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GELATIN: A VERSATILE BIODEGRADABLE BIOPOLYMER FROM COLLAGEN HYDROLYSIS–PROPERTIES, EMERGING APPLICATIONS, CHALLENGES, AND FUTURE PROSPECTS

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Abstract

Gelatin is a natural, biodegradable biopolymer derived from the partial hydrolysis of collagen obtained from animal sources such as mammals, fish, and poultry. Owing to its biocompatibility, low immunogenicity, and versatile physicochemical properties, gelatin has gained significant attention across multiple industries, including food, cosmetics, pharmaceuticals, and biomedical engineering. Structurally, gelatin consists of shorter polypeptide chains formed by the breakdown of collagen's triple-helix configuration, which imparts unique gelling, emulsifying, and stabilizing characteristics. Recent advancements have expanded gelatin's applications in tissue engineering and regenerative medicine, particularly in wound healing, drug delivery systems, and scaffold fabrication. The source of gelatin plays a crucial role in determining its physicochemical properties and acceptability, with fish gelatin emerging as a promising alternative due to fewer religious restrictions and lower risk of disease transmission. Furthermore, innovations in gelatin-based edible films and coatings have contributed to sustainable food packaging solutions. Despite its advantages, challenges such as low mechanical strength and thermal stability remain, necessitating modifications like cross-linking and composite formation. Overall, gelatin continues to be a highly valuable biomaterial with expanding applications. Future research should focus on enhancing its functional properties, safety evaluation, and large-scale industrial implementation to meet growing global demand.

1. Introduction

Gelatin is a colourless, without flavour or pale-yellow coagulating agent derived from animal collagen. It is a high molecular weight biopolymer, formed by the process of hydrolytic degradation of proteins. Gelatin typically originates from collagen from pigs, cattle, fish and poultry. It is found in the skin, bones, tendons and ligaments of animals [1]. It is a partially hydrolyzed collagen, is biodegradable and has an extensive variety of possibilities for use in food, which includes texture, water-binding and creamy providers, foaming, emulsifier and fining agents, colloid stabilizers, biodegradable materials for packaging, vehicles for encapsulating probiotic living cells, and micro-encapsulating agents [2]. It is a white or yellowish, readily apparent glossy solid, is a partially degraded component of collagen which is present in animal connective tissue [3].

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Figure 1. Powdered Gelatin

As a product of anthropogenic activity gelatin is known since very ancient times. In any case, it had already been observed then that meat and fish broths are sometimes able to automatically solidify in the air without any preliminary cooling with the formation of a specific substance, this was something in the middle of a liquid and solid body. Primarily in the 10th century B.C. the book "Kitab al-Tabikh" described the recipe for the preparation of fish jelly, the primary ingredient of which is in fact one of the different kinds of gelatin, by boiling fish heads [4].

1.1. Difference Between Collagen And Gelatin

Collagen and gelatin are both natural biomaterial s that are frequently utilized in therapeutics, parti cularly for skin wound healing [5]. However, certain differences exist that make one more advantageous to the other. Collagen is the most important structural protein in skin, tendon and bone and is an important protein produced by the body. The word collagen is derived from a Greek word, where "kola" means gum, and "gen" means producing. Collagen is one of the most useful of the biomaterials. It has been extensively researched as polymer for the use in many different biomedical goods such as cosmetic and pharmaceutical products due to its low immunogenicity and high biocompatibility [6].
Gelatin is manufactured by hydrolysis of collagen, and the most found sources are pig skin, cow skin, pig bone, and bovine bone. Gelatin is frequently employed as both a pharmaceutical delivery mechanism for bone-forming active compounds and a scaffolding material. The uncommon arginine-glycine-aspartate (RGD) sequence of gelatin enhances cell adhesion, proliferation, and differentiation [7].Some of the differences between gelatin and collagen are shown in the Table 2.1.

Table 2.1 Differences between collagen and gelatin

Sr No.	CATEGORY	COLLAGEN	GELATIN
1	Chemical structure	Triple helix with thousands of amino acids	Degraded form of collagen with shorter amino acid chains
2	Digestibility	Very easy to digest when taken as collagen peptides	Easy to digest
3	Solubility	Dissolves in hot and cold liquid	Only dissolves in hot liquid
4	Gelling properties	No	Yes
5	Forms	Powder, granulated	Powder, granulated, sheet form
6	Uses	Mostly as a nutritional supplement	Mostly as a gelling agent in cooking

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7	Benefits	For skin, joint, gut, and bone health	For skin, joint, gut, and bone health

1.2. New Advances In The Production Of Gelatin Based Constructs

The most recent developments in tissue engineering have produced innovative solutions that address the drawbacks and make gelatin an acceptable alternative. Because of this, clinical translation may proceed more quickly. The progress made in overcoming the aforementioned constraints is discussed in this section [8]. Illustrative images of the latest advances in the design process of gelatin-based systems are showing in Figure 2.

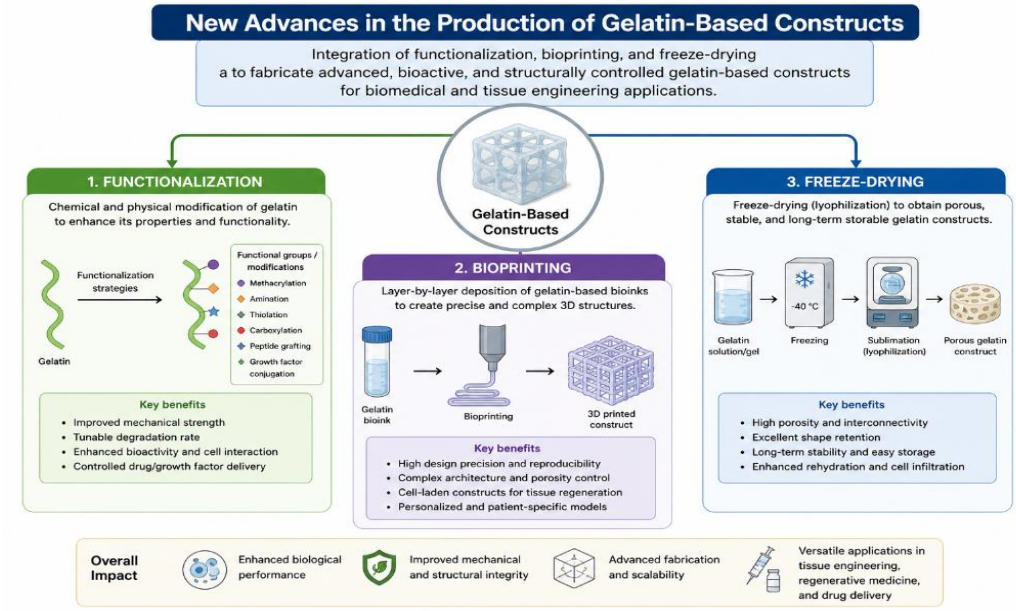

Figure 2. Illustrative image of the latest advances in the design process of gelatin-based systems.

1.3. Structure Of Gelatin

Gelatin is a natural biopolymer that is naturally b iocompatible and biodegradable, has a low immu nogenicity, and has been categorized as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration [9]. It is made up of an amphoteric polymer produced from collagen through alkaline, acidic, or heat hydrolysis [10]. There are two different kinds of gelatin accessible commercially: Type A cationic gelatin, which is produced by partial acid hydrolysis of collagen, and Type B gelatin. During this treatment, the amide groups of glutamine and asparagine undergo modifications to carboxyl groups, prompting the protein isoelectric point to move to higher values (pI = 7-9) [33]. Type B anionic gelatin is derived from collagen processed with an alkali. During alkaline hydrolysis, the asparagine and glutamine amide groups are substantially removed, which leads to an increase in the aspartic and glutamic acid concentrations [11, 12].

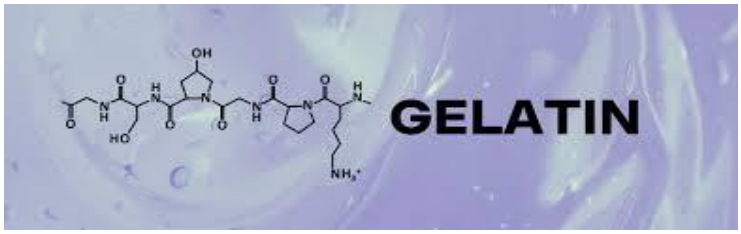

Figure 3. Structure of Gelatin

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2. Applications Of Gelatin

2.2. Cosmetic Applications

Many cosmetic products, which include face crea ms, body lotions, shampoos, hair sprays, sun prot ection products, and bathing salts and bubbles, i mplement gelatin as a gelling ingredient [13]. The skin's fundamental purpose is to act as a barrier against external environmental variables that include sunlight. Skin ageing is an oxidative process that causes the release of free radicals. It includes both inherent (age) and exogenous factors (exposure to UV radiation (A and B). However, the skin has its own antioxidant defense system. The skin's natural pigment, melanin, absorbs ultraviolet (UV) light [14].

Fish gelatin hydrolysates (proteins and peptides) were employed to prevent UV-induced skin damage. They fixated the damage done to the skin's structure by protecting the skin's lipids balanced due to their antioxidant characteristics' light induces an impairment in antioxidant enzymes such as total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), which contribute to an endogenous system that protects the skin from oxidative stress. Polypeptides synthesized from Pacific cod (Gadus macrocephalus) have been investigated on mice to see how they counteract photoaging and oxidative damage to the skin. The study concluded that utilizing gelatin hydrolysates greatly boosted the activity of T-SOD, CAT, and GSH-Px. Hydrolyzed gelatin inhibited NF-κB expression, resulting to reduced lipid peroxidation and a lower concentration of inflammatory cytokines in experimental mice. Another study observed the protective benefits of gelatin and hydrolysates extracted from salmon skin on mouse skin. Again, gelatin and its hydrolysate were found to reduce the harmful consequences of UV radiation by strengthening T-SOD, CAT, and GSH-Px. Another technique that makes use of gelatin and its hydrolysates in the protection against UV radiation is strengthening the immunity by boosting the thymus index and boosting the immune increasing hydroxyproline in the skin, which is an indication for the collagen content. Similarly, gelatin hydrolysates that comes from tilapia gelatin (Oreochromis niloticus) have been demonstrated to have a scavenging action against reactive oxygen species of UV, which causes skin damage and premature ageing. Thus, fish gelatin/hydrolysates could be considered a novel source of component with future potential for use in skin anti-aging therapy applications [15, 16]. Figure 4. Shows the illustration of the cosmetic applications of the gelatin.

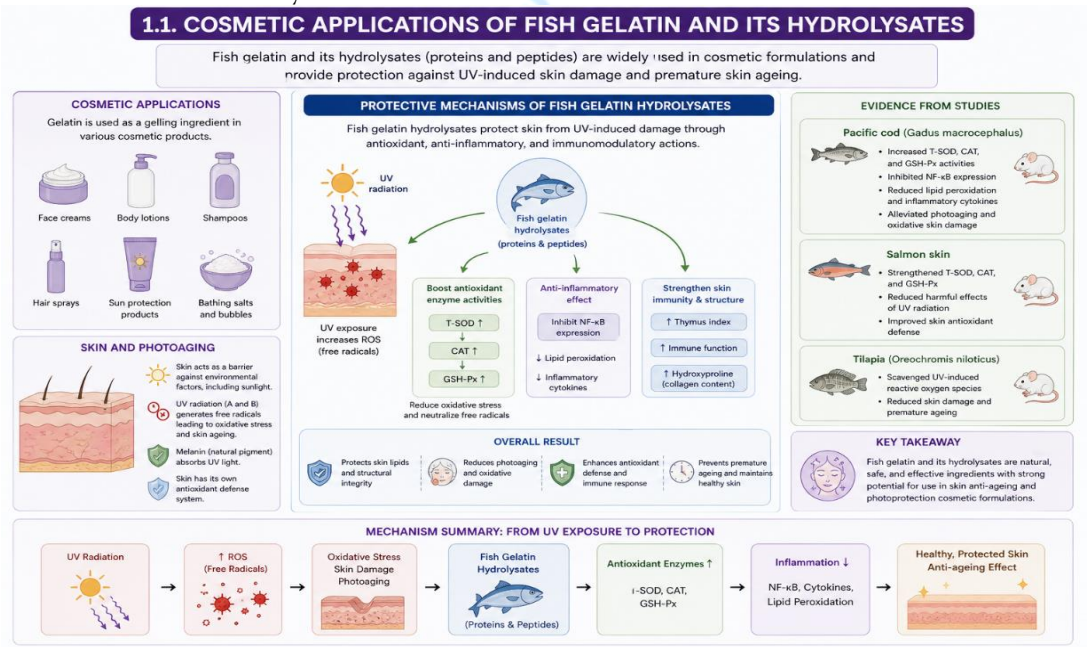

Figure 4. Cosmetic applications of the gelatin.

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2.3. Biomedical Applications

2.3.1. Antihypertensive Activities

A particular type of treatment for hypertension is angiotensin-converting enzyme (ACE) inhibitor. Initiatives to develop novel ACE inhibitors ended up resulting in medication candidates with a variety of undesirable effects, including dizziness, headache, dysgeusia, cough, and rash. Biologically active peptides, such as those in fish gelatin (mostly obtained from skin and fish scales), are comparatively priced, abundant, and safe, and could be employed as antihypertensive ingredients in functional suppers in order to help treat persons with high blood pressure [17].

Antihypertensive peptides obtained from fish gelatin acted in two ways: competitive inhibition and noncompetitive inhibition. The presence of C-terminal tryptophan, tyrosine, phenylalanine, or proline, in addition to N-terminal branched-chain aliphatic amino acids, typically indicates a competitive inhibitory mechanism in peptides. These structural components competed with angiotensin-I to bind ACE, prohibiting the synthesis of angiotensin-II. When the noncompetitive inhibitory peptides bound to ACE, they produced an inactive complex, preventing the enzyme from attaching to another substrate. ACE inhibitory peptides with a non-competitive mechanism of action usually display a high hydrophobicity at their N terminus. The most efficient ACE inhibitory peptides contained short-chain peptides with hydrophobic or positively charged residues at the C-terminus. Antihypertensive peptides have been extracted from fish gelatin by enzyme digestion, mainly employing alcalase, pepsin, trypsin, pronase E, and collagenase [17]. Figure 5 Shows the illustration of the biomedical applications of the gelatin.

2.3.2. Antioxidant Activity

Natural antioxidants are starting to gain popularity since they are both safe and readily accessible. Peptides produced from collagen may have varying levels of antioxidant activity. The peptides isolated from fish gelatin hydrolysed protein displayed free radical scavenging action, decreased lipid peroxidation, protected DNA from hydroxyl radical degradation, and worked as chelating agents [17]. Figure 5 Shows the illustration of the biomedical applications of the gelatin.

2.3.3. Antimicrobial Activity

To exert antibacterial effects, peptides ought to have less than 50 amino acids, roughly half of which are hydrophobic, and have a molecular weight of less than 10 kDa. As the primary innate immune mechanism, numerous species produce Antimicrobial Peptides (AMPs). AMPs played an important part in natural immunity by directly interacting with bacteria and eradicating them. Compared when compared to conventional bactericidal antibiotics, they killed germs faster and were not influenced by antibiotic resistance mechanisms. Figure 5 Shows the illustration of the biomedical applications of the gelatin.

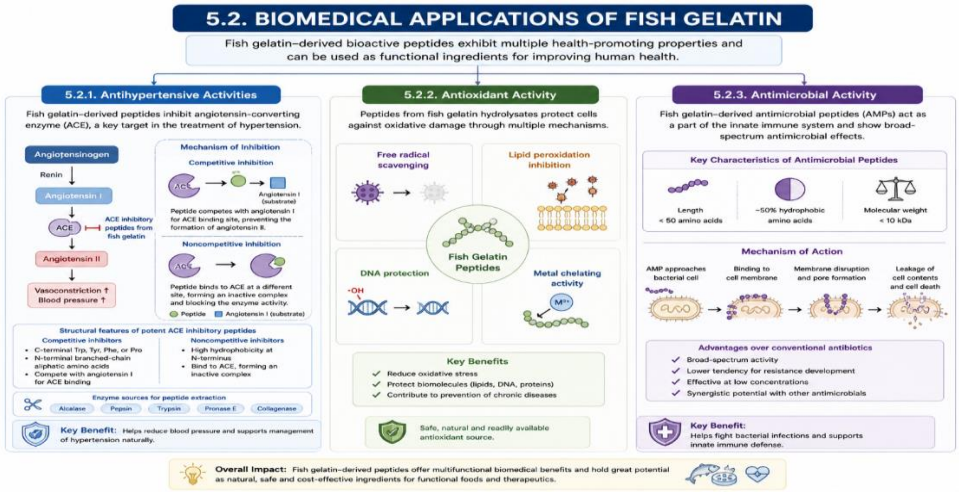

Figure 5. Biomedical applications of gelatin

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2.4. Pharmaceutical Applications

2.4.1. Production Of Drug Capsules

Capsules are frequently employed orally simply because of their availability, practicality, and ability to mask unpleasant odours and tastes of pharmaceutical products [18]. There are two different kinds of gelatin capsules: (i) hard gelatin capsules, which are frequently applied for powders, and (ii) soft gelatin capsules, which are typically used for liquids. These capsules should be sturdy and sufficiently flexible to withstand high-speed filling apparatus, as well as contain softening characteristics that allow the capsule to seal quickly. The film-forming properties of fish gelatin, which is employed in capsule manufacture, have been extensively studied. The main issue was the low gelling temperature caused by low proline and hydroxyproline levels. This particular problem was resolved by crosslinking with trans-glutaminase [19]. Figure 6 Shoes the illustration of the pharmaceutical activities of the gelatin.

2.4.2. Drug Delivery

Natural biomaterials, that includes collagen [20] and gelatin [21], are chosen for drug delivery via various channels due to their greater biocompatibility than synthetic polymers [22]. Gelatin has been used significantly in research [21, 22]. Gelatin is nontoxic and biodegradable. Its qualities can be further improved by modification [23]. For example, cross-linking gelatin can improve its stability and prolong its circulation time in vivo [24]. It can also block its expansion in water, reduce its porosity to cell membranes, and decrease its solubility at high temperatures [25]. Figure 6 shows the illustration of the pharmaceutical activities of the gelatin.

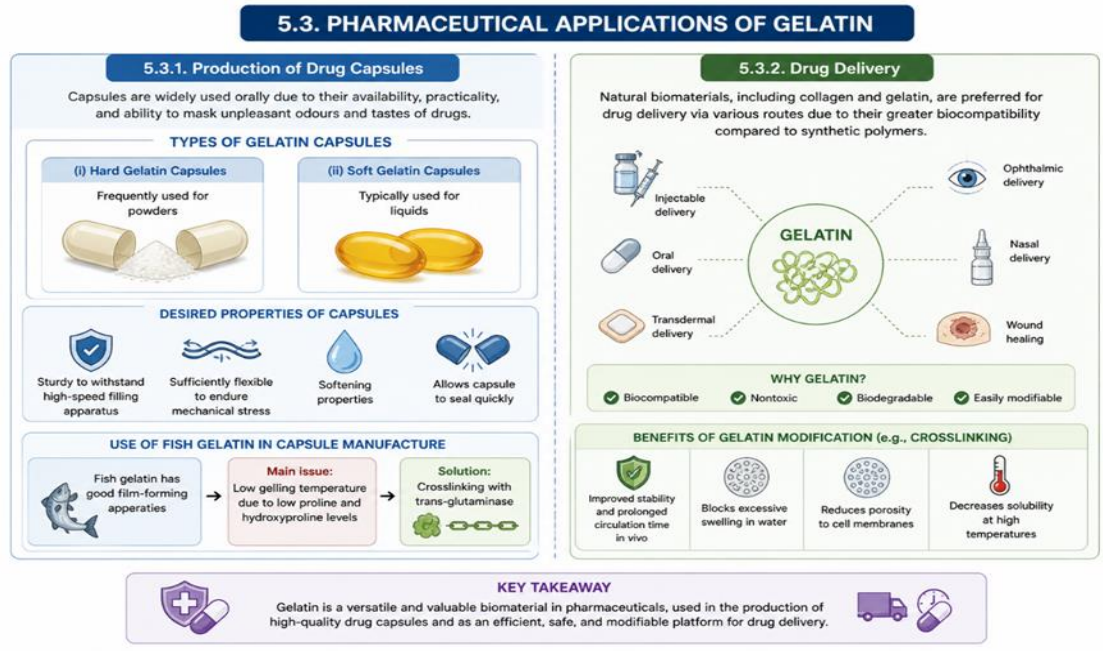

Figure 6. Pharmaceutical applications of the gelatin

3. Gelatin And Gelatin Nanoparticles

Gelatin, a biodegradable polymer, has gained greater attention in recent years due to its wide range of potential uses and environmentally friendly attributes [26]. Gelatin is a natural and renewable chemical that can be exploited throughout numerous sectors, including bioengineering, pharmaceuticals, and the food industry [27, 28]. Gelatin originates from the incomplete hydrolysis of collagen, which is present in tissue from animals like bones, hide, and pigskin [29]. Porcine skin accounts for the majority of gelatin manufacturing (46%), while bovine hide and bones contribute 29.4% and

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23.1%, correspondingly [30]. Fish are responsible for only about 1.5% [31]. Gelatin can be separated into two types: Type A, which is cationic and has an isoelectric point (IEP) of 7 to 9, and Type B, which is anionic and has an IEP of 4.8 to 5. The United States Food and Drug Administration (FDA) likewise perceives gelatin a safe polymer [32].

4. Fish Gelatin

Fish gelatin is a potential source for the creation of natural-based polymers, including hydrogels. Fish gelatin-based hydrogels are made from pure fish gelatin or by rearranging its functional group to create fish gelatin hybrid hydrogels. Fish gelatin composite hydrogels combine fish gelatin with other polymers or materials. Modifying fish gelatin will create a network precursor that is easier to cross-link and polymerize. Fish gelatin has been explored for hydrogel and composite hydrogel fabrication. It is mostly derived from cold-water fish, particularly cod, pollock, haddock, sea bream scales, and marketed cold-water species [33]. Research on fish gelatin-based hydrogels and composites provides several reasons for selecting this material. Fish gelatin is generally selected based on social, religious, and cultural considerations, as well as health risks, economic factors and physicochemical features of fish gelatin [33]. Fish gelatin is generally tolerated across socioreligious and cultural groups, but mammalian gelatin is considered illegal in some places. For example, porcine gelatin is not approved for ingestion by muslims and the bulk of the Hindu population is against beef gelatin, although Jews do. Fish gelatin is not a source of zoonotic infections or viruses, making it safe for consumption. Fish gelatin is a cost-effective option as it is primarily produced from by-products of fish processing, including skin and bones. Fish gelatin has a lower hydrophobic amino acid concentration compared to mammalian gelatin and other natural protein-based hydrogels, which produces a liquid and viscous consistency at room temperature. Such a characteristic is crucial for creating 3D bioprinting ink and injection technology [33].

5. Clinical Application for Wound Healing

In today's world therapeutic settings incorporate absorbable gelatin hemostatic sponges [34]. Gelatin is frequently administered in intraoperative and postoperative patients and is most used in clinical practice simply because of its hemostatic action. Gelatin sponge exhibits good restorability and histocompatibility. Its porous structure can absorb blood expansion, destroy platelets, release coagulation-activating enzymes, accelerate coagulation, enhance the development of blood clots, and achieve hemostasis. It is a loose, porous sponge-like substance with an excellent capacity to absorb water [35]. Clinicians have publicly acknowledged the success rate of gelatin hemostatic sponges, even if they come from different manufacturers. When gelatin materials are incorporated together, the hemostatic impact is greater than when gelatin is applied alone [36]. There have been reports of gelatin-based material combination medical treatment. In 2015, Naoki Morimoto et al. employed a combination of platelet-rich plasma (PRP) and gelatin for the treatment of persistent skin ulcers. Vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), and platelet-derived growth factor (PDGF) are all brimming in PRP and may accelerate the healing of chronic wounds [37]. Because the hemostatic agent's cascade response with coagulation brings about in a biochemical cross-linking effect that has greater adhesion, flexibility, and hemostatic affect than SURGIFLO®, it is advantageous [38].

6. Sources Of Gelatin

6.1. Gelatin From Mammalian Sources

Mammals, primarily cattle and pigs, are the main manufacturer of gelatin, accounting for 46% of pig skin, 29.4% of bovine hide, and 23.1% of pork and cow bones [39, 40]. Since they are so readily available, bovine and pig skin gelatins are frequently employed in the food manufacturing sector. Type B gelatin is often generated by alkaline treatment of cow skin, whereas type A gelatin is produced by acidic treatment of pig skin [30], with isoelectric points of pH 4.8–5.5 and pH 7–9.4, respectively [41]. Glycine, proline, and arginine equates have been observed to be higher

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in porcine gelatin's amino acid composition than in bovine gelatin [42]. Because of its robust film-forming capabilities and outstanding gel characteristics (viscosity and strength), mammalian gelatin is more commonly employed than that from other sources. On top of that, a lot of research has been done on intelligent and active mammalian gelatin, particularly active bovine gelatin/nano chitin/corn oil composite film [43] and intelligent bovine/curcumin composite film [44].

On the other hand, because they cannot be used or consumed by Muslims, Jews, or Hindus for a variety of reasons, mammalian gelatins pose significant negative consequences and problems with relation to religious concerns and Halal issues [45]. Furthermore, alternative gelatin sources for porcine and bovine gelatin substitution have been prioritised and taken into consideration due to the potential hazards of spreading harmful pathogens derived from bovine spongiform encephalopathy (BSE), also known as mad cow diseases and food and mouth disease (FMD) [46]. The food and beverage industry has benefited enormously from the utilisation of alternative gelatin from other sources because of the rapidly growing demand for halal-certified products on a global scale [45].

6.2. Gelatin from marine source

Mammalian gelatin has bloom values around 130 and 308 g, however fish gelatin usually has a lower bloom value between 0 and 270 g. Because of differences in proline and hydroxyproline content in collagens from various species and environment temperature, marine gelatins may display a wider spectrum of bloom values. Viscosity values (cP) for the gelatin skin of various freshwater fish species have been observed to range from 1.87 to 3.63 cP [47]. According to Muyonga et al. [48], the levels of proline and hydroxyproline in warm-water and cold-water fish were typically 22–25% and 17%, respectively. According to a study by Sila et al. [49], a significant amount of glycine and imino acid residues were identified in the amino acid profile of the gelatin derived from the skin of European eels (Anguilla anguilla). Fish gelatin does, in general, show excellent properties in films; it is translucent, nearly colorless, soluble in water, and very extensible [50]. Active and intelligent marine gelatin films, such as active fish skin gelatin/peppermint essential oils composite films [51] and intelligent fish gelatin/haskap berry extracts composite films, have been the subject of numerous examinations [52]. Sarbon discovered that the viscosity value of chicken gelatin was 150 mL/g, while that of bovine gelatin was 127 mL/g. Additionally, it was discovered that amino acids like glycine (33.70 and 32.84%, respectively), proline (13.42 and 12.09%, respectively), hydroxyproline (12.13 and 9.65%, respectively), and alanine (10.08 and 11.06%, respectively) were present in the gelatin from chicken skin and duck feet, which played a role to the heightened gel strength and stability [53].

6.3. Gelatin From Poultry Source

As a replacement to mammalian resources, new gelatin sources such chicken skin, feet, and bone sparked interest [53-55]. Duck, chicken, and turkey species are among the poultry species utilized. In accordance with reports, avian gelatin has molecular weight (285,000 g/mol) [53, 55], secondary structure, and amino acids that are roughly comparable to those of mammalian gelatin (350.00 g/mol). In the opinion of Sarbon et al. [53] and Rahman and Jamalulail [56], the bloom value of chicken skin and chicken foot gelatin is substantially higher (355.00 g and 264.33 g, respectively) than that of bovine gelatin (229.00 g) [57].

7. Properties of Gelatin in Wound Dressing Application

Wound dressings are frequently constructed with a variety of natural polymers. Gelatin, cellulose, alginate, collagen, elastin, chitosan, chitin, dextran, and other polymers are among them. Good biocompatibility and biodegradation, non-toxicity, non-immunogenicity, and affordability are all intriguing characteristics of natural polymers. Additionally, certain natural polymers have a great affinity for tissues that have been damaged, promote blood coagulation, speeds up the healing of infections, and stimulate skin regeneration [58]. One biopolymer that is frequently employed in the creation of wound

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dressings is gelatin. Furthermore, it is implemented in pharmaceutical and biological applications. A significant number of biomedical researchers have been interested in using gelatin primarily due to its high biocompatibility, good biodegradability, cell-interactivity, non-immunogenicity, exceptional processability, ready availability, and cost-effectiveness. Gelatin is a well-known biopolymer that has numerous biological applications because of its comparatively low antigenicity. Nevertheless, since gelatin is a hydrophilic protein and crosslinking is typically needed to improve its mechanical performance and long-term reliability, gelatin products are insoluble in biological settings. There are multiple mechanisms to crosslink gelatin, including chemical methods utilizing fructose, diepoxy, genipin, dextran dialdehyde, formaldehyde, diisocyanates, glutaraldehyde, or carbodiimides, or enzyme-based approaches employing transglutaminase [58]. Some properties of gelatin are in figure 7.

Figure 7. Showing some of the properties of the gelatin

8. Current And Future Trends

In order for it to satisfy customer requirements and meet the growing demand for gelatin worldwide, numerous strategies and studies have been conducted focusing on the creation of gelatin film packaging utilized from alternative sources, such as seafood and poultry. Additionally, a lot of research has been done recently to further develop and broaden the functioning and features of gelatin-based films by adding different kinds of active ingredients such metal oxides, natural extracts, and essential oils. It has been discovered that introducing these active ingredients improves the intended products' sensory qualities and microbiological safety while continuing to maintain their quality [59].Improvements in the future should concentrate more on the toxicity, migration evaluations, and risk assessment associated with the use of active or intelligent agents in gelatin packaging film, as well as their possible effects on the environment and human health. Additionally, while these films are still far behind the superior barrier function offered by synthetic packaging, more study is required in the search for components that can enhance the gelatin film barrier and functional features, including the incorporation of nano-engineered materials. Furthermore, research on the realistic use of gelatin-based film on an industrial scale is at present lacking. Consequently, more application testing for gelatin-based films should be conducted, including the use of these films in conventional packaging machines and as flexible, intelligent, and active packaging for a variety of

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food goods that satisfy the needs of consumers. Additionally, greater research on labelling details employing modern technology for example, flexography printing for gelatin-based film should be conducted [40].

9. Gelatin Based Hybrid Wound Healing

9.1 Hydrogels

The high retention of considerable quantities of water and other biological fluids within their three-dimensional network is made achievable by the hydrophilic composition of hydrogels, which are considered polymeric materials [60]. In the event of extended interaction with biological fluids, they can be transformed for improved stability or loss of function. These polymeric materials' biodegradation, biocompatibility, porosity, ability to contain and release biologically active compounds, flexibility, and high-water content contribute to making them useful in wound healing applications [61]. Patient compliance, an accelerated wound healing mechanism, the high adsorption capacity of physiological fluids which deliver moisture to the wound bed, their ability to effectively protect the wound from microorganisms, and specific environmental stimuli-responsiveness (e.g., pH, temperature, and ionic strength) are additional benefits of polymer-based hydrogels that have been attracting a lot of interest from biomedical researchers in the field of wound management. The wound dressings' responsiveness to environmental cues facilitates drug release into the infected wound region in a sustained profile, which diminishes the frequency of prescribed doses [62, 63]. Figure 8 Showing crosslinked hydrogel on skin wound.

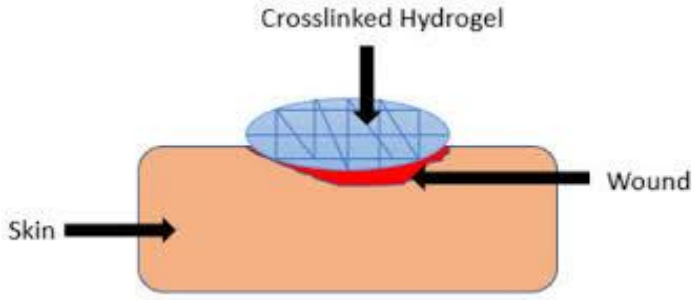

Figure 8. Illustrating the crosslinked hydrogel on skin wound.

For the medical management of wounds caused by diabetes, Hsu et al. created gelatin-hyaluronic acid (HA) hybrid hydrogels encapsulated with recombinant thrombomodulin via a process of chemical cross-linking and freeze-drying [64]. The scanning electron microscopy (SEM) photographs demonstrated porous morphology, implying that an increase in the HA standard concentration reduced the hydrogels' porosity. Within the first 30 minutes, the hybrid hydrogels' water absorption quickly increased, and within 24 hours, they swelled more than 11 times. This is highly beneficial for drug absorption, wound exudate absorption, and provide a moist environment for the injury bed. When compared to pristine hybrid hydrogels, the thrombomodulin-loaded hybrid hydrogels considerably accelerated wound contraction after two days of wounding, as demonstrated in in vivo wound closure tests conducted with streptozotocin-induced mice [64]. Gelatin-oxidized starch hybrid hydrogels were established by Mao et al. for use in wound healing. The hydrogels' high cell viability of skin fibroblasts (L929 cells) was observed in the in vitro cytotoxicity assays, suggesting their good biocompatibility and non-toxicity. When the wounds in the rabbit model were treated with hybrid hydrogels, the in vivo wound healing experiments showed an accelerated wound healing process with decreased scar formation [65]. Zheng et al. developed injectable hydrogels for antimicrobial wound dressings using gelatin and gellan covered with tannic acid. Tannic acid-loaded hybrid hydrogels shown more effective antibacterial activity against S. aureus, E. coli, and drug-resistant bacteria (methicillin-resistant S.

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aureus [MRSA]) in an in vitro antimicrobial examination utilizing agar disc diffusion. According to in vivo research on full-thickness wounds in a mouse model, wounds treated with hydrogels loaded with tannic acid healed significantly more quickly and were completely closed on the twelfth day following surgery without leaving behind scars [66].

9.2. Films And Membranes

Films are translucent, resilient, semi-permeable bandages. Films establish a moist environment, help to promote cell motility, encourage autolysis, are partially permeable to oxygen and water vapour, and prevent the growth of germs [67]. They can be utilised for treating superficial wounds, mild exuding wounds, and chronic wounds (for example, such as those on the elbow, heels, and flat parts of the body). Films are inexpensive, waterproof, rid themselves of necrotic debris, and allow for periodic wound inspections [68]. Most film dressings are either non-absorptive or less absorptive, which is a negative aspect. They might result in skin maceration when removed. The film dressings are changed once a week, depending on an excessive amount of exudates [69].

A number of investigators have reported adopting hybrid film wound dressings based on gelatin. For wound healing applications, Taheri et al. came up with gelatin-chitosan hybrid films encapsulated with tannic acid and/or bacterial nanocellulose [70]. Sakthiguru and Sithique invented gelatin-chitosan biocomposite films that contained allantoin for application during wound healing [71]. Allantoin-incorporated hybrid films illustrated improved water absorption capacity in the water absorption tests. Excellent biocompatibility and non-toxicity have been revealed by the in vitro cytotoxicity tests of all the films evaluated using the MTT assay, which exhibited over 80% cell viability when incubated with L929 fibroblasts. When comparing to free allantoin and plain films, the antimicrobial investigations showed that allantoin-loaded films proved to have better antibacterial activity against S. aureus and E. coli, suggesting that allantoin-incorporated dressings are promising antibacterial wound dressing materials [71] as shown in Figure 9.

Figure 9. Transparent/adhesive film dressing on skin wound

For administering lupeol for use as a wound dressing, Patel et al. created gelatin-chitosan films. The hybrid films' SEM micrograph images demonstrated a comparatively smooth, fibrous, and porous morphology that is suitable for expanding the oxygen supply to the lesion for

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quicker wound healing. Lupeol was discharged from the films in a biphasic pattern, with an initial burst release and an ongoing release of 90.99 ± 1.27% lupeol after 24 hours, according to the in vitro drug release studies. The in vitro antioxidant tests showed that adding lupeol to the gelatin-based hybrid films substantially enhanced their capacity to scavenge free radicals, exposing the wound-healing capabilities of lupeol-loaded films during the inflammatory stage of wound healing [72]. Cahú et al. manufactured hybrid films of gelatin, chitosan, and chondroitin 4-sulfate which contained zinc oxide (ZnO) nanoparticles. Excellent biocompatibility and a strong antibacterial impact against S. aureus and E. coli in vitro have been demonstrated by the nanoparticle-loaded films' lack of toxicity towards skin fibroblasts (3T3) or keratinocytes (HaCaT) cell lines. Gelatin-based hybrid films dramatically boosted the percentage of wound reduction from 65% to 86% in full-thickness excision after six days, compared to only 51% for the control, as determined by in vivo wound healing examinations utilizing the rat model [73].

9.3. Sponges

Soft and malleable sponges have a micro-pore structure that is complexly connected. They have strong fluid absorption ability, cell interaction, and hydrophilicity because of their distinguishing structural characteristics [74]. They are recommended for preventing the accumulation of exudates due to their great swelling capacity and rapid hemostatic abilities. In conjunction with creating a moist environment, sponges that sufficiently absorb wound exudates shield the wound bed in opposition to bacterial invasion [75]. Biomedical dressings have made considerable use of porous sponges made of both natural and synthetic biological polymers. Tissue advancement was accelerated by sponges with interconnected channels and pore diameters of pore size ranging from 10 to 100 microns in diameter. Biomedical dressings have made considerable use of porous sponges made of both natural and synthetic biological polymers. Tissue advancement was accelerated by sponges with interconnected channels and pore diameters of pore size ranging from 10 to 100 microns in diameter [76].

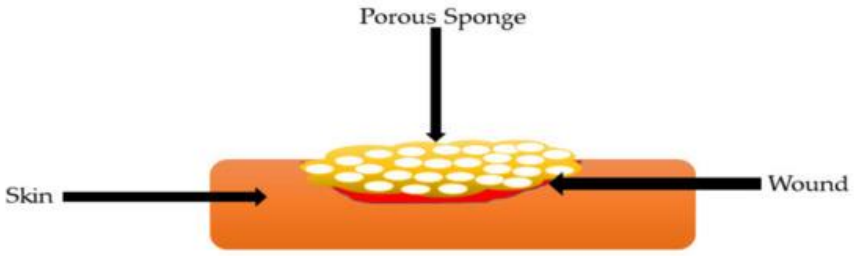

Figure 10. Microporous Sponge on Skin Wound

Gelatin-konjac glucomannan hybrid sponges wrapped with gentamicin sulphate and gold (Au) nanoparticles were reported by Zou et al. to treat bacterial wounds. The transmission electron microscopy (TEM) images revealed that Au nanoparticles exhibited an average particle size of 3.55 0.26 nm and were spherical or elliptical in a single dispersed mode. These dual drug-loaded sponges were exhibited to be non-toxic by the in vitro cytotoxicity analysis of the hybrid sponges, which revealed a cell survival value of more than 88% on L929 cells. The dual drug-loaded sponges showed greater antibacterial efficacy against E. coli, S. aureus, and MRSA in the in vitro antimicrobial tests, but the plain hybrid sponges showed no antibacterial properties against these bacterial species. When compared to other groups, the full thickness wounds treated with dual drug-loaded hybrid sponges nearly entirely healed on day 14, according to in vivo experiments utilising the rabbit model [77].

9.4. Nanofibers And Nanofibrous Materials

Materials that are used to heal wounds with diameters ranging from several nanometres to a few microns are called nanofibers [78].

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Electrospinning is a commonly employed method for preparing these materials (Figure 8) [75]. Because of their potential to transport drugs, electrospun nanofibers are regarded as suitable wound dressings for chronic wounds. By imitating the extracellular matrix, nanofibers encourage the growth of new tissues and the multiplication of epithelial cells in the wound area [79]. High-gas permeation, cell respiration, high surface area to volume, high porosity, enhancing fluid absorption, encouraging dermal drug delivery, stimulating haemostasis of damaged tissues, and maintaining a moist environment—all of which prevent microbial infections are among the many advantages of nanofibers and nanofibrous dressings [80, 81].

Electrospun hybrid nanofibers based on PCL and gelatin cephalexin were created by Bakhsheshi-Rad et al. Cephalexin has been included to the nanofibers in order to treat wounds antimicrobially [82]. For wound treatment, İnal and Mülazımoğlu stated electrospun nanofibers made from gelatin and poly([2-(methacryloyloxy) ethyl] trimethylammonium chloride) (PMETAC). Over 80% of the gelatin-PMETAC nanofibers decomposed quickly in the first week of the degradation experiments conducted in a similar skin-like environment. When nanofibers were cultured with L929 fibroblast cells, the in vitro cytotoxicity tests utilising the MTT assay revealed minimal toxicity. High inhibition of more than 90% against E. coli, S. aureus, and Acinetobacter baumannii was demonstrated by the in vitro antibacterial tests of nanofibers. Methicillin-resistant Staphylococcus aureus (MRSA) showed a 75% inhibition. The presence of PMETAC produced these superior antibacterial properties [83].

Astragaloside IV-encapsulated gelatin-silk fibroin electrospun nanofibers were created by Zhang et al. for the management of wounds. The drug-loaded and plain hybrid nanofibers have porosities of 89% and 88%, respectively, which are suitable for a perfect wound dressing. Astragaloside IV-loaded nanofibers had a rapid drug release profile for the first 12 hours, followed by a gradual release that is appropriate for wound healing. Comparatively to the blank nanofiber dressing and pure astragaloside IV solution groups, the drug-encapsulated nanofiber dressings significantly accelerated the rate of healing during the beginning phases of damage, according to the wound healing experiments. The aforementioned results are due to the astragaloside-loaded hybrid nanofibers' effective barrier against pathogenic organisms and good biocompatibility [84].

9.5. Gelatin-Based Microspheres

Traditionally made of resorbable or biodegradable polymers, microspheres are spherical shells [85]. They generally have a diameter of only a few micrometres. High drug load capacity, site-specific action, regulated drug release, and outstanding resilience (chemically, physically, and thermally) constitute some of the distinctive characteristics of this kind of device. They are also cheap, straightforward to make, and environmentally friendly [86]. To increase mechanical characteristics, Che et al. incorporated gelatin microspheres to a composite hydrogel. Before being added to the hydrogel, the gelatin microspheres were created using an emulsion cross-linking technique. The hydrogel with outstanding mechanical strength had a fast gelation time and little capacity for swelling when the microsphere ratios were increased to 40 mg/mL. However, the hydrogel containing 30 mg/mL of the gelatin microspheres demonstrated strong bacterial growth suppression effects against S. aureus and Escherichia coli, and it also demonstrated good stability and mechanical properties deemed suitable for wound healing [87].

Ciprofloxacin hydrochloride-loaded gelatin microspheres were introduced to chitosan/gelatin composites by Fang et al. The wound dressings showed outstanding mechanical qualities, high water absorption capacity, suitable porosity, and biocompatibility. Both in vitro and in vivo, the drug release profile was maintained with a strong antibacterial impact against S. aureus, P. aeruginosa, and E. coli. Collagen deposition had been accelerated up, making microspheres a viable wound dressing for bacterially infected and seawater-immersed wounds [88]. Thyagarajan et al., prepared wound dressings that will inhibit

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overexpression of matrix metalloproteinase, a family of endopeptidases involved in the remodelling of ECM, which is also capable of degrading ECM when over-expressed. Gelatin and siderophore were used that establishes microspheres. Siderophores are iron chelators that minimise the bacterial load and inhibit matrix metalloproteinase at the wound site. The microspheres had a mean diameter of 7.0 ± 0.52–25.3 ± 0.31 µm, were stiff, and had of significance porosity. The microspheres encouraged adhesion between cells and proliferation, were biocompatible with NIH 3T3 fibroblast cell lines, and had a quick drug release profile [89]. The wound dressings might have been used to treat burn wounds that are infected with microorganisms. Kirubanandan et al. manufactured porous collagen scaffolds which incorporated gelatin microspheres loaded with ciprofloxacin. Ciprofloxacin was delivered from the scaffolds under control for two days, with a 27% drug burst release in the first 5 hours of administration. In vitro, scaffolds significantly inhibited pseudomonas pathogens. An in vivo investigation of the scaffolds in full-thick wounds showed quicker recuperation within 20 days. Regeneration of the dermis and epidermis at the wound site verified the closure of the wound. By breaking down gelatin in the infected wound environment, the ciprofloxacin-loaded gelatin microspheres in the scaffold facilitated a prolonged release profile of the medication [90].

10. The Preparation Methods of Gelatin - Based Edible Composite Films and Coatings

10.1. Solution Casting

The food packaging industry makes use of the solution casting method, a moulding technique, in order to produce gelatin-based composite films by dissolving biopolymers and mixing them with plasticisers or additives [91]. Food preservation and packaging are just two of the industrial uses for chitosan, a soluble derivative of chitin. Research revealed that the solution casting procedure is used to create composite films based on chitosan gelatin [92]. Steps for gelatin solution casting employing chitosan were described by Roy et al. Equal amounts of chitosan and gelatin were added to a 1% acetic acid aqueous solution, mixed, heated to dissolve, and then refrigerated to room temperature. After dispersing Tween 80 and 2 weight percent cinnamon essential oil (CEO) in distilled water and vigorously stirring, 1.0 weight percent rutin (based on biopolymers) was added to the membrane-forming solution. Before peeling, the film-forming solution was cast onto a 24 cm by 30 cm flat Teflon film-coated glass plate and allowed to dry at room temperature for 48 hours. Functional films based on chitosan and gelatin have potent antibacterial and antioxidant properties and can be used in active packaging [93].

10.2. Extrusion

Extrusion moulding is a plastic processing technique capable of producing a range of cross section products or semi-products by transferring the material through the role between the extruder barrel and the screw, with one side being heated and formed into plastic and the other side being pushed forward by the screw. Conventional commercial plastic packaging films are mostly made using the extrusion method. In general, it is more commonly utilized than solution casting in the processing of rubber, plastic, and fiber due to its high production efficiency and low energy usage [94]. Cheng et al. homogenized glycerol, deionized water, beeswax, and Tween 80 with the produced starch/gelatin mixture for an additional 10 minutes after mixing gelatin with the lake for a period of five minutes at room temperature. The extrusion temperature from barrel to die was set at 90, 100, 105, 110, 100, and 90 °C, while the screw speed was set at 125 rpm. The extrudates were broken up into particles and kept at 23 ± 2 °C and 53 ± 2% relative humidity for at least 72 hours before blowing the film [95].

10.3. Coating

Fruits and vegetables typically have a coating with edible coatings by spraying or dipping them in liquid [96]. In most cases, film-forming materials and additives make up edible coatings. Proteins, polysaccharides, and lipids are just a few of the naturally occurring polymers that work effectively as film-forming ingredients in food coatings. Fresh

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persimmons, tomatoes, cherries, bread, and other fresh foods are preserved using gelatin, a biopolymer derived from collagen, as a film-forming ingredient in edible coatings [97, 98]. Gelatin at a 2% (w/v) solution and carboxymethyl chitosan (CMCS) solution were made simultaneously in distilled water in a water bath at 60 °C. Before cooling to 23 ± 1 °C, the liquid was constantly agitated for 30 minutes until it was completely dissolved. At 23 ± 1 °C, CMCS and gelatin were brought together in a 2:1 ratio. 1% glycerol and 0.1% Tween 20, which served as plasticizer and surfactant, respectively, were added to the mixture and agitated for a period of thirty minutes. To prepare the film, the mixture was centrifuged to eliminate air bubbles and particles. The supernatant was then collected [98].

Chin et al. mixed the 6% wt/v gelatin dispersion with 30% wt/wt glycerol. To create coating films, aloe vera extraction was added to the gelatin dispersion at various proportions (1–9% wt/wt). The solubility and plasticity of the composite coating greatly improved. Apart from that, as the concentration of aloe gel increased, the coating demonstrated improved mechanical capabilities [99]. In accordance to a different study, furcellanan and gelatin were incorporated with water (0.5:1:98.5 v/v/v) for 30 minutes at 50 °C. Tea extract or water (for control samples) with strong antibacterial and antioxidant qualities was subsequently incorporated along with glycerin (1% v/w). In order to generate an edible coating solution, the mixture was agitated for 15 minutes at 300 rpm [99].

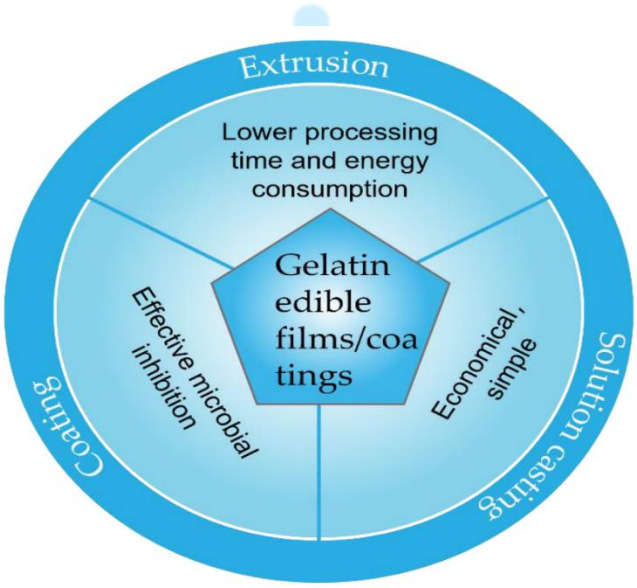

Figure 11. Illustrating three different preparation methods of gelatin based edible composite films and coatings

11. Bone Regeneration

Bone is a complex hard connective tissue that gives the body form, protects various internal organs, preserves structural integrity, and makes getting around easier. Furthermore, it helps maintain homeostasis, store minerals, and control blood pH. Bone remodelling is an ongoing endeavour that depends on the balance between osteoclasts' bone resorption and osteoblasts' bone deposition, which are crucial for bone regeneration, healing, and the preservation of the tissue's structural integrity. It is widely acknowledged that bones can heal itself in cases of small fractures without the need for surgery. Nevertheless, due to a lack of coordinated bone regeneration, patients with extensive bone defects lack the ability to mend themselves. The most common bone problems that impact an individual's longevity and quality of life are bone injuries, tumours, diabetes, abnormalities, and ageing. According to statistics, bone tissue loss from illness or trauma affects over twenty million people globally each year. It's

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important to remember that the most difficult and pressing issues in bone surgery are thought to be complex fractures and fixing bone abnormalities [100]. One of the most effective materials for creating a bone scaffold is gelatin. In addition to its low mechanical strength, gelatin possesses excellent biodegradability and non-antigenicity. It's interesting to note that guided bone regeneration (GBR) has demonstrated an important purpose in bone tissue engineering. GBR is a popular and efficient technique for augmenting alveolar bone abnormalities. GBR may enhance the cell adhesion and proliferation required for bone repair. In addition to having excellent mechanical qualities, GBR is known to be biocompatible and biodegradable. For improved tissue restoration, nanofibrous GBR membranes are more appropriate. Electrospinning has been utilised to create PCL and Gel nanofibrous membranes with various weight ratios. PCL/Gel hybrid nanofiber was successfully produced using acetic acid. Nanofibers with a diameter of 200 to 600 nm had homogenous, smooth structures. Cell viability and wettability, which encourage adherence of cells and proliferation, may be enhanced by the addition of gelatin. Hybrid PCL/Gel membranes may improve the capacity for osteogenesis. This demonstrated that the nanofibers improve the development of bone [101].

12. Conclusion

Gelatin is a versatile natural biopolymer derived from collagen, known for its biocompatibility, biodegradability, and low immunogenicity. Its functional properties, including gelling, film-forming, and water-binding abilities, enable wide applications in food, cosmetics, pharmaceuticals, and biomedical fields. The source of gelatin mammalian, marine, or poultry affects its properties and acceptability, with fish gelatin emerging as a favorable alternative due to safety, cost-effectiveness, and fewer religious concerns, despite some limitations in mechanical strength and thermal stability. Recent advances in gelatin-based materials, such as hydrogels, films, sponges, nanofibers, and microspheres, have enhanced its applications in wound healing, drug delivery, and tissue engineering. These systems offer controlled drug release, improved cell interaction, and effective healing performance. Gelatin is also increasingly used in biodegradable packaging through edible films and coatings. However, challenges such as low mechanical strength, thermal instability, and solubility issues remain. These limitations can be improved through crosslinking and composite development. Overall, gelatin remains a promising biomaterial, with future research needed to enhance its performance and expand its large-scale, sustainable applications.

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