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REVIEW ARTICLE

Collagen – Zinc Oxide Nanoparticles (ZnO NPs) Composites for Wound Healing – A Review

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ABSTRACT:
Fish Collagen which is also called as Marine collagen has gained immense attention in the recent years as an appropriate alternative to mammalian collagen. Fish collagen is essentially the superhero of collagen sourced from animals. Fish collagen is made up of mostly Type 1 collagen, which makes up to 70 percent of total skin. Collagen is found to heal wounds by attracting new skin cells to the wound site. It promotes healing and provide platform for new tissue growth. Zinc Oxide Nanoparticles are nanoparticles of ZnO that have diameter less than 100 nanometres. Recently, ZnO Nanoparticles have shown to disrupt bacterial cell membrane integrity, reduce cell surface hydrophobicity and enhancement of intracellular bacterial killing. The ZnO nanoparticles were synthesized from the plant. The paper will describe the potency of fish skin waste, problems in healing burn injuries, collagen extraction, green synthesis of ZnO NPs, collagen and ZnO NPs applications in wound dressing. Since ZnO nanoparticles have much anti-bacterial activity it can be combined with collagen and characterized for the application in wound healing management.

KEYWORDS: Collagen, Zinc Oxide Nanoparticles, Antibacterial analysis, Wound healing management.

INTRODUCTION:
Wound healing, is a notable problem for healthcare systems worldwide, accounting over 1.5% of the total world population.[1] Collagen is found to be a fibrous protein which contributes as a major part in connective tissue of animal skin and bones. Generally, it has been applied in medical, pharmaceutical and cosmetic industries.[2 3] Collagen is one of the most used polymers in biomaterials fields due to its excellent properties in biodegradability, biologic profile and in vivo response.[4] Fish skin can be used as an alternative source for the collagen extraction since mammalian collagen extraction has been reported with several problems.[5] Therefore collagen from various fishes have been extracted and characterized.[678] Being a natural protein, collagen itself cannot heal the infected tissue because bacteria use it as a substrate. On the other hand, ZnO nanoparticles have been found to be non-toxic to animals, and also proved to be efficient antimicrobials.

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Nanoparticles, and their combinations have gained a lot of attention in wound healing applications.[9] This review demonstrates the extraction and characterization of fish skin collagen from fish and efficacy of combined zinc oxide nanoparticles – fish collagen in wound healing application. Collagen is known for its excellent stimulatory effect on new tissue growth, which makes the preparation developed in this study to have promising clinical applications. Hence, Zinc oxide – fish collagen composite can expect to have improved antimicrobial and wound healing properties. The paper will describe the potency of fish skin waste, problems in healing burn injuries, collagen extraction, green synthesis of ZnO NPs, and collagen and ZnO NPs applications in wound dressing. Since ZnO nanoparticles have much anti-bacterial activity it can combined with collagen and characterized for the application in wound healing management. This study confirms its feasibility as an alternative therapeutic agent and excellent.

Fish skin wastes:
Fish skin waste was found to be potentially utilized as a high economic value product. Various studies have been performed to check the potential of fish waste, of all fish,

skin waste was considered to be having much yield value of collagen, which is highly dependent on the extraction material, extraction procedure, type of fish, and collagen extraction techniques.[10] In wound dressing, collagen can be used with high economic value. Normally fish skin consists of two layered tissues, i.e. Outer layer dermis tissue and inner epidermal tissue. The two layers differ in structure, function and origin. The dermis layer consists of most of the collagen fibers. The composition of fish skin is presented in table 1.[11]

Table 1. Chemical composition of fish skin.[11]

Composition	Total (%)
Water	69.6
Protein	26.6
Fat	0.7
Ash	2.5

Collagen:

Collagen is the main structural protein found in various connective tissues in the body. It is the main component of connective tissues and most abundant protein in mammals. It makes up to 25% to 35% of the whole protein content in the body. To date, 30 different types of collagen have been identified, i.e. type 1 to type XXVIII. Type 1 collagen comprises of over 90% of human body.[12] It involves in maintaining tissue shapes. Collagen is found in fibrous tissues such as ligaments, joints, teeth, bones, cartilages, tendons, blood vessels, and skin. Collagen has been utilized in various applications, such as food industry, leather manufacturing, pharmaceutical, and cosmetic industries.[13-16]

Collagen Extraction:

Commercial collagen is obtained from various sources like chicken, pork skin, cows. Whereas the extraction from these sources causes various problems considering religious beliefs and biological contamination such as TSE (Transmissible spongiform Encephalopathy), FMD (Food and Mouth Disease) etc.[17] Currently research focusing on extraction of collagen from fish have been appreciated and welcomed. Collagen can be obtained by two methods either by chemical hydrolysis or enzymatic hydrolysis.[18] The extraction procedures includes three methods, Acid- soluble Collagen(ASC), Pepsin Soluble Collagen (PSC) and hydro extraction.[19] Chemical hydrolysis method is the most commonly performed method of collagen extraction, but enzymatic extraction method produces better collagen with higher nutritional value. The first step in collagen extraction includes, pretreatment with NaOH, to eliminate non-collagen substances to obtain high yield of collagen. There are several types of acids such as acetic acid, lactic acid, citric acid are used in acid extraction process, however organic acids are better in total collagen dissolving. The most commonly used acid for collagen extraction is acetic acid.[20] Various research on collagen extraction includes the combination of different extraction methods. Singh et al [21], extracted collagen from the skin of *Pangasinodon hypophthalamus* using ASC and PSC methods.[21]

Zinc Oxide Nanoparticles:

ZnO is a type of metal oxide. Zinc oxide nanoparticles has found to be most used in past two to three years due to its wide range of applications in the field of electronics, biomedical systems, wound healings, etc.[22-28] There are numerous types of metal oxides have been synthesized till date such as TiO_2, CuO, and ZnO. Of all these, ZnO nanoparticles is widely used since it is less expensive to produce, it is safe and can be produced easily.[29] They have huge range of biomedical applications like drug delivery systems, anti-bacterial, anti-cancer, anti-fungal, anti- inflammatory and anti-diabetic properties.[30-36] It has been used in cosmetics like sunscreens also. It is found to have very strong antibacterial effect on both gram positive (+) and gram negative (-) bacteria even at very low concentration.

Green Synthesis of ZnO NPs using plant extract:

Several plant parts like leaf, stem, root and seed have been utilized for ZnO NPs blend as a result of the elite phytochemicals that they produce. Utilizing regular concentrates of plant parts is an ecofriendly, modest procedure and it does not include utilization of any intermediate base gatherings. It is very cheap and does not require any costly equipment. Plants are most preferred source of NPs synthesis is because they lead to large scale production of stable NPs.[37]

The most common method for ZnO NPs synthesis is made from leaves or flowers of plants. The plant part is made clean by washing it thoroughly with running tap water and it is sterilized using double distilled water. Then the plant part is kept undisturbed at room temperature followed by weighing and crushing it using a mortar and pestle. The plant part is boiled using Milli Q water, and continuous stirring is done using magnetic stirrer.[38-42] The solution is filtered using Whatman filter paper, where the filtrate is used for further studies and residue is discarded. After this, small volume of the filtered extract was taken, mixed with 0.5Mm of zinc nitrate, or zinc sulphate or zinc acetate. After incubation, color change of white or yellow was obtained which is a visual confirmation of the presence of nanoparticles (synthesized NPs).[43-44] This is then followed by UV-Visible spectrophotometry, to confirm the synthesis of NPs. The NPs are then centrifuged and the pellets are taken and dried to get crystal NPs. The synthesized NPs are further characterized using, Fourier Transform Infrared spectroscopy(FTIR), X-Ray Diffractometer (XRD), Scanning Electron Microscopy(SEM), Transmission Electron Microscopy (TEM), Atomic

Force Microscopy (AFM), Photoluminescence Analysis (PL), and Energy Dispersion Analysis of X-Ray(EDAX).[45-47] Azam et al. synthesized ZnO NPs from the plant Aloe Vera (Liliaceae),[48] Jafarirad et al. synthesized ZnO NPs from the plant *Rosa canina* (Rosaceae),[49] Ramesh M et al. synthesized ZnO NPs from the plant *Solanum nigrum* (Solanaceae),[50] Ambica S et al. synthesized ZnO NPs from the plant *Pongamia pinnate* (Legumes).[51 52]

Table.1. Plant mediated synthesis of ZnO NP

S. No.	Plant (family)	Common Name	Part taken for extraction	Reference
1	*Azadirachta indica* (Meliaceae)	Neem	Fresh leaves	53
2	*Agathosma betuline* (Rutaceae)	Buchu	Dry leaves	54
3	*Coptidis rhizoma* (Ranunculaceae)	Coptis Rhizome	Dried Rhizome	55
4	*Aloe Vera* (Liliaceae)	Aloe Vera	Leaf extract	48
5	*Trifolium pretense* (Legumes)	Red clover	Flower	56
6	*Phyllanthus niruri* (Phyllanthaceae)	Stone beaker	Leaf extract	57
7	*Pongamia pinnate* (legumes)	Indian beech	Fresh leaves	51
8	*Rosa canina* (Rosaceae)	Dog rose	Fruit extract	49
9	*Ocimum basilicum* L. var	Red Rubin basil	Leaf extract	58
10	*E. crassipes*	Water hyacinth	Leaf extract	59
11	*Aloe Vera* (Liliaceae)	Aloe Vera	Freeze dried	60
12	*Solanum nigrum* (Solanaceae)	Black nightshade	Leaf extract	50
13	*Anisichils carnosus* (Lamiaceae)	Kapurli	Leaf extract	61
14	*Azadirachta indica* (Meliceae)	Neem	Leaf	62
15	*Coccus nucifera* (Arecaceae)	Coconut	Coconut water	63
16	*Gossypium* (Malvaceae)	Cotton	Cellulosic fibre	64
17	*Moringa oleifera* (Moringaceae)	Drumstick tree	Leaf	65
18	*Azadirachta indica* (Meliaceae)	Neem	Fresh leaves	52
19	*Calotropis gigantean* (Apocynaceae)	Crown flower	Fresh leaves	66
20	*Vitex negundo* (Lamiaceae)	Nochi	Leaf	67

Problems in healing burn injuries:

Burn injuries commonly involves in long term disability. This involves various phases like coagulation, inflammation, granulation, proliferation, matrix synthesis, angiogenesis, re-results in reactions such as drug resistance and allergies. To cope up with this, the drugs are to be derived from natural available sources. [68]

Wound healing phase:

Wound healing phase involves 4 phases, i.e. Haemostasis phase, Defensive or inflammatory phase, Proliferative phase, Maturation phase. Haemostasis is the very first phase of wound healing, which begins at the onset of injury, the aim of this is to stop the bleeding. In this phase, our body will activate its emergency repair system, which is the blood clotting system and will form a dam to block the drainage. The second phase, defensive phase, involves in destroying bacteria and removing debris. This phase prepares the wound bed for the growth of new tissue. Third phase is proliferative phase, involves in 1) filling the wound; 2) Contraction of the wound; 3) covering the wound and the last phase is the maturation phase, where the new tissue slowly gains strength and flexibility.[69]

Wound dressing:

Wound, whether it is a minor cut or major incision, it is very important to care for it properly. Wound dressing is defined to be in contact with the wound. Historically, wet to dry dressings have been extensively used for wound. In 1600 BC, Linen strips were soaked in grease or oil was covered with plasters were used for wounds. Then further treatments were practiced for wound healing like, usage of honey, oil, wine, resin, vinegar, wool boiled in water to the wound healing site.[70] During 19th century, antibiotics were introduced for the control of infections. In 20th century, modern wound dressings arrived.[71] Through this dressing, wounds are exposed to complements, proteinases and growth factors. These dressings help in faster re-epithelialization, collagen synthesis, promotes angiogenesis. During the mid-1990, synthetic wound dressings, expanded into various products including alginates, hydrogels, synthetic foam dressings, silicone meshes, tissue adhesives, hydrogels, collagen containing dressings.

Collagen and ZnO nanoparticles application as wound dressings:

Yang et al.[72] made a research work on collagen dressings. Collagen is extracted from pig skin and made into collagen sheets. These collagen sheets are applied to the burns. The dressing designed for this study is referred as Young Collagenous Wet table membrane (YCMW). These sheets were made of both type 1 and type 2 collagen, initially wet followed by drying when applied to wounds. YCMW sheets are cheap and can help in wound healing without forming antigenicity. The weakness of YCMW sheets involves maceration and difficulty in separation of eschar. These sheets are susceptible to infections, where additional material is needed to overcome this deficiency. YCMW sheets are suitable only for dry and clean wounds.[72] YCMW illustration is given below in the figure (1).

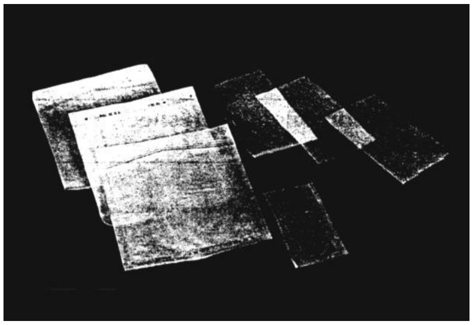

Fig.1. YCMW products and packaging

Xie et al.[73] made a research on wound dressing but developing composites from chitosan, collagen and alginate. This is commonly referred to CCA creation. CCA creation involves in wound healing by inhibiting the sea water. Chitosan are biocompatible, non-toxic, haemostatic, and antibacterial. Collagen has low antigenicity, high antibacterial activity, low inflammation, better biocompatibility, and cell proliferation. Alginate has higher absorbing efficiency of water, and triggers wound healing. CCA creations has higher wound healing capacity.[73] The process and rate of wound healing by CCA in mice are represented in figure (2).

Rabia et al.[74] made a research on ZnO-NPs embedded biodegradable bandages. They developed bandages by composites from alginate, thiolate chitosan, zinc oxide nanoparticles which is commonly referred as (TCS- Alg -ZnO) and chitosan, alginate and zinc oxide nanoparticles, referred as (CS- Alg- ZnO). The ZnO NPs bandages are analysed for electron microscopic analysis, where the bandages impart fibrous nature of the lyophilized bandages. Thus, the porous nature results in wound healing activity. The porosity of CS-Alg-ZnO bandage was 5%, while the porosity of TCS-Alg-ZnO bandages resulted in 45% which purely involves in the healing, cellular organization, angiogenesis of the tissues. Lysozyme degradation of the bandage was carried out for 21 days, which shows that TCS-Alg-ZnO bandage possesses the higher innate ability of lysosomal degradation compared to the CS-Alg-ZnO bandages. In-vivo wound healing capacities of both bandages were carried out for 28 days. This shows that TCS-Alg-ZnO bandages are more enhanced when comparing to CS-Alg-ZnO bandages.[74] The in-vivo analysis of wound healing bandages are illustrated in figure (3).

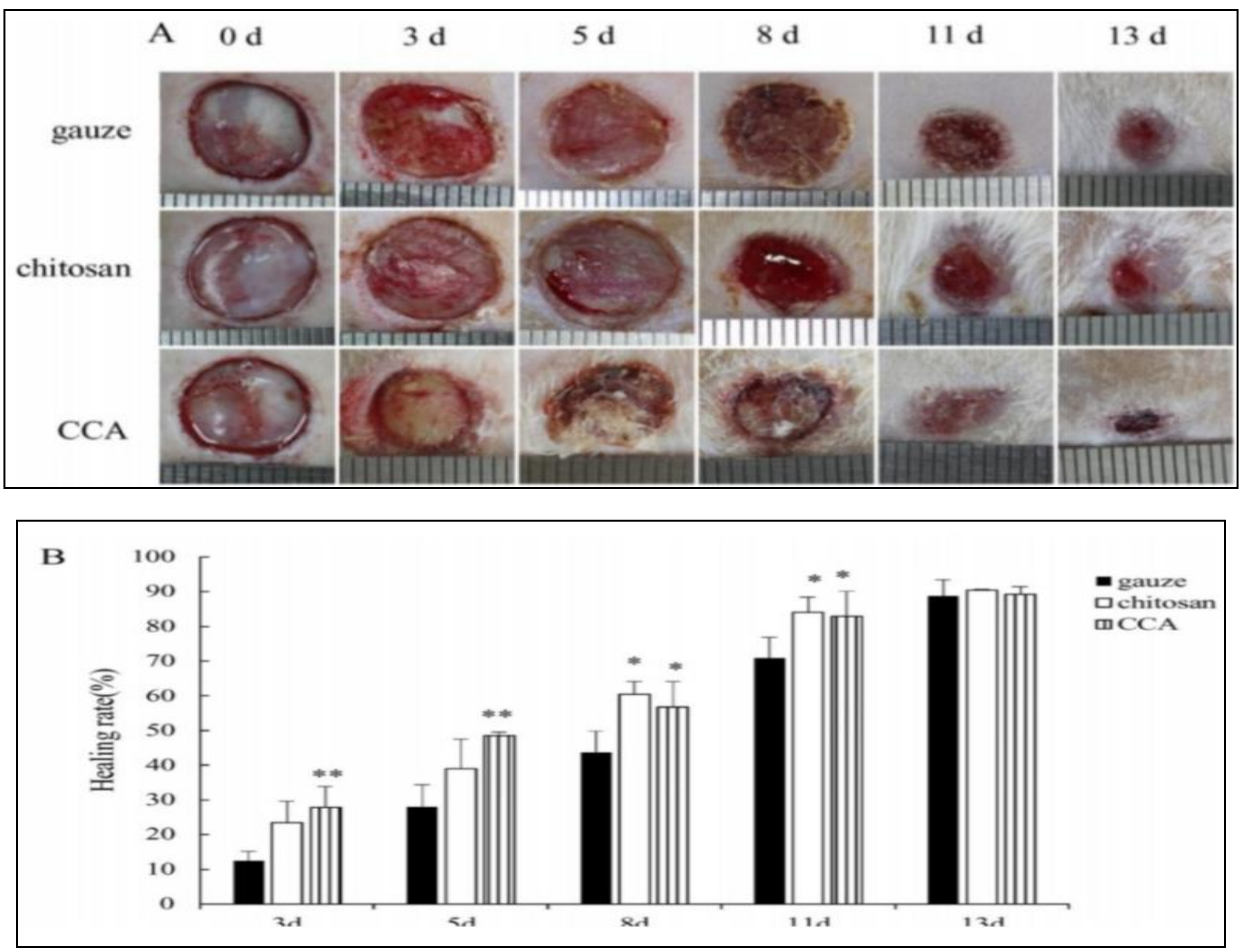

Fig.2. (A) The wound healing process by CCA, chitosan and gauze. (B) The wound healing ratio by CCA, chitosan and gauze.

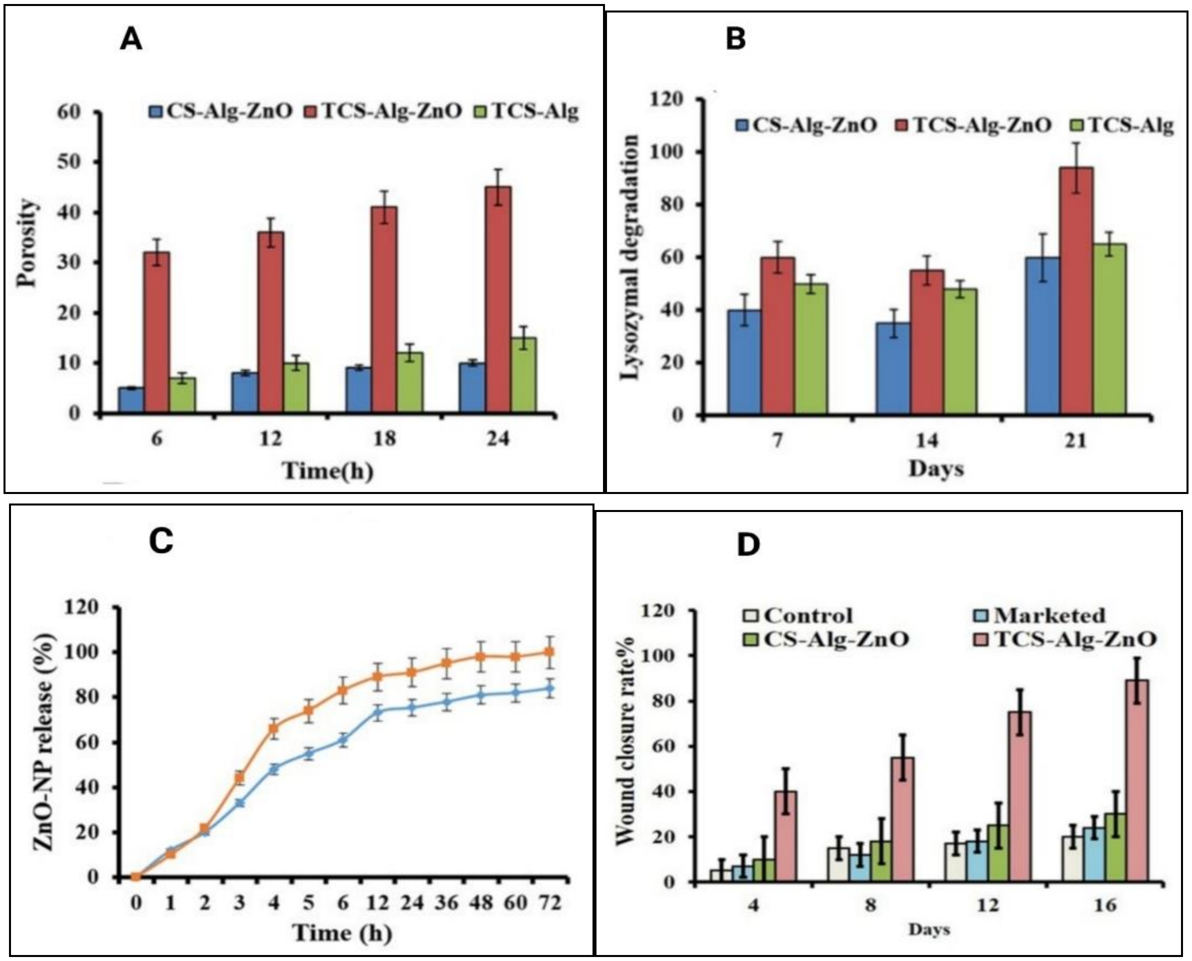

Fig.3. (A) Porosity analysis of CS- Alg- ZnO, TCS- Alg- ZnO and TCS- Alg. (B) Lysosomal degradation analysis of CS- Alg- ZnO, TCS- Alg- ZnO and TCS- Alg. (C) In vitro analysis of ZnO- NPs from CS- Alg- ZnO and TCS- Alg- ZnO bandages. (D) In vivo wound healing analysis graph showing speed of wound closure in terms of reduction in wound size after the application of bandages.

CONCLUSION:

The utilization of fish skin wastes can produce products with high economic values. Biosynthesis of nanoparticles using eco-friendly approach has gained numerous applications in various fields. Wound dressing with collagen and ZnO nanoparticles individually has higher levels of antibacterial activity, non-toxicity, highly antigenic when compared to conventional wound dressings. The wound healing process is very faster and does not cause any pain using the collagen and ZnO NPs. Wound healing treatment using collagen will make the wound heal faster than the use of conventional wound dressing.

The future prospect includes the conjugation of both collagen and ZnO NPs in wound healing applications. Thus, collagen wound dressing combined with other material like ZNO NPs may be used to heal wounds, burns in a better way.

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CONFLICT OF INTERESTS:

There is no conflict of interests.

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