ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 17, Issue, 07, pp.33871-33874, July, 2025
DOI: https://doi.org/10.24941/ijcr.49273.07.2025

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ADVANCES AND EFFECTS OF COLLAGEN MEMBRANE

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ARTICLE INFO

Article History:
Received 09th April, 2025
Received in revised form
21st May, 2025
Accepted 19th June, 2025
Published online 30th July, 2025

Keywords:
Collagen Cross-linking, Guided bone regeneration, Glutaraldehyde, Genipin, EDC/NHS, Chitosan, UV light, Dehydrothermal treatment, Enzymatic crosslinking.

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ABSTRACT

Background: Collagen membranes are widely used in guided bone regeneration (GBR) due to their biological properties, but their rapid degradation and poor volume stability limit their effectiveness. Cross-linking methods are employed to enhance the mechanical properties and degradation time of collagen-based materials. **Methods:** This review examines various collagen cross-linking techniques, including glutaraldehyde, genipin, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), chitosan, temperature, UV light, enzymatic methods, and nanoparticles. The structure, preparation, and properties of collagen-based materials are discussed, with a focus on comparing these cross-linking approaches. **Results:** The review highlights that each cross-linking method, such as glutaraldehyde, genipin, EDC/NHS, and others, modifies collagen's mechanical strength and degradation resistance differently. Physical, chemical, and enzymatic cross-linking techniques are analyzed, showing variations in their impact on biocompatibility and structural stability. **Conclusion:** Cross-linking enhances the properties of collagen membranes, but no standardized method exists for creating a robust, biocompatible collagen matrix. The choice of cross-linking technique depends on specific application needs, and further research is needed to optimize these methods for tissue engineering applications.

Citation: Dr. Sarath Jayanth Vinod, Dr. Biju Balakrishnan and Dr. K V Arun. 2025. "Advances and effects of collagen membrane". *International Journal of Current Research, 17, (07), 33871-33874.*

INTRODUCTION

A clinically accepted method for treating bone deficiencies is guided bone regeneration (GBR). By preventing soft tissue invasion from leaving the mucosa and establishing an underlying area to assist bone formation, barrier membranes play a crucial part in this process. (1) The following characteristics should be considered by the GBR membrane: Biocompatibility means that it doesn't harm the surrounding tissue or the healing process; cellular occlusion means that non-osteogenic cells can't invade bone defects from the mucosa; easy handling means that it's not too stiff without compromising its ability to maintain space; bio-activation means that it encourages wound healing and tissue integration. Barrier membranes need to stand for 16–24 weeks to improve bone and 4–6 weeks to regenerate periodontal tissue. Due to their superior biological qualities and the ability to promote bone regeneration without the need for a second surgery to remove them, collagen membranes have garnered a lot of attention (1). Still, significant limiting considerations include the majority of collagen barrier membranes' poor volume stability and quick rates of breakdown. This material class's quick fragmentation and degradation during gingival dehiscence with membrane exposure and the resulting reduced bone repair is another significant drawback. In order to overcome the present material deficiencies, a variety of techniques, including physical, chemical, and enzymatic crosslinking strategies, ultraviolet (UV) radiation, genipin (Gp), and glutaraldehyde treatments, have been examined to prolong the collagen membranes' mechanical properties and degradation time. Additionally, to improve their regenerating capabilities and to avoid membrane collapse and volume stability, collagen membranes are frequently mixed with other substances like bone grafts or resorbable stabilising structures like magnesium meshes.(1)

RESULTS

Collagen fibres' mechanical strength is increased by adding more cross-links, which stop collagen molecules from sliding against one another under stress. Moreover, the structural intricacy and biocompatibility requirements of collagen restrict its possibilities for modification. Unfortunately, there aren't any established cross-linking techniques for creating a robust, biocompatible collagen matrix at the moment. To create a cross-linked collagen matrix, physical cross-linking with ionising radiation, ultraviolet (UV) light treatment, dehydrogenation heat treatment (DHT), or photo-oxidation is typically regarded as an easy and secure process. To enhance

the characteristics of collagen casings (a type of collagen film), Wang et al. employed UV light and DHT. The tensile strength and elongation at break of collagen casings were significantly enhanced by UV treatment, DHT, and their combination; DHT had the most significant effect. The simple process of cross-linking collagen molecules using dehydrothermal (DHT) includes exposing them to high temperatures (>90 °C) in a vacuum. (2) Additionally, it was hypothesised that extra cross-links could form as a result of the interaction between lysine and alanine if DHT treatment was prolonged.(3) DHT delivers sterilisation and is not cytotoxic because it doesn't require any chemical reagents. Collagen denaturation, which involves the reorganisation of the tertiary structure into a less organised structure, can be brought on by high temperatures. (4) Collagen fibres' shrinkage temperature was raised by DHT cross-linking as opposed to UV cross-linking, and their solubility in a solution containing enzymes that break the collagen peptide bond was reduced. According to the statement, DHT cross-linking is most likely more effective than UV cross-linking. Additionally, temperature affects cross-link density; when the temperature rose, the amount of amino groups became less. As a result, denaturation rose with temperature and exposure time. That means a reaction at a certain temperature could result in a scaffold with a certain degree of denaturation.

DHT-cross-linked collagen is important because it promotes the adhesion and growth of human fibroblasts. In comparison to DHT treatment, the UV-induced cross-linking reaction is quicker and more efficient. Free radicals are produced on the aromatic groups of tyrosine and phenylalanine during this process. Chemical bonds are formed when radicals engage with one another. (5) High cross-link density cannot be created by UV light alone.(6) Currently, there are other factors besides UV light that can cause the creation of cross-links. Numerous research groups have expressed interest in the new combined approaches, which seem to be more successful. This method typically creates intra- and intermolecular bonds inside the collagen fibres by reacting a photo-activatable reagent with UV light. Another illustration of UV-based combination techniques is the UV-induced cross-linking reaction of collagen caused by EDC/NHS. The effect of UV irradiation combined with EDC/NHS on the integrin-mediated cellular response to collagen-based scaffolds was ascertained by Bax et al.(7) According to their findings, collagen cross-linking at greater EDC/NHS concentrations results in the creation of material devoid of biological activity. To preserve the biological activity of collagen-based composites, UV irradiation of low-concentration EDC/NHS collagen seems to be a more appropriate technique. Glutaraldehyde (GA) is an aliphatic molecule containing five carbons and an aldehyde group at each end. One of the chemical substances utilised to enhance the mechanical qualities of collagen-based biomaterials is this one. (8) This substance is applied to collagen to prevent enzymatic or thermal deterioration. The ε-amine groups of lysine or hydroxylysine residues react with the aldehyde group of GA to create GA-protein cross-links, as is well known (9). Another significant factor is the concentration of collagen involved in the reaction; if it is too low, no improvement in any significant properties (such as mechanical strength or thermal stability) is seen; conversely, if it is too high, an inhomogeneous reaction may take place. GA is frequently used as a cross-linking agent because of its low cost, high reactivity, and high solubility in water solution Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) can be used to chemically cross-link biological materials. Collagen cross-linking with EDC-NHS causes a covalent bond to develop between the carboxylic acid groups of glutamic and aspartic acid. This composite considerably improves the physicochemical characteristics of collagen and is non-cytotoxic and biocompatible. It is important to note that EDC is transformed into o 1-ethyl-3-(3-dimethylaminopropyl)-urea rather than being released into the matrix (10). Furthermore, EDC-NHS effectively imitates the enzymes that maintain the collagen structure in vivo. (11) Yang (2012) described the cross-linked collagen matrices' shape, swelling characteristics, thermal and enzymatic stability, and effect on cell proliferation. The organised structure of cross-linked collagen promotes cell adhesion and proliferation in addition to increasing its thermal stability. Composites like collagen: gelatin hydrogels cross-linked with EDC-NHS are biocompatible and readily adhere to specific cell types due to their highly porous and interconnected structure. (12) Chitosan is a linear polysaccharide made up of 2-acetamido-2-deoxy-D-glucan (N-acetyl D-glucosamine) and 1-amino-2-deoxy-D-glucan (D-glucosamine) units that are randomly distributed. It is created by subjecting the chitin to an alkaline agent, such as sodium hydroxide. Collagen and chitosan work together to enhance the mechanical and biological characteristics of scaffolds, including their resistance to enzymatic degradation, compressive strength, and degree of water absorption.(13) Chitosan is added to collagen to strengthen its structure and enlarge its pores. The amount of cross-linking agent and the weight ratio of the constituents determine the swelling characteristics of chitosan/collagen composites, it is important to note (14). The impact of collagen-chitosan sponges on the development of preosteoblast cells (MC3T3-E1 cell line) was examined by Arpornmaeklong in 2007. They examined the differences in differentiation and cell growth between chitosan and chitosan-collagen:MC3T3-E1 sponges and found that these characteristics are enhanced when mouse preosteoblast cells adhere to chitosan-collagen:MC3T3-E1.(14) An essential part of Gardenia jasminoides, genipin is a chemical molecule derived from the iridoidglucoside (geniposide). This material has long been utilised in alternative medicine because of its antibacterial, anticancer, and anti-inflammatory qualities.(15) One possible explanation for genipin's ability to cross-link proteins with amino groups is (i) the quick nucleophilic attack of amine groups on lysine and arginine on the genipin's C3 atom, which creates a heterocyclic compound of genipin linked to basic protein residues, and (ii) the gradual nucleophilic substitution of genipin's ester groups, which results in a secondary amide bond (16). Numerous research groups studied genipin as a stabiliser of collagen structure and collagen-based tissue equivalents. According to Shreiber et al. (2008), genpin alters collagen gels.(17) After a concentration of 5 mM and above, genipin became cytotoxic. Since it was established that 10 mMgenipin killed L929 fibroblasts, researchers recommended using 1 mMgenipin in investigations using cellular collagen constructs, but greater genipin doses are acceptable for acellular constructs. It is also well known that genipin and scaffolds containing it promote cell proliferation and differentiation. Enzymatic post-translational changes stabilise collagen fibrils under physiological circumstances (18). Collagen is able to preserve its biological activity, flexibility, and stability as a result. Among these enzymes is lysyl oxidase (LOX), which converts lysine and hydroxylysine's ε-amino groups into aldehyde groups. Microbial transglutaminase (MTG) is currently one of the most used enzymes that strengthens collagen structure. Chen et al. used MTG to study the collagen cross-linking reaction and

describe the characteristics of this composite in 2005(19). In addition to improving collagen's mechanical strength and stability, the MTG cross-linking procedure produced a material that was cytocompatible and promoted the survival of human fibroblasts. (20) The mechanism of action of MTG is based on calcium ions and involves an acyl transfer reaction between a primary amine and a glutamine residue's γ-carboxyamide group. This reaction results in the creation of intramolecular or intermolecular ε-(γ-glutamyl) lysine bonds (21). A new technique called crosslinking with functionalised nanoparticles has made it possible to stabilise and strengthen collagen tissues. Collagen has been crosslinked by using metal oxide nanoparticles to directly bind its side-chain moieties. (22) Crosslinking functional nanoparticles to collagen may improve its physicochemical and biological properties. EDC coupling has been used to crosslink collagen matrices to gold and chitosan nanoparticles. Collagenase recognition sites on collagen molecules are blocked by nanoparticle bonding, protecting scaffolds from enzymatic breakdown. (23) The rough surface of crosslinked nanoparticles encourages cell adhesion and motility on the scaffold surface, resulting in better biological properties. (24) If organic groups are attached to the surface of nanorods, collagen side groups may interact with them over a great distance. The collagen-(3-mercapto-1-porpanal)-ZnO scaffold not only has mechanical properties that are significantly better than native collagen, but it also encourages cell growth. (25) When dendrimer nanoparticles are added, peptide crosslinking can more easily access collagen scaffolds with amine groups. By immobilising the biomolecules on its surface, collagen crosslinked with the nanoparticles shows a prolonged release of the biomolecules. Intelligent biocomposites that can yield beneficial biomolecules are created by this approach. (26)

DISCUSSION

Despite the fact that several crosslinking techniques are available to stabilise the mechanical and degrading characteristics of in-service materials, the crosslinking agents' negative impacts on the scaffolds' biological activities restrict their use in tissue engineering. The migration and adhesion of cells are necessary for tissue regeneration.(27) Although collagen crosslinking may have an impact on both of these characteristics. Integrins allow the triple helical array's receptor recognition motifs to connect with cells in natural type I collagen (11). In regenerative medicine, recombinant collagen scaffolds engineered with integrin-binding motifs may be employed as cell carriers due to their improved cell retention capabilities. EDC-induced crosslinking, regrettably, consumes cell-reactive carboxylate anions (on glutamate or aspartate residues) and free primary amino groups (on lysine residues).(28) As a result, the collagen scaffold has fewer vital cell-binding motifs available and is a less desirable surface for cell attachment. Scientists have been focussing on varying the degree of crosslinking to enhance the quantity of cell-reactive carboxylate anions following EDC/NHS crosslinking, in order to stimulate chondrocyte proliferation and matrix secretion.(29) There has also been research into other crosslinking techniques that do not use cell-reactive carboxylate anions. For instance, collagen's biological functioning can be preserved while undergoing relatively low-level crosslinking between the nuclei of aromatic residues (tyrosine and phenylalanine) thanks to UV irradiation. In order to stabilise protein chains and increase the effectiveness of UV light, glucose is utilised in UV crosslinking (6). Collagen scaffolds incorporated with cells is a potentially promising technique for regenerating tissues such as the dental pulp. Proanthocyanidin-crosslinked collagen scaffolds have the potential to promote proliferation of human periodontal ligament cells. Epicatechin, a natural collagen crosslinking agent, induces odontogenic differentiation of human dental pulp cells. This finding represents an impressive advancement in regenerative dental biotechnology. Collagen structure is maintained, cell infiltration is enhanced, and angiogenesis is accelerated with the use of Traut's reagent and sulfosuccinimidyl 4-(Nmaleimidomethyl)cyclohexane-1-carboxylate crosslinking in mice (30). More strongly aligned collagen networks with greater resistance to proteolytic digestion are produced by the crosslinking that transglutaminases induce. Better fibre alignment is the reason for the overall beneficial effect of enzymatic crosslinking on osteoblast migration, which is caused by the activity of certain matrix metalloproteinases that specifically break down fibrillar collagen (31).

CONCLUSION

The structure of collagen is strengthened by three different kinds of polymerisation techniques: enzymatic, chemical, and physical cross-linking. In order to regulate the biopolymer scaffolds' mechanical stability, rate of degradation, and immunogenicity after implantation, crosslinking techniques have included chemical, physical, and enzymatic approaches. Due in large part to variations in crosslinking methods, concentrations, and exposure times, each crosslinking technique exhibits a distinct level of structural and mechanical stability. As of yet, there isn't a gold standard procedure for crosslinking materials made of collagen. Future studies may focus on the combination of collagen crosslinking and some contemporary technologies, like nanotechnology and biofabrication, rather than just collagen crosslinking.

Abbreviations

- **GBR** - Guided Bone Regeneration
- **UV** - Ultraviolet
- **DHT** - Dehydrothermal Treatment
- **GA** - Glutaraldehyde
- **EDC** - 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
- **NHS** - N-hydroxysuccinimide
- **Gp** - Genipin
- **LOX** - Lysyl Oxidase
- **MTG** - Microbial Transglutaminase

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