). This section will discuss collagen association with polymers and nanomaterials to understand the most recent applications of collagen scaffolds.

Association of collagen with natural and synthetic polymers

Several cross-linking strategies have been performed using bonds through reagents or mixtures with other polymers to increase collagen versatility and mechanical strength. Polymers can be of natural origins, such as chitosan and hyaluronic acid (Lin et al. 2009; Li et al. 2019; Andonegi et al. 2020), or synthetic, such as poly(ε-caprolactone) (PCL) and polylactic acid (PLA) (Vonbrunn et al. 2020). These materials are biocompatible, biodegradable, non-toxic, and do not cause immunogenicity (Khor and Lim 2003; Andonegi et al. 2020; Vonbrunn et al. 2020).

Chitosan is obtained by deacetylated chitin, abundant in crustaceans, insects, fungi, and yeasts. It interacts with extracellular matrix components (Nilsen-Nygaard et al. 2015; Abd El-Hack et al. 2020). Introducing chitosan into collagen scaffolds improves structural and mechanical strength, affecting the biological properties of the material (Tan et al. 2001; Hua et al. 2020) and showing improvements in the early stages of wound healing. Chitosan properties reduce blood clotting time and rapid thrombin formation because chitosan-based dressings attract more red blood cells and platelets and adsorb plasma proteins and fibrinogen (Zhao et al. 2017; Biranje et al. 2019; Wang et al. 2021). They also have antimicrobial activity, inhibiting bacterial growth in the first days after injury due to ionic interactions between the positive charges of amino groups and the negative charges of bacterial cells, causing the lysis of these cells and reducing exacerbated immune responses that could hinder the repair process (Moon et al. 2020; Rathinam et al. 2020; Wang et al. 2021). These dressings are biocompatible and bioactive, showing good cell affinity and affecting cell adhesion, growth, and proliferation. Chitosan also promotes granulation tissue formation through the action of cytokines, such as TGF-β, PDGF, and IL-1, increasing the production of granulation tissue, which mainly mediates macrophage and fibroblast proliferation and capillary formation (Li et al. 2019; Nadi et al. 2020; Feng et al. 2021).

Hyaluronic acid (HA) is a linear polysaccharide composed of glucuronic acid and N-acetylglucosamine. It is an extracellular matrix (ECM) component in all body tissues. It interacts with other ECM components, influencing structure and malleability. Therefore, HA is involved in numerous cellular activities, such as maintaining tissue homeostasis and signaling and remodeling of cells (Fraser et al. 1997; Toole 2004). Biomaterials incorporated with HA have better biostability and bioactivity, mediating cell proliferation and growth (Kirk et al. 2013). In wound healing studies, HA-based biomaterials affected all repair process stages, increasing re-epithelialization, improving granulation tissue formation, reducing the wound area, and presenting a higher rate of wound closure and contracting. Also, the formed scar tissue showed an improved tissue architecture with more collagen fibers. Therefore, these biomaterials can maintain an optimal microenvironment for wound healing (Gokce et al. 2017; Seong et al. 2019; Makvandi et al. 2020; Mittal et al. 2020).

This characteristic of HA in modulating wound healing is due to immunological properties related to inflammation, which can promote or attenuate this process according to the molecular size of the biomaterial. Therefore, HA fragments initially promote fibrinogen deposition, causing hemostasis of the injured site. Then, HA influences the recruitment of inflammatory cells and secretion of inflammatory cytokines, such as TNF-α, IL-1β, and IL-8. Subsequently, at the end of the inflammatory phase, HA reduces the production of pro-inflammatory cytokines. Wound healing is optimized during the inflammation phase, improving the rate of closure, re-epithelialization, and angiogenesis (Gao et al. 1992a; Tavianatou et al. 2019; Graça et al. 2020).

Studies also report collagen association with synthetic polymers. In these cases, polymers provide mechanical and structural resistance to scaffolds, and collagen works in cell adhesion, differentiation, and proliferation (Khoshnoodi et al. 2006). There are numerous synthetic polymers today, but this review will mention poly (ε-caprolactone) (PCL) and polylactic acid (PLA).

PCL is a highly prominent polymer in the biomedical field because it is biocompatible, biodegradable, and resistant. However, its chemical structure and lack of functional groups make it commonly incorporated with other compounds to improve bioactivity (He et al. 2020; Vonbrunn et al. 2020). Among these materials is collagen, which despite different biological properties that optimize the repair process, it does not present good mechanical characteristics. Thus, PCL is responsible for improving these features. Combining these biomaterials provided good mechanical properties, such as the tensile characteristic. They also accelerated the repair process due to increased cell proliferation, higher wound contraction rate, higher neovascularization and collagen deposition, and lower scar formation (Ehterami et al. 2018; Ghorbani et al. 2020; Jafari et al. 2020; Miele et al. 2020).

PLA is an aliphatic polyester widely explored in the biomedical field due to its excellent biocompatibility, biodegradability, and mechanical properties. These traits make it a promising material for developing wound healing therapies. PLA increases cell proliferation, accelerating the wound closure rate and increasing angiogenesis and extracellular matrix deposition in the injured area (Sharma et al. 2014; Bi et al. 2020). The combination of PLA and collagen produces scaffolds that provide a favorable structure for cell growth and proliferation, highlighting the biocompatibility of these materials (Kang et al. 2018; Vonbrunn et al. 2020; Hajikhani et al. 2021). Moreover, this polymer can be associated with other compounds, such as chitosan, cellulose acetate, and hydroxyapatite, adding relevant properties, such as antibacterial activity and other potential physicochemical properties for biomedical use (Gomaa et al. 2017; Ren et al. 2020; Donya et al. 2021).

Association of collagen with nanomaterials

Therapeutic development has advanced, especially regarding materials from 1 to 100 nm, called nanomaterials (Weng et al. 2018). These materials have been studied and present applications in different areas of knowledge, such as biology, physics, chemistry, medicine, and computing. Nanomaterials are effective in penetrating tissues and adaptable to new technologies, present high cell selectivity, and are associated with different compounds, such as protein, RNA, DNA, and others (Weng et al. 2018). In this context, associations of nanomaterials with collagen – a natural polymer – can benefit wound healing.

Collagen alone is a natural composite with scaffold applications such as gel, hydrogel, and sponge, associated or not with other materials for wound healing. Amiri et al. (2021) demonstrated that electrospun collagen nanofibers or nanoparticles produced different behavior in Normal Human Epidermal Keratinocytes (NHEK) cells, in which collagen nanoparticles showed better results in morphology and viability than collagen nanofibers. However, it is worth considering how to cross-link the nanocomposite or nanostructure.

Gold nanoparticles (AuNPs) are suitable nanomaterials for therapeutics, such as drug delivery. After this nanomaterial sets in human tissues, it Interacts with proteins in the extracellular matrix, and collagen is the most common protein. Tang et al. (2019) showed, in a computational study, the interaction of collagen triple helices with AuNPs with diameters from 3 nm to 8 nm up to atomic levels. Collagen protein adsorbed on both AuNPs, showing good interaction. Golden nanoparticles support interchain hydrogen bond interactions between carboxyl oxygen atoms of collagen. Besides the residues occupying the X position on the Gly-XY triplet, carbonyl oxygens of AuNPs point towards the gold surfaces, preventing bonds with other collagen proteins (Tang et al. 2019b).

Collagen adsorption to the AuNP surface occurs in three steps: (1) Biased diffusion – proteins go toward the water/AuNS interface until approaching the gold surface; (2) Anchoring – proteins engage with the water layer adjacent to the gold surface; (3) Stepwise adsorption – collagen forms a direct contact with the gold surface. Tang et al. (2019a) demonstrated that Lys is a significant amino acid prone to anchoring on gold surfaces with hydrogen bonds (Figure 3).

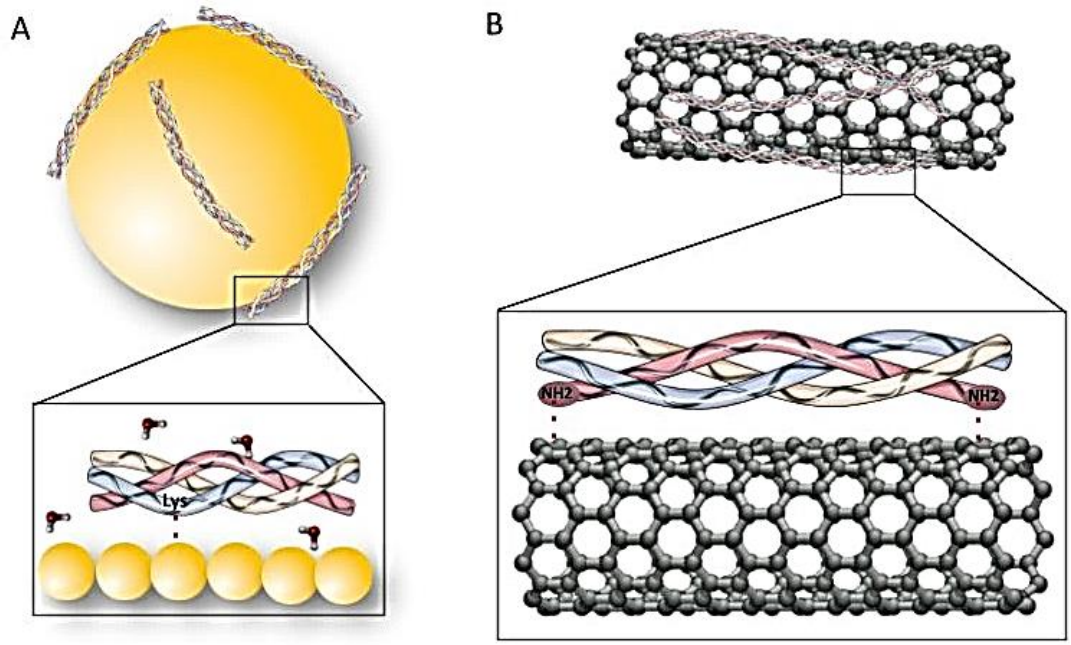

Figure 3. Schematic illustration of A) Gold nanoparticles functionalized with collagen; B) Carbon nanotubes encapsulated with collagen.

Carbon nanotubes (CNT) are a great nanomaterial because of their low density and favorable mechanical, electronic, and biological properties (Negri et al. 2020). CNT functionalization with functional groups, such as oxygen content to link with proteins, is vital to associate collagen with CNT by the amide bond. These processes allow collagen cross-linking around CNT, resulting in collagen-encapsulated CNTs (Figure 3). The Col-encapsulated CNTs showed that good cell compatibility improved the mechanical properties of the composite and was less susceptible to thermal damage during functional use in applications (Chi and Wang 2018; Fielder and Nair 2020; Li et al. 2020). Incorporating CNTs into type I collagen hydrogel improved the electrical performance required for stimulating cells and restoring the electrical sensitivity of tissues (Yu et al. 2017).

Wound healing must consider CNT alignment, which reduces hypertrophic scar formation by suppressing cell proliferation and ECM deposition, and stretches and grows parallel to ACNT arrays. Furthermore, the cytoskeleton of fibroblast cells was rearranged by F-actin and α-tubulin parallel to CNT (Weng et al. 2018) (Figure 3).

Collagen scaffolds without associations

Collagen sponges

Sponges are scaffolds with high porosity produced with drying by lyophilization, a dehydration process of an aqueous collagen solution in which sponge porosity can change depending on the shape and freezing time (O'Brien et al. 2004). There are studies combining sponges with elastin, fibronectin, and glycosaminoglycans (GAGs), among others, providing scaffolds with higher strength and promising results in skin wound healing (Doillon and Silver 1986; Lefebvre et al. 1992). Their use improves skin healing by absorbing exudates from the tissue, allowing adhesion to the wound while maintaining environment moisture, thus protecting against mechanical impacts and reducing bacterial infection in rats and *in vitro* (Chvapil et al. 1986; Geesin et al. 1996).

Other studies have been performed to verify the effects of collagen sponges on healing, using different skin injuries in animal models (Table 1). Wistar rats with excisional wounds showed a restructuring of skin appendages with type I bovine collagen (Sedlarik et al. 1991), and Sprague-Dawley rats provided better re-epithelialization (Chvapil et al. 1986). Using type I porcine collagen in deep burns on rabbit skin resulted in 28 days of treatment after applying the collagen sponge (Shi et al. 2019).

In recent years, different collagen sources, such as from fish and other animals, have gained space in research due to their abundance, low price, and non-immunogenicity (Zhou et al. 2016), as shown in Table 1. Shi et al. (2019) found that collagen from grass carp fish produced accelerated re-epithelialization and might represent alternative collagen to porcine and bovine collagen. There were similar results for goat collagen sponge scaffolds applied to Swiss mice (Banerjee et al. 2012).

Collagen membranes and dressings

Another way of using collagen is from films produced through the evaporation of collagen solutions, which join these films and form multilayer membranes (Sorushanova et al. 2019). Collagen membranes, alone or combined with other compounds, have been used as wound dressings and damaged tissue reinforcement because they provide an environment for fibroblast infiltration, reducing wound contraction. They have represented a drug delivery alternative due to their ability to adhere to tissue (Yoshizato et al. 1988; Bradley and Wilkes 2009).

Studies with type I/III bovine collagen membranes (Geistlich Pharma, Wolhusen, Switzerland) in full-thickness wounds of domestic pigs showed improvements in keratinocyte proliferation and fixation and the development of a functional epidermal layer (Wehrhan et al. 2010). Fibroblast proliferation and better re-epithelialization for burns occurred in partial- and full-thickness rabbit wounds using a bovine atelocollagen fiber dressing (Takeda et al. 1983).

Studies with partial-thickness burns in Sprague-Dawley rats reported good re-epithelialization using porcine dermal collagen membrane but without significant difference in the wound contraction rate (Gao et al. 1992a). Aoki et al. (2015) produced, with porcine atelocollagen, an artificial skin Vitriband dressing with film, silicone-coated polyethylene terephthalate (PET), and xerogel collagen membrane that, when applied to full-thickness wounds of C57BL6 mice, induced epithelialization and inhibited fibroblast differentiation.

Using collagen-only membranes and dressings is also advocated in a clinical setting with human patients (Table 2). Shah and Chakravarthy (2015) applied a natural type I bovine collagen dressing (microscaffold) to 21 chronic wounds with no results from numerous previous treatments. They found 15 healed wounds, and ten were not treated with a concomitant antimicrobial agent within 90 days.

In patients with venous leg ulcers (VLUs) without previous results for at least six weeks, ProHeal collagen membrane (MedSkin Solutions, Germany) showed smaller wound size and the absence of side effects and pain within 12 weeks (Romanelli et al. 2015). Gao et al. (1992a) applied porcine dermal collagen membrane to burns and noticed faster re-epithelialization than spontaneous healing.

Hydrogels

Hydrogels are cross-linked polymers like a gel in water, usually hydrophilic, which approximate them to the characteristics of soft tissue (Zhu and Marchant 2014). The union of inherent properties of collagen hydrogels, such as flexibility and high absorption allows its extensive application, mainly associated with growth factors to boost cell differentiation, drug administration, and controlled release of growth hormones, among others (Bell et al. 1981; Cascone et al. 1995).

Collagen-only hydrogels have poor mechanical properties and provide good shrinkage. However, they exhibit good cell integration and colonization at a higher concentration of type I bovine collagen (Helary et al. 2010).

Telocollagen extracted from the skin of tilapia is among the sources of hydrogel manufacturing, reducing the wound area and inducing re-epithelialization and regeneration of skin appendages in second-degree burns in Sprague-Dawley rats. That demonstrates that collagens extracted from animal sources other than conventional ones can be a new source of pure hydrogels (Ge et al. 2020) (Table 1).

Another study that corroborates such a statement applied a gel based on recombinant human collagen (rhCollagen, Collplant) from modified tobacco plants to complete incisional and excisional wounds in the ears of Sprague-Dawley rats and domestic pigs, resolving exacerbated inflammation and wound closure acceleration (Shilo et al. 2013).

Table 1. Experimental studies on unassociated collagen application analyzed as a research object.

Author	Date	Scaffold/ Treatment	Animal model	Collagen type	Wound type	Treatment days	Results
Takeda et al	1983	Collagen dressing	Male rabbits, 3 kg	Fine fibers of bovine atelocollagen	Excisional wounds with an area of 4x4 cm and depth of 0.51 mm, gauze dressings, partial and full thickness, and burns.	8, 12, and 14 days for partial-thickness wounds; 3, 7, 14, and 21 days for full-thickness ones; and 7, 14, and 21 days for burns.	All wound models showed fibroblast proliferation, wound closure, and re-epithelialization with consequent degradation. Soft, no need for removal, less immunogenic, and good adhesion.
Chvapil et al	1986	Common collagen sponge laminated with polyurethane film	Sprague-Dawley rats, male, 250-280 g	Not specified	1) Excisional wound, superficial, 0.15-mm deep, 2 cm in diameter; 2) Excisional wound, full-thickness, 2 cm in diameter, depth to the dermal panniculus skin; 3) Excisional wound, full-thickness inoculated with *E. coli.*	2, 5, 6, 8, and 11 days	Collagen sponges reduced the *E. coli* bacterial count, had better adhesion to the wound, reduced wound contraction, had better epithelialization, higher capillary density, and more spindle cells on the granulation tissue surface. However, the laminated collagen sponge showed better results than the common sponge.
Brown et al	1990	Regular, denatured, and acellular dermal collagen grafts (DCGs)	Sprague-Dawley rats, 300-350 g	Autologous collagen. The skin removed from the wound was used to produce the graft	Excisional wound, full-thickness, square-shaped, 2.5 cm x 2.5 cm, and covered with dressings.	0, 3, 6, 9, 12, 15, and 18 days	There was no significant difference in wound closure and higher resistance to contraction. Denatured grafts significantly lost their ability to resist wound contraction.

Sedlarik et al	1991	Collagen sponge cross-linked with hexamethylene diisocyanate and not	Wistar rats, female, 6 months old, 300-343 g	Type 1 bovine collagen	Excisional wounds, 1.5 x 1.5 cm, on the back, full-thickness, sutured dressing, changed every 3 days.	3, 6, 12, 24, 27, and 30 days.	Reduction of the wound area with lower macroscopic scar depression, better re-epithelialization with reticulated collagen, and restructuring of skin appendages.
Gao et al	1992	Porcine dermal collagen membrane for both studies	1) Sprague-Dawley rats, female, 250-300 g; 2) Twelve patients aged 3-38 years.	Collagen extracted from pig skin	1) 50-cm^2 deep burn, partial-thickness, shaved epidermis; 2) Burn, exposure to water at 75° for 15 s, covered with Vaseline gauze.	1) 3 and 10 days; 2) Time required for complete re-epithelialization.	1) Wounds with 69% re-epithelialization and no significant difference between contraction and open wounds. 2) Significantly accelerated re-epithelialization compared to spontaneous healing. The skin rejected the substitutes with both collagens and was excluded from the study.
Van Luyn et al	1995	A dermal substitute with two layers of connected cross-linked sheep dermal collagen (H/HDSC) and one substitute with one layer of non-cross-linked collagen and one layer of disconnected cross-linked collagen (N/HDSC).	Albino Oxford (AO) rats, male, 3 months old, 350 g	Non-cross-linked sheep dermal collagen and cross-linked hexamethylene diisocyanate	Excisional, full-thickness, 15-mm wounds, sutured wound substitutes, non-adhesive dressings changed every 7 days.	1, 2, 4, 6, and 8 weeks.	The substitute only with layers of cross-linked collagen showed dermis and epidermis repair, inhibited contraction, epidermal cell infiltration, and angiogenesis.
Banerjee et al	2010	Goat collagen sponge scaffold. The same bovine calf collagen scaffold was used as a control group	Swiss albino mice, male	Type I goat collagen	Excisional wound, full-thickness, 1.5 cm in diameter, dorsal.	7 and 14 days	The physicochemical properties of the two collagens have comparable characteristics. Goat collagen has better cytocompatibility than bovine collagen. Both

							groups showed almost complete re-epithelialization on day 14. The epidermis was thicker with bovine collagen.
Helary et al	2010	Collagen hydrogel	Wistar rats, male, 250 g	Type I collagen unspecified	A vertical incision on the midline of the back, incisional wounds.	15 and 30 days	Good integration with the skin and cell colonization after 15 days for the highest collagen concentrations.
Wehrhan et al	2010	Bovine collagen membrane (Geistlish)	Adult domestic pig, 6 months old, 80-110 kg	Bovine collagen types I/III	Excisional wounds, full-thickness, 2x2 cm, membrane sutured to the injury, no secondary dressing.	1, 3, 5, 7, 14, 21, and 28 days	Rapid epithelialization, improved keratinocyte proliferation and fixation, similar structure to the basal lamina, functional epidermal layer, increased vascularization 7 days after the injury.
Shilo et al	2013	Recombinant human collagen-based gel (rhCollagen)	Sprague-Dawley rats, male, 200-250 g. Two 37.3 kg and 38.9 kg domestic pigs.	rhCollagen (Collplant) from modified tobacco plants.	1.4 cm X 1.4 cm, full-thickness, square, excisional wounds, bandage.	1, 3, and 6 days for rats; 7 days for one pig and 21 days for the other.	Accelerated wound closure, better epithelialization, and improved inflammation resolution in rats. Faster healing and evolution of these phases in pigs.
Aoki et al	2015	VitriBand, an artificial skin made with film dressing, silicone-coated PET* film, and xerogel collagen membrane	C57BL6 mice, male, 10 weeks old, 25-30 g.	Porcine atelocollagen	Excisional, full-thickness, 15-mm diameter wounds.	7 and 14 days	It induces epithelialization, inhibits myofibroblast differentiation, and reduces inflammation and damage to scar tissue by removing it.
Zhou et al	2016	Tilapia collagen sponge and Tilapia collagen nanofibers	Male Sprague-Dawley (SP) rats, 6-8 weeks old, 200-250 g.	Type I tilapia collagen and tilapia collagen nanofiber	Incisional wound, longitudinal section, sponge added, and the skin sutured. Three full-thickness excisional wounds, 1.8 cm in	7 and 14 days	Non-immunogenic collagen sponge. The nanofibers induced keratinocyte differentiation to form the epidermis *in vitro*.

					diameter, covered with collagen nanofiber, and dressing to close the wound.		Improved healing, lower inflammatory response, and better environment for new epidermis growth because of differentiated epidermal cells, organized basal cells, horny layer, and living keratinocytes.
Shi et al	2019	Porous porcine skin collagen (PSC) and fish scale collagen (FSC) sponge scaffolds were applied 24 hours after the burn	Two white rabbits	PSC and FSC corresponding to type I collagen in rats	Deep burns made with gauze boiled in hot water and applied to the skin for 20 seconds, totaling 20 wounds. The dressing was changed every 3 days.	7, 14, 21, and 28 days	Accelerated re-epithelialization, better scar closure, macroscopically higher presence of hair in the FSC groups, and PSC substitute potential.
Ge et al	2020	Collagen hydrogel containing 10 mg/ml telocollagen.	Sprague-Dawley rats, adults, 250-280 g, male and female.	Telocollagen (PSC) extracted from the skin of tilapia	Second-degree burn with 1.3 cm in diameter, circular-shaped, performed with a small temperature-controlled electric iron.	7, 14, 21, and 28 days	Reduction of the wound area and induction of epithelialization and regeneration of skin appendages.
Lai et al	2020	Application of Sturgeon cartilage collagen (SCC) *in vivo* and *in vitro*	Male C57BL/6J mice, 8-10 weeks	Sturgeon cartilage collagen similar to rat type II collagen	Two 8-mm excisional wounds on the back. The dressing was changed every 2 days.	5 and 10 days	Increased fibroblast proliferation, migration, and invasion, and ECM synthesis with accelerated wound repair.
Sohutskay et al	2020	Oligomeric collagen scaffold as a permanent dermal replacement and integrator	Male Sprague-Dawley rats, 200-250 g, 7-9 weeks old	Type I oligomeric collagen from pig dermis	Two excisional wounds, made with a 15-mm diameter punch, covered with gauze, changed every 7 days, full-thickness, scaffold sutured with silk.	7 and 14 days	Production of collagen-fibrillar structures recapitulates the normal dermis, persistence in the wound bed facilitates the reconstruction of the dermis integrating the tissue, and resistance to wound contraction, but delayed vascularization.

Sumiyoshi et al	2020	Hybrid dermal graft with collagen film in the upper layer and sponge in the lower one. Experiments were performed only with the hybrid film or sponge.	C57BL/6J female mice, 6 weeks old, and transgenic strains with the Col1a2 gene coupled to an EGFP reporter gene (COL/EGFP).	Moon jellyfish type I collagen and type I porcine collagen	Excisional, full-thickness wounds made with a 6-mm punch, covered with a dressing, and sutured with the graft.	6 and 21 days	Higher keratinocyte migration, infiltration of inflammatory cells and fibroblasts, accelerated epidermal re-epithelialization, presence of molecules that maintain the epidermal barrier, and no excessive scar formation.

* Polyethylene terephthalate (PET).

Table 2. Clinical studies with unassociated collagen application analyzed as a research object.

Author	Date	Treatment	Patient group	Collagen type	Wound type	Treatment days	Results
Gao et al	1992	Porcine dermal collagen membrane for both studies	1) Sprague-Dawley rats, female, 250-300 g; 2) 12 patients, 3-38 years old	Collagen extracted from pig skin	1) Deep burn, partial excision, scraped epidermis; 2) Burn, maintained covered with Vaseline gauze.	1) 3 and 10 days; 2) Time required for complete re-epithelialization	1) Wounds with 69% re-epithelialization and no significant difference between contraction and open wounds. 2) Significantly faster re-epithelialization than spontaneous healing.
Uygur et al	2008	Bovine collagen gel-fix spray gauze dressing (gel-fix collagen spray, Euroresearch)	26 patients, male and female, 14-42 years old	Type I bovine collagen	An excisional wound in the partial donor area, patients underwent autologous burn graft, the donor area was the object.	4, 30, 60, and 90 days	Epithelialization of 7-11 days, lower than control, significant pain reduction, no significant difference for the scar at days 30, 60, and 90.
Romanelli et al	2015	ProHeal collagen membrane (MedSkin Solutions, Germany), as a primary dressing, covered with a secondary one changed twice a week	Forty patients with venous leg ulcers but who were not diabetics or smokers, without autoimmune and arterial diseases, or with signs of infection	Collagen of unnamed origin	Venous leg ulcers (VLUs), no healing improvement for 6 weeks with the conventional treatment.	12 weeks	Higher granulation tissue, reduced wound size, some wounds healed before 12 weeks, no side effects and pain during dressing changes.

Shah and Chakravarthy	2015	Collagen dressing (microscaffold) concomitant with antimicrobial agents for topical use in some cases, 2 to 3 times a week with dressing changes.	Patients aged 53-92 years, 15 women and 5 men, with comorbidities	Natural type 1 bovine collagen	Chronic wounds with no results from previous treatments, a total of 21 wounds, biopsy wounds, burns, dehiscence, radiation, surgical site, trauma, and venous stasis.	12 weeks	Within 90 days, 15 of the 21 wounds closed and ten had no concomitant treatment.
Strong et al	2016	Fetal bovine collagen (Primatrix, TEI Biosciences, Boston, MA), antibiotic application to wounds	A 48-year-old patient underwent the first usual treatment, and the burns progressed to a third degree after 7 days	Type III bovine collagen	Large surgically excised TBSA burn, full-thickness, second-degree and deep second-degree, dressing changed every 3 days.	Clinical observation on days 10, 18, and 30. Postoperative examination at 6, 9, 12, and 26 months.	Perceived repopulation and revascularization of cells that allowed migration and re-epithelialization of epidermal cells, complete wound closure, repigmentation, and healthy, soft, and flexible skin without signs of hypertrophy or fibrosis.
Amstrong et al	2020	Acellular matrix of the purified reconstituted bilayer (Geistlish, Derma-Guide)	Ten patients, 65-76 years old, women and men, with comorbidities and foot ulcers with conventional treatment attempts without healing for more than 4 weeks	Porcine collagen	Diabetic foot ulcers closed with a dressing after applying the matrix, changed every 7 days	12 weeks	Wound closure in 9 of 10 patients within 4 weeks. One subject did not follow the protocol. No adverse events or complications during the study. Material with easy application and low cost compared to others.

Collagen-based skin substitutes

One of the possibilities of using collagen involves directly replacing damaged skin, which can occur via collagen grafts or matrices. Grafts can be autologous, allogeneic, and xenogeneic, and the gold standard in the clinic is allogeneic or xenogenic skin and other sources, such as the intestinal submucosa (Sandor et al. 2008). Wounds with collagen-based substitutes show higher granulation tissue formation and re-epithelialization and lower contraction (Leipziger et al. 1985).

Brown et al. (1990) developed acellular dermal collagen grafts (DCGs) from autologous collagen in excisional wounds in Sprague-Dawley rats to achieve the best collagen-based skin substitute. The authors did not find significant differences in wound closure, and denatured grafts lost the ability to resist wound contraction.

Also regarding excisional wounds, van Luyn et al. (1995) evaluated the efficiency of a dermal substitute with two layers of cross-linked sheep collagen (H/HDSC) and another mixed group with a cross-linked and non-cross-linked (N/HDSC) collagen layer in Albino Oxford rats, achieving dermis and epidermis repair and contraction inhibited only with composite substitutes (N/HDSC).

Attempting to find the best collagen source, Lv et al. (2022) developed an acellular dermal matrix of tilapia. The matrix healed excisional wounds in female SPF rats, decreased inflammation, and showed good biocompatibility and biodegradability compared to commercial fetal bovine matrices.

Another approach is mixing different collagen sources to test whether scaffolding options consist of jellyfish and pig collagen, with collagen film in the upper layer and collagen sponge in the lower layer. This hybrid graft was applied to excisional wounds in the skin of C57BL/6J mice, showing a higher keratinocyte migration and molecules maintaining the epidermal barrier (Sumiyoshi et al. 2020).

Type I oligomeric collagen is fibril-forming, highly purified, and has an inherent ability to form fibrillar matrices, an excellent characteristic for scaffold formation (Bailey et al. 2011). Hence, Sohutskay et al. (2020) developed a pig-derived type I oligomeric collagen scaffold to work as a permanent and integrated dermal substitute in excisional and full-thickness wounds in Sprague-Dawley rats, resulting in the production of collagen-fibrillar structures that recapitulate the mechanobiology of the skin and dermal reconstruction improvement by the non-degradation of the material.

Primatrix (TEI Biosciences, Boston, MA) with fetal bovine collagen was applied to a patient with extensive second-degree burns that, after conventional treatment, evolved to third-degree burns with cell repopulation and revascularization that allowed the migration of epidermal cells and complete wound closure with consequent repigmentation within 26 months, in soft and flexible skin (Strong et al. 2016).

The purified reconstituted bilayer acellular matrix (Geistlich, Derma-Guide, Wolhusen, Switzerland) from porcine collagen was applied to patients with diabetic foot ulcers plus comorbidities unresponsive to conventional treatments for more than four weeks. Wound closure occurred in nine out of ten patients within four weeks without adverse effects (Armstrong et al. 2020) (Table 2).

Other techniques

Besides the discussed possibilities of scaffolds and collagen-only substitutes, Lai et al. (2020) applied Sturgeon cartilage collagen (SCC) to excisional wounds of C57BL/6J mice, showing an increased fibroblast proliferation, migration, and invasion in the wound area and ECM deposition that culminated in faster wound repair. In the study, tilapia collagen worked as a positive control and showed better results in fibroblast infiltration because Sturgeon collagen is similar to the denser type II collagen.

Zhou et al. (2016) evaluated the effects of tilapia collagen nanofibers on excisional and full-thickness wounds in Sprague-Dawley rats, noting an induction of keratinocyte differentiation for epidermis formation *in vitro*, a higher healing rate, a lower degree of the inflammatory response, organized basal cells, and horny layer of keratinocytes *in vivo*.

In clinical studies, Uygur et al. (2008) used type I bovine collagen spray (gel-fix collagen spray, EuroResearch, Milano, Italy) with conventional gauze in patients with partial-thickness donor area wounds who underwent autologous grafting to recover from burns. Epithelialization occurred between seven and 11 days and significantly reduced the pain.

Collagen matrices have been studied as potential implants. Rennert et al. (2013) evaluated acellular bovine collagen matrix (PriMatrix) as a subcutaneous implant in C57BL/6J mice. Effectively, the implants underwent progressive remodeling, maintaining tissue with reduced inflammation.

5. Conclusions

Advances in science have allowed the development of grafts considered the gold standard in the clinic, but they also enabled the progress and improvement of collagen-based scaffold materials. There are many possible combinations of scaffolds, collagens, and associations to achieve skin wound regeneration. This article discusses the most recent and revealing associations of collagen with polymers and nanomaterials, which can provide substance delivery and considerable wound repair improvement.

However, using unassociated collagen scaffolds is also feasible and can produce less immunogenic and allergenic materials, which are cheaper and more efficient for skin repair in experimental and clinical models. Moreover, graft materials without needing degradation have been efficient for long-term tissue replacement.

Therefore, this research analyzed the most discussed scaffolds clinically and experimentally, serving as a basis for future studies with collagen materials and assisting in developing science on the subject.

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