

Original Article

Extraction and Characterization of Fish Skin Collagen for the Fabrication of Mangiferin-Enhanced Biomaterial for Wound Healing Application

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Collagen is a major component of the extracellular matrix, a biocompatible and biodegradable protein which is useful in wound healing biomaterials. Mammalian collagen sources are, however, associated with potential zoonosis and immunogenicity and this has resulted in increased interest in the recovery of collagen from fish processing waste. The skin of *Coryphaena hippurus* (mahi-mahi) was used as an induced source of type I collagen, which was isolated by an acid-assisted extraction method which includes the following stages: alkali treatment, defatting, digestion in acetic acid, salting-out, dialysis and lyophilization. The morphology, chemical structure and conformation of the extracted collagen was determined by SEM, FTIR and CD spectroscopy, which revealed that the native triple-helical structure of collagen was retained following extraction. The antioxidant, anti-inflammatory and anti-microbial property of mangiferin made it an ideal choice to be incorporated at two different concentrations (1mg/mL and 5mg/mL) in the collagen matrix with the tricalcium phosphate (TCP) to fabricate a wound healing composite scaffold. The surface morphology, functional group composition, crystallinity, thermal stability, porosity and cell viability of the fabricated scaffolds were evaluated using SEM, FTIR, XRD, TGA/DSC, ethanol infiltration method and MTT assay, respectively. The results showed that the native structure of mahi-mahi skin collagen did not change after extraction, and mangiferin was incorporated without affecting the integrity of the scaffold. The mangiferin-loaded scaffolds showed a dose-dependent reduction in porosity as the amount of mangiferin was increased, and had good thermal stability and cytocompatibility, suggesting possible usage as a sustainable, bioactive material for wound healing applications. This research fills a significant gap in the literature by assessing the physicochemical compatibility of fish-skin collagen and mangiferin as a basis for the development of an affordable marine biomaterial for tissue engineering and regeneration-based wound care.

Introduction

Collagen is the most abundant structural protein in the human body, secreted by fibroblasts, osteoblasts, chondroblasts, and several epithelial cells, and comprising a family of at least 30 genetically distinct sub-type [1,2]. It is synthesized as precursor procollagen polypeptide chains in the rough endoplasmic reticulum of fibroblasts, with the polypeptide chains being translated from mRNA, which is rich in glycine, proline and hydroxyproline residues. After post-translational hydroxylation and glycosylation that stabilizes the triple-helical structure, procollagen

is passed through the Golgi apparatus and then released into the extracellular matrix [3,4]. There, the procollagen is processed by procollagen peptidases to produce the mature collagen which self-associates into fibrils stabilized by cross-linking by lysyl oxidase, that is, by producing tensile strength and elasticity characteristic of the mature collagen network [5,6]. This matrix is not fixed and is continually remodelled through a continuous process of regulation by matrix metalloproteinases and their inhibitors, which is a key mechanism of tissue maintenance, repair and wound healing [4].

Among collagen subtypes, Type I is the major collagen isoform in

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the body (~90% of body's collagen) and is the most important isoform for wound-care biomaterials, and is the most relevant to this application. Type II is involved in elastic cartilage and joint function, Type III found in muscle, arteries, and organs, Type IV found in skin basement-membrane networks, and Type V found in the cornea, select layers of skin, hair, and placental tissue [7,8].

Mammalian (bovine and porcine) sources have long been the source for the production of collagen for biomedical applications, but have well documented zoonotic disease transmission, religious/cultural restrictions, and immunogenic reactions in sensitive individuals [9,10]. Such concerns have led to an increase in interest in marine collagen from fish, which is derived from the skin, scales, bones, and fins that are typically used as low value by-products in the fishing industry [11]. The species that are most widely studied sources include *Coryphaena hippurus* (mahi-mahi), *Gadus morhua* (Atlantic cod), and *Salmo salar* (Atlantic salmon) due to their preferred collagen content and structure [12]. Marine-based collagen poses no risk of disease transmission, has no religious restrictions, is economically processible and has a smaller peptide fragment size compared to mammalian based collagen, making it more easily absorbed by the human body, which makes it a sustainable option to assess fishery waste [10,13].

But the methods of extraction are of importance to the quantity and purity of collagen recovered. Acid-soluble extraction using dilute acetic acid is generally the mildest method for preserving native collagen structure but is comparatively low-yielding. For example, acid-soluble collagen (ASC) isolated from the skin of striped catfish (*Pangasianodon hypophthalmus*) yielded only 5.1%, wet-weight basis, compared with 7.7% for pepsin-soluble collagen (PSC) extracted from the same skin, giving a combined accumulated yield of 12.8% [14]. Enzymatic (pepsin-assisted) extraction, when optimized properly, can greatly improve the yield: response surface optimization of pepsin concentration, solid to liquid ratio and hydrolysis time in giant croaker (*Nibea japonica*) skin resulted in a yield as high as 84.85% [15]. A practical drawback is the increased complexity of the enzymatic process, which relies on pepsin from pigs. A lack of consistency across species and protocols makes it difficult to find an extraction method that is standardized, and high yielding for many underutilized fish species such as mahi-mahi [12].

Further, extracted collagen must be characterized using several techniques for biomedical applications: morphological and microstructural analysis (SEM); chemical analysis (SDS-PAGE for protein and molecular weight profiling, FTIR for secondary structure, UV-Vis for purity and concentration); crystallographic evaluation (XRD); thermal and mechanical testing (TGA, tensile/compressive analysis). Many reported extraction studies only address a subset of these, thus only a limited number of parameters are considered for the structural integrity of the resulting biomaterial [12,16].

Another drawback is the fact that although collagen is structurally biocompatible, it does not provide much active pharmacological effect to the wound microenvironment. This has led to the development of interest in the functionalization of collagen scaffolds with bioactive natural compounds [4,17]. Mangiferin, a C-glucosylxanthone polyphenol isolated from the bark, leaves and fruit peel of *Mangifera indica* (mango), is a strong candidate: flavonoids and mangiferin found in mango by-products are highly associated with the wound-healing process and have antioxidant, anti-inflammatory, and antibacterial properties [18,19]. The activity of COX-1 and COX-2 is inhibited, leading to reduced production of PGE₂, the activation of the NLRP3 inflammasome is inhibited and the migration and invasiveness of macrophages are increased via activation of Nrf2, thus promoting improved wound healing [20,21]. For applied dressing systems, mangiferin containing formulations have been correlated with greater re-epithelialization, improved neo-vascularization, free-radical scavenging and decreased inflammation when compared to untreated controls [18,19].

Although the single effects of marine collagen and mangiferin were documented individually, the combination is still not studied much, especially the physical and chemical compatibility of the combination of mangiferin and fish skin collagen, and the concentration of mangiferin that can be added without destroying the integrity of the collagen scaffolds. This is a clear and readily attainable research opportunity that is

missing in the field of wound-care biomaterials [22].

Therefore, the present study is focused on to extract collagen from fish skin and characterize its morphology and structural properties using SEM, FTIR, and CD, along with evaluation of relevant biochemical properties such as collagen content. In addition, the prepared collagen matrix was loaded with mangiferin at selected concentrations, and its physical and chemical compatibility was evaluated to assess its potential for improving the therapeutic efficacy of the resultant wound-healing biomaterial.

Materials and Methods

Raw materials and reagents

Fresh skin of *Coryphaena hippurus* (mahi-mahi) was used as the raw material for collagen extraction. All the chemicals (sodium hydroxide, n-butanol, acetic acid and sodium chloride) used for pretreatment, extraction and purification were of analytical grade and distilled water was used throughout. Beakers (100, 500, 1000 and 2000 mL), Falcon tubes (5, 20 and 50 mL), a spatula, a thermometer, a muslin cloth, a dialysis tubing, a magnetic stirrer, a homogenizer, and a centrifuge were used for processing.

Pretreatment and extraction of collagen

Mahi-mahi skin was subjected to an acid-assisted extraction protocol including multiple steps to obtain type I collagen. The fish skin was initially peeled off manually and was cleaned in detail with deionized water to get rid of any scales and impurities on the surface. This was then divided into smaller portions to enhance the surface area for treatment. The cleaned skin was rinsed in 0.8 M saline solution for 10 minutes to remove water-soluble proteins and contaminants, in preparation for alkali treatment [23,24].

The skin was then treated with sodium hydroxide solution and left to incubate for 4 days to eliminate non-collagenous proteins, fats, and pigments without destroying the collagen fibres. After alkali treatment, the samples were washed extensively to remove the alkali and homogenized at 2000 rpm, 4°C to further release the tissue into smaller particles for further downstream extraction. The homogenized skin was then treated with n-butanol and homogenized for 48 hours at 4°C at 2000 rpm to remove the lipids [23,25].

Collagen was then acid digested for three days in an acetic acid solution which caused the collagen fibres to detach from the skin matrix of its surroundings and allowed for the isolation of type I collagen. The filtered mixture was clarified by centrifugation to collect the soluble fraction, which contained the collagen, from the solid fraction [26].

Desalting was performed by stirring the collagen solution with 5% (w/v) sodium chloride at 5-minute intervals until a white precipitate appeared, indicating salting-out of the collagen [27]. The precipitate was left to settle for 24 hours before being centrifuged and purified.

Purification by dialysis

The collagen precipitated by salting-out was dialyzed for purification. Dialysis tubing was first soaked in distilled water at 100°C for 10 minutes to remove preservatives and then cooled to room temperature before use. The collagen solution was poured into the dialysis bag with both ends tightly closed to prevent any leakage and was then placed in a beaker containing acetic acid solution on a magnetic stirrer to allow continuous stirring [28]. To ensure the removal of salts and low molecular weight impurities, the acetic acid dialysate was changed every 4–6 hours, which created a concentration gradient [29]. The dialysis was performed for four days; the first two days with acetic acid as the dialysis solution, and the last two days with distilled water, and then the dialysis bag was opened and the purified collagen solution was collected [28].

Lyophilization

The purified collagen solution was freeze dried into a stable and storable product. The solution was first quickly frozen at -80°C to enable water in the sample to crystallize in fine crystals without disrupting the native molecular structure of the collagen. Primary drying was then per-

formed under vacuum conditions allowing the ice to sublime directly from the solid state to the gas state, without causing any chemical changes in the collagen, mild heat was applied to speed up the sublimation process [30]. Secondary drying was then performed in which the temperature was increased a little more to remove some of the water held in the bound form, leaving the final moisture content below 5%. The freeze-dried collagen thus recovered was light, porous and easily rehydrated, and stored at 4°C until needed, so that it would be stable and not degrade.

Characterization of extracted collagen

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The chemical composition and secondary structure of the extracted collagen was investigated by using FTIR spectroscopy. The characteristic amide band (amide I, II, and III) that represents the peptide bonds of the protein backbone was analysed in the spectra, and these bands are used as markers of triple-helical integrity. Changes or reduction in these characteristic peaks was considered as an indication of the preservation or degradation of native collagen structure after extraction and processing [31,32].

Scanning electron microscopy (SEM) analysis

SEM was employed to examine the surface morphology of the extracted collagen. The high-resolution imaging showed the fibrous architecture, fibre alignment and porosity of samples, which are directly linked to mechanical and biological performance of collagen in tissue engineering products. SEM analysis helped to visualize the characteristic fibrillar network of native collagen and helped to identify any microstructural changes because of the processing or crosslinking [33].

Circular dichroism (CD) spectroscopy analysis

The secondary structure and conformational stability of collagen obtained by extraction was analysed by circular dichroism (CD) spectroscopy, especially for triple-helical conformation [34]. CD is a property that depends on the chiral architecture of the secondary structure of the protein, which is very sensitive to the difference in the absorption of left- and right-handed circularly polarized light. The native, structurally intact collagen has positive peak at 221-222nm and negative peak at 197-200nm which is characteristic of a preserved triple helix [35]. Peak intensities and shapes were used to determine the helical content and to detect structural perturbations due to thermal denaturation, pH changes or chemical modification, to compare native and denatured collagen and evaluate the crosslinking efficacy.

All three methods (SEM, FTIR, and CD) contributed complementary structural and functional information for the complete characterization of collagen for biomedical and industrial applications.

Fabrication of unloaded and mangiferin-loaded collagen scaffold

Fish collagen was extracted and used as the main biomaterial on the scaffold, for cell adhesion and tissue regeneration. To improve the antioxidant and wound healing properties of the scaffold, a natural polyphenol, mangiferin, was added to the extracted collagen. To stimulate mineralization and bone-regenerative properties, a bioactive ceramic, tricalcium phosphate (TCP) was added to the formulation. A magnetic stirrer was used to homogenize the formulation, and well plates were used for subsequent gelation and cell-based assays.

Scaffold preparation

The extracted fish collagen solution was then well mixed with the tricalcium phosphate powder in a predetermined ratio where collagen would act as the matrix for the interaction between cells and TCP as the mineralization component to imitate the native bone composition [36]. The scaffold formulation was then divided into three experimental groups: COL/ β -TCP (control, without mangiferin), COL/ β -TCP/1 mg·mL⁻¹ mangiferin and COL/ β -TCP/5 mg·mL⁻¹ mangiferin, which enabled to compare the performance of the scaffold under the three bioactive loading conditions. All three formulations had been dispensed

into well plates and were allowed to gel at room temperature or by incubation at 37°C for several hours, to allow for stabilization of the matrix and uniformity of structure. The gelled scaffolds were then frozen at -80 °C and lyophilized for 24-48 hours, which resulted in the removal of any residual solvent while maintaining the porous microarchitecture necessary for infiltrating cells and diffusing nutrients within the scaffold [37,38].

Characterization of unloaded and mangiferin-loaded scaffolds

Scanning electron microscopy (SEM)

Scanning electron microscope (SEM) was used to characterize the surface morphology and microstructure of scaffolds. Samples were dried and then coated with gold using an E1010 ion sputter (Hitachi, Japan) for conductivity before imaging, which was conducted on a ZEISS SEM (Seron Technology, India). The dimensions and size distribution of the fibre and pores were quantitatively analysed with the help of imageJ software [39].

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the prepared scaffold were taken to determine the functional group. The spectra were measured at a resolution of 4 cm⁻¹ in the frequency range of 4000 to 400 cm⁻¹ at room temperature [40].

X-ray diffraction (XRD)

The X-ray diffraction (XRD) pattern of the samples was obtained by X-ray machine at 40 kV, 30 mA, Cu Ka radiation ($\lambda = 1.54060\text{\AA}$). XRD pattern was collected for a definite period [41].

Thermogravimetric analysis (TGA)

Thermal stability of the scaffolds were measured using a thermogravimetric analyser. Dry samples (5–20 mg) were put into a suitable pan and then heated from 25°C up to 600°C at a constant heating rate of 10°C/min under an inert nitrogen atmosphere. The thermogravimetric curves were analysed in terms of weight loss vs. temperature, and various thermal transitions and decomposition peaks were observed [42].

In-vitro Cytocompatibility Assessment (MTT Assay)

The cytocompatibility was evaluated by NIH3T3 mouse fibroblast cells cultured in Dulbecco's Modified Eagle Medium (DMEM) under standard cell culture conditions (37°C, 5% CO₂, humidified atmosphere). NIH3T3 cells were plated at a density of 5×10^3 cells/well into 96-well plates and subsequently cultured for 24 h. Cells were then incubated with conditioned media prepared from hydrogel samples (10 $\mu\text{g/L}$) for 24 hours before performing MTT assay. Cells were then treated and incubated for 4 h after adding MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The formazan crystals were then dissolved in dimethyl sulfoxide (DMSO) and the absorbance measured at 570 nm to assess cell viability and cytocompatibility [43,44].

Porosity measurement

The porosity of the scaffolds was assessed using Archimedes' principle in combination with the ethanol displacement method. Scaffolds were cut into uniform sizes and submerged in a known volume of absolute ethanol (v1). After allowing the scaffolds to soak and become fully saturated, the total volume of ethanol plus the saturated scaffold was recorded as v2. Following overnight immersion, the scaffolds were removed, and the residual volume of ethanol (v3) was measured. Porosity (%) was calculated as: [45]

$$\text{Porosity (\%)} = [(v1 - v3) / (v2 - v3)] \times 100$$

All measurements were performed in triplicate to obtain an average porosity value for each formulation.

Results and Discussion

Characterization of extracted collagen

The yield of collagen extracted from mahi-mahi skin was 140.72 mg/g

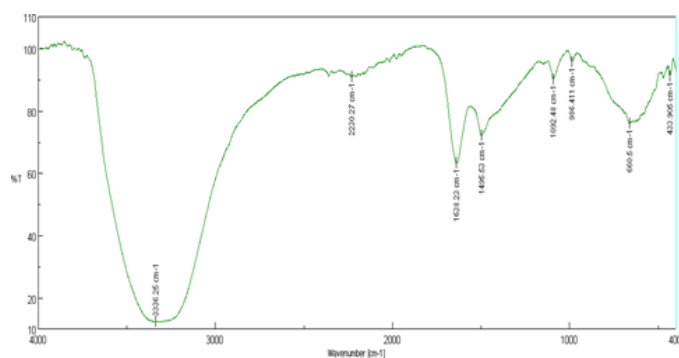


Figure 1: FT-IR spectra of extracted collagen

(14.07% on a wet weight basis) using the acid-assisted method. The acid-only protocol, and the high yield of acid-soluble collagen (ASC), with no animal-derived enzymes used, as compared to other fish species, which typically have 5–8% yields of ASC, demonstrate that the acid-only procedure was efficient, making mahi-mahi skin an economically viable and technically practical source of collagen for large-scale production, versus other fish species whose skins have been less investigated as a source for collagen.

FTIR analysis

The extracted collagen was analysed by FTIR spectroscopy in the range of 4000–400 cm^{-1} and the resulting spectrum is presented in figure 1, as is typically done with protein biomaterials. The Amide I band was well visible in the spectrum at 1653 cm^{-1} , indicating that the protein's peptide backbone structure, and hence its overall structure, was retained; an important parameter for the intended functionality of the biomaterial. The hydroxyl (–OH) group interactions were confirmed by a broad absorption band at 3335 cm^{-1} , suggesting strong intermolecular hydrogen bonding that helped maintain the structure stability of the collagen network. Furthermore, a peak at 1092 cm^{-1} , which is indicative of C–O stretching, indicated that glycosidic interactions were present in the sample, which could be important in certain drug-release applications, such as wound healing. These spectral features together validate that the extraction method was able to maintain the key chemical and structural characteristics of the native collagen.

SEM analysis

Figure 2A reveals, the SEM images of the collagen sample at 250x magnification showed a fibrous and layered structure of the collagen, revealing the presence of interconnected fibrils that displayed a well-defined surface texture, which could be considered as having good structural integrity for biomedical applications. The even distribution of these fibrils implied good porosity and bioactivity, which are crucial for cell attachment and tissue ingrowth in tissue engineering. The imaging was done at an accelerating voltage of 3.00 kV and a working distance of 7.2

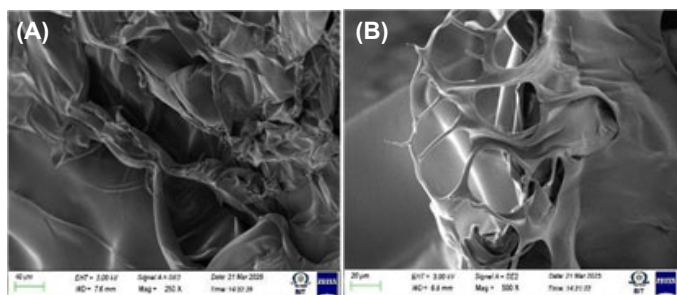


Figure 2: A) SEM image of collagen at 250x; B) SEM image of collagen at 500x

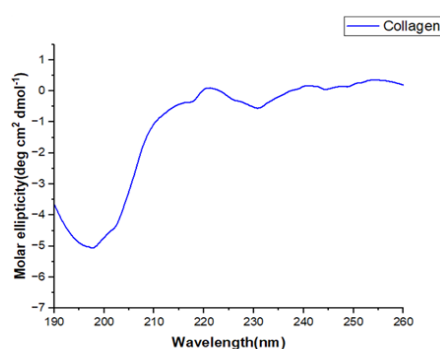


Figure 3: CD spectrum of the extracted collagen showing characteristic peaks of the intact triple-helical structure

mm to maximize surface contrast and resolution. When imaged at higher magnifications (500x), the lyophilized collagen scaffold was seen to have a thin web like structure and interconnected pores measuring approximately 10–40 μm (figure 2B). The observed scaffold surface was smooth and without external damage or collapse, suggesting that the freeze-drying protocol did not cause any visible structural changes or damage to the scaffold. This highly porous and interconnected structure is advantageous for cell infiltration and nutrient diffusion, and thus makes the collagen extracted appropriate for tissue engineering and wound healing.

Circular dichroism analysis

The extracted collagen was tested via CD spectroscopy, and the spectrum shown in figure 3 displayed an intense negative peak around 200 nm and a less intense positive peak around 220–225 nm typical of intact collagen triple helix. It was found that there were no significant changes in the peak shift or band broadening, which meant that the secondary structure of the collagen remained unchanged throughout its extraction and purification process, with little loss of structure. Concluding from the CD data, FTIR and SEM data, it is observed that the extracted collagen maintained its native conformational integrity which validates its suitability for further processing of the scaffold for the delivery of mangiferin to heal the wounds.

Characterization of unloaded and mangiferin-loaded scaffolds

Scanning electron microscopy (SEM)

The SEM images presented in figure 4 revealed that the COL/ β -TCP scaffold consisted of highly porous and interconnected structure which would be beneficial for nutrient transport, cell infiltration and diffusion of drugs. The overall structure of the scaffold was identified to be well organized with a network of fibres having uniform distribution of pores, which is desirable for tissue engineering applications. The morphology of the two drug loaded scaffolds were observed after incorporating mangiferin. The scaffolds loaded with COL/ β -TCP/1mg/ml and COL/ β -TCP/5mg/ml had a denser fibrillar network with smaller pore size and fewer pores than the unloaded scaffold. The scaffold matrix was successfully loaded with mangiferin, and no scaffold collapse and disruption of the collagen fibres were observed, with a higher density observed at a higher concentration of mangiferin, possibly due to the interactions between the collagen network and polyphenol, which diminishes the free interstitial space for pores to be formed during lyophilization. Moreover, high magnification micrographs showed no aggregation of the drug, supporting the uniformity of drug distribution within the scaffold structure although the porosity has decreased.

Fourier transform infrared spectroscopy (FTIR)

To study the chemical interactions between collagen, β -TCP and mangiferin, FTIR spectroscopy was used, and the spectra are presented in figure 5. The presence of all the characteristic absorption bands of the functional groups of collagen and of β -TCP, suggests that the scaffold

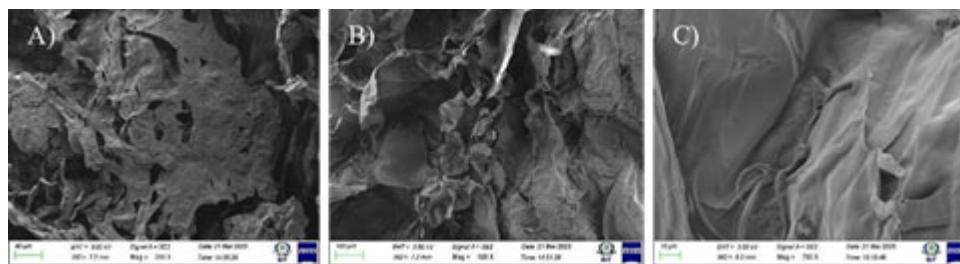


Figure 4: A) SEM image of COL/β-TCP; B) SEM image of COL/β-TCP/1mg/ml; C) SEM image of COL/β-TCP/5mg/ml Scaffold

fold matrix was retained in all the formulations. The peak intensity was found to be higher at the regions of 1000–1700 cm^{-1} and 3200–3500 cm^{-1} for the drug loaded scaffolds when compared with the control scaffold, which shows the successful incorporation of mangiferin into the composite scaffold. The interactions between the scaffold components and mangiferin were more evident at higher concentrations in the COL/β-TCP/5mg/ml scaffold, which indicated more pronounced spectral modifications.

X-ray diffraction

Figure 6 showed the XRD patterns used to evaluate changes in crystallinity after drug incorporation in both COL/β-TCP and drug loaded scaffolds. The unloaded COL/β-TCP scaffold showed the broad diffraction peak between 20° and 35° (2θ), which is typical of a semi-crystalline structure. After the loading of mangiferin the peak intensity and sharpness decreased, especially in the COL/β-TCP/5mg/ml formulation. The reduction in crystallinity suggests that mangiferin is well dispersed in the scaffold matrix, and also the increase in intermolecular interactions between collagen, β-TCP and mangiferin. The higher amorphous component of the scaffolds loaded with the drugs could be the reason for the better drug dissolution and controlled release properties.

Thermogravimetric analysis

The TGA thermograms shown in figure 7 were used to assess the thermal stability of the scaffolds. All formulations showed a multi-stage degradation profile. The weight loss before 150°C was attributed to the

evaporation of moisture physically adsorbed and bound water molecules. The major degradation step was observed between 200°C and 500°C, which is related to the degradation of collagen and other organic components of the scaffold. The COL/β-TCP/5mg/ml formulation had the greatest mass retention at the higher temperatures than the other formulations, reflecting greater thermal stability than the unloaded and lower dose formulations. This enhanced thermal resistance can be explained as the increased intermolecular interactions between mangiferin and the scaffold matrix. Results indicate that the incorporation of mangiferin has a positive effect on the thermal behaviour of the scaffold that is beneficial for biomedical processing and applications for sterilization.

Such spectral and thermal transformations during FTIR, XRD and TGA, take place simultaneously in two modes of interaction and not in one mode. The first part of the explanation is that the carbonyl and amine groups of the collagen backbone (amide I and II bands) could form hydrogen bonds with the phenolic hydroxyl groups in mangiferin, leading to increased absorption intensities in the drug loaded scaffolds. Second, the hydroxyl groups of mangiferin, which have a catechol-like structure, could also have coordinate or electrostatic interactions with the Ca^{2+} ions present on the surface of the β-TCP, which could prevent the occurrence of long-range crystalline order, thus accounting for the gradual decrease in the sharpness of the XRD peaks at higher concentrations of mangiferin. In both cases, the mobility and packing of chains in the matrix would be limited, as observed from TGA, at higher mangiferin loading. Complementary methods like isothermal titration calorimetry or Raman spectroscopy of the phenol band and phosphate band are suggested as future directions in order to confirm the relative contribution of each interaction.

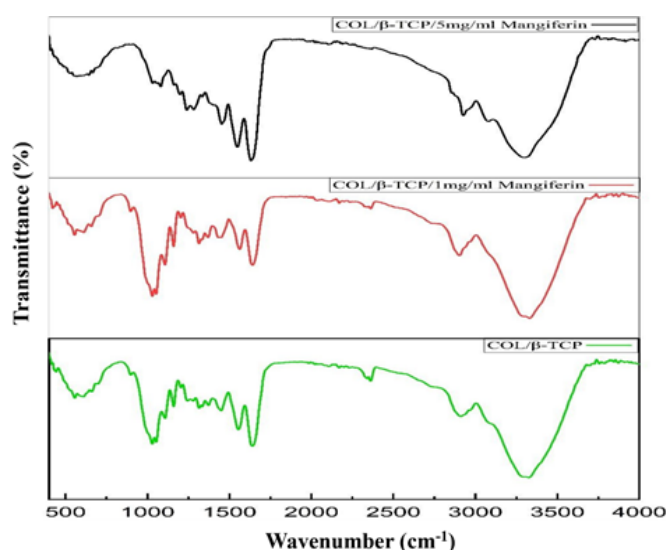


Figure 5: FT-IR spectrum of COL/β-TCP, COL/β-TCP/1mg/ml, COL/β-TCP/5mg/ml scaffolds showing characteristic functional groups and spectral changes after drug incorporation

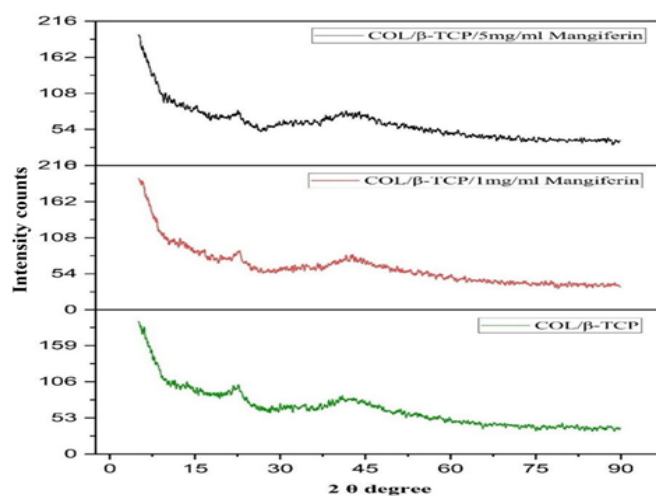


Figure 6: XRD analysis of COL/β-TCP, COL/β-TCP/1mg/ml, COL/β-TCP/5mg/ml scaffold

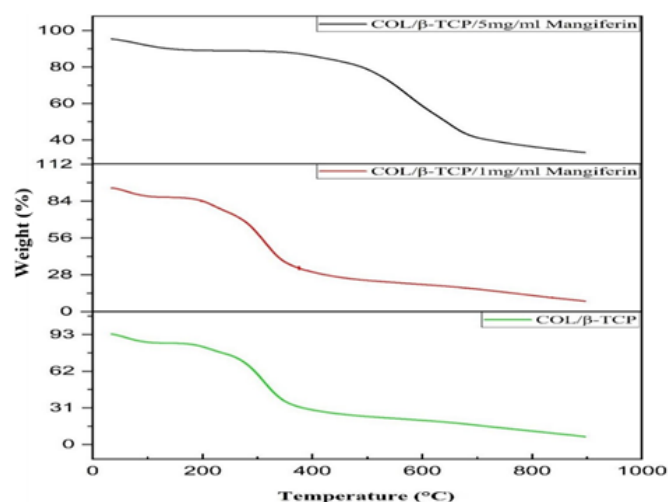


Figure 7: TGA analysis comparison of COL/β-TCP, COL/β-TCP/1mg/ml, COL/β-TCP/5mg/ml scaffolds showing the thermal degradation behaviour and stability

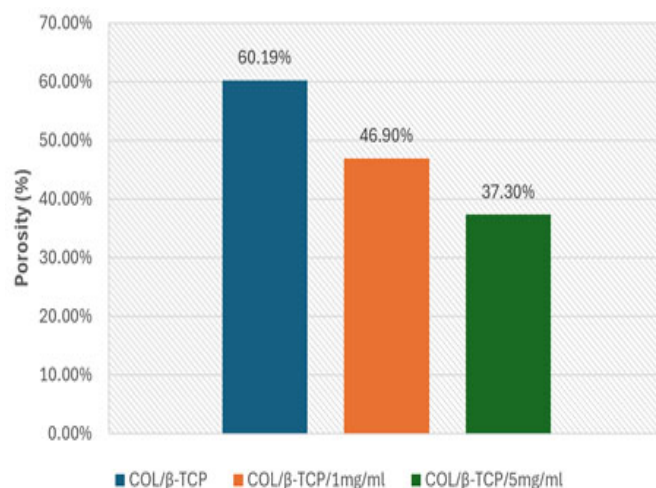


Figure 9: Porosity (%) of COL/β-TCP, COL/β-TCP/1mg/ml, COL/β-TCP/5mg/ml scaffolds showing the effect of mangiferin incorporation on scaffold pore structure

In-vitro cytocompatibility assessment

The MTT assay data in figure 8 tested the cytocompatibility of fabricated scaffolds after 48 hours of incubation. The cell viability of the unloaded scaffold (COL/β-TCP) did not show any significant variation with respect to concentration, with the variation between replicates being relatively small, suggesting that the base scaffold material was not cytotoxic and cell response was consistent. The viability of the COL/β-TCP/1mg/ml scaffold decreased slightly at the lowest concentration used (125 µg/mL, ~80%), before increasing gradually in a concentration-dependent manner, reaching ~121% at 1000 µg/mL. For COL/β-TCP/5mg/ml, cell viability significantly increased from ~98% at 125 µg/mL to a maximum of ~148% at 250 µg/mL and then slowly decreased with increasing concentrations of the formulation (up to 128–134% at 1000–500 µg/mL). There was a proliferative or mitogenic effect of mangiferin at observed concentrations on cultured cells, as evidenced by viability values exceeding 100% for mangiferin loaded scaffolds, especially COL/β-TCP/5mg/ml. Generally, all three formulations exhibited cytocompatibility, with scaffolds loaded with mangiferin, particularly 5mg/mL, exhibiting positive cell proliferation.

Porosity measurement

The porosity data in figure 9 indicated that the COL/β-TCP scaffold

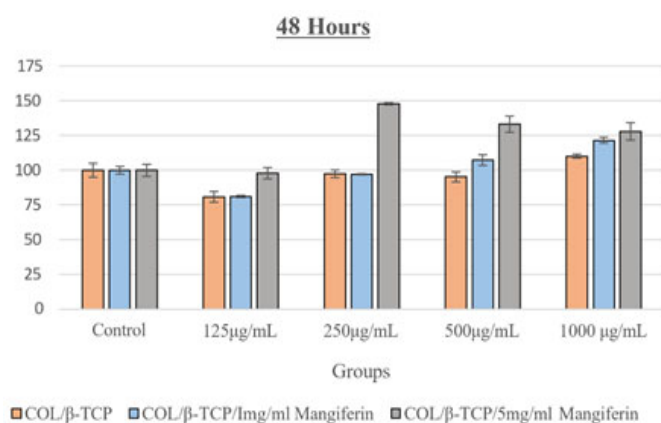


Figure 8: MTT assay results showing the cell viability of COL/β-TCP and mangiferin-loaded scaffolds at different concentrations

had the highest porosity of 60.19%, which had a good, interconnected pore network suitable for cell infiltration and nutrient diffusion. The porosity of the scaffolds was significantly reduced in a concentration-dependent manner, with a porosity of 46.90% and 37.30% for COL/β-TCP/1mg/ml and COL/β-TCP/5mg/ml, respectively. The incorporation of polyphenol molecules (mangiferin) would occupy the space between the collagen molecules and the TCP, which as a result would make the scaffold more compact, resulting in a reduction in available pore space as seen with the increase in mangiferin concentration. Although all three formulations exhibited a significant number of pores (>35%), the decrease should be taken into consideration when optimizing the loading of mangiferin into application where cell infiltration is a critical requirement for the successful delivery of the drug.

An interesting finding from the cell viability study was that the mangiferin scaffold at 5 mg/mL, which had the lowest total porosity (37.30%), showed the best cell proliferation. In the traditional paradigm of tissue engineering, higher porosity is directly associated with higher cell proliferation, due to increased surface area for cell attachment and better nutrient diffusion. However, in an *in vitro* monolayer culture, cells are exposed to an excess of nutrients from the surrounding culture medium, which is often replenished. Under these static, nutrient rich conditions, limitations of physical pore space and transport are minimized. Rather, the cell kinetics are strongly modulated by chemical cues [46,47].

Mangiferin is a natural polyphenol and potent mitogenic agent. The high concentration of mangiferin released from the dense scaffold matrix is likely to compensate for the reduced architectural spacing, by triggering intracellular signaling cascades, such as the MAPK/ERK or PI3K/Akt pathways, actively accelerating progression through the cell cycle. The concentrated polyphenol provides a biochemical stimulus which, in essence, overcomes the structural limitations of the lower porosity matrix, leading to the observed increase in MTT metabolic activity [48,49].

Conversely, we predict that this compensatory effect will be very different when translating to a three-dimensional *in vivo* environment. In living systems, it is difficult to overcome the structural constraints of a low-porosity matrix solely through biochemical signaling. The *in vivo* matrix is wholly dependent on host neovascularization and interstitial fluid flow to provide oxygen and nutrients to the inner core of the construct and to remove metabolic waste. 37.30% porosity builds dense physical barriers to prevent cell migration into the core of the scaffold and limit space for new blood vessel infiltration (angiogenesis). While mangiferin's mitogenic signaling can enable initial localized host cell activation at the scaffold periphery, the lack of interconnected macroscopic pores will ultimately result in either a necrotic core or premature

fibrous encapsulation. Therefore, although a low-porosity, high-polyphenol matrix is excellent under well-nourished *in vitro* conditions, optimising the architectural interconnectivity and porosity remains paramount to translate these proliferative benefits into successful *in vivo* tissue regeneration [50,51].

In this study, mechanical testing, degradation/swelling analysis and scaffold-level functional bioactivity assays (DPPH, zone-of-inhibition, scratch-migration tests) were not conducted and are recognized as limitations and not future embellishments. With an increasing trend in porosity shown above, increasing the amount of mangiferin would be expected to increase the compressive modulus and reduce the rate of degradation and fluid uptake, both of which are important parameters to consider for a wound dressing that must have a balance between conformability and structural gain. Similarly, the antioxidant, anti-inflammatory and antimicrobial activity of mangiferin has not been properly demonstrated on the scaffold. The gaps are identified as the immediate next steps in front of the further translation.

Conclusion

By successfully extracting type I collagen from the skin of mahi-mahi (*Coryphaena hippurus*) and retaining its native triple-helical structure in both FTIR, SEM and CD analyses, this study shows the potential for this new material to be used as a sustainable alternative from non-mammalian sources for biomedical applications. The fabricated scaffolds showed good physicochemical compatibility between the components of the scaffold, as incorporation of mangiferin did not alter the structural integrity and the functional group composition of the fabricated scaffolds. The MTT results indicated that the scaffolds and mangiferin-loaded scaffolds were both cytocompatible, and the scaffold with the lowest porosity (37.30%) also showed higher cell proliferation, a result that could be explained by the mitogenic signalling of the drug mangiferin that overcame the decrease in porosity under *in vitro* conditions. This porosity reduction will have mechanical and degradation consequences, which were not measured directly in this study, and the overall antioxidant, anti-inflammatory and antimicrobial activities of mangiferin have been reported in literature, but have not been directly proven on the scaffold, which will be the next steps. Overall, these results suggest that the developed platform of mahi-mahi collagen, mangiferin and TCP may be a promising biomaterial for wound healing applications and most importantly, the porosity needs to be tuned to achieve the highest possible porosity and mechanical integrity needs to be confirmed by testing before moving forward toward clinical translation. Ongoing research and development efforts, with a specific emphasis on mechanical improvements to the product, optimized extraction procedures for collagen, customized adjustments of the concentration of mangiferin and cutting-edge manufacturing processes such as 3D printing and lyophilization, will pave the way for broader clinical applications and eventually better results for patients. The incorporation of such biocompatible materials could lead to the development of scaffolds that offer structural support and actively promote tissue regeneration, enhancing the effectiveness of regenerative medicine therapies. Overall, the potential of generating advanced scaffolding materials inspired by the combination of mahi-mahi collagen and mangiferin, consistent with the modern requirements in the field of tissue engineering, represents a groundbreaking idea. The synergy may inspire development of new applications such as skin regeneration and other important repair capabilities, thus forging the way for the next generation of biomaterials used in regenerative therapies.

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References

1. P. Balasubramanian, M.P. Prabhakaran, M. Sireesha, S. Ramakrishna, Collagen in Human Tissues: Structure, Function, and Biomedical Implications from a Tissue Engineering

- Perspective, in: A. Abe, H.H. Kausch, M. Möller, H. Pasch (Eds.), *Advances in Polymer Science*, Springer, Berlin, Heidelberg, 173-206 (2012).
2. A.A. Jaziri, R. Shapawi, R.A.M. Mokhtar, W.N.Md. Noordin, N. Huda, Extraction and Characterization of Type I Collagen from Parrotfish (*Scarus sordidus* Forsskal, 1775) Scale Solubilized with the Aid of Acetic Acid and Pepsin, *Int. J. Biomater.*, 2023, 7312447 (2023).
3. I.N. Amirrah, Y. Lokanathan, I. Zulkiflee, M.F.M.R. Wee, A. Motta, M.B. Fauzi, A Comprehensive Review on Collagen Type I Development of Biomaterials for Tissue Engineering: From Biosynthesis to Bioscaffold, *Biomedicines*, 10(9), 2307 (2022).
4. S.S. Mathew-Steiner, S. Roy, C.K. Sen, Collagen in Wound Healing, *Bioengineering*, 8(5), 63 (2021).
5. E.G. Canty, K.E. Kadler, Procollagen Trafficking, Processing and Fibrillogenesis, *J. Cell Sci.*, 118(7), 1341-1353 (2005).
6. M. Yamauchi, M. Sricholpech, Lysine Post-Translational Modifications of Collagen, *Essays Biochem.*, 52, 113-133 (2012).
7. R. Parenteau-Bareil, R. Gauvin, F. Berthod, Collagen-Based Biomaterials for Tissue Engineering Applications, *Materials*, 3(3), 1863-1887 (2010).
8. N. Zhang, S. Guo, Y. Zheng, W. Li, Isolation and Characterisation of Acid Soluble Collagens and Pepsin Soluble Collagens from Eel (*Anguilla japonica* Temminck et Schlegel) Skin and Bone, *Foods*, 14(3), 502 (2025).
9. D. Coppola, M. Oliviero, G.A. Vitale, C. Lauritano, I. D'Ambra, S. Iannace, et al., Marine Collagen from Alternative and Sustainable Sources: Extraction, Processing and Applications, *Mar. Drugs*, 18(4), 214 (2020).
10. T. Silva, J. Moreira-Silva, A. Marques, A. Domingues, Y. Bayon, R. Reis, Marine Origin Collagens and Its Potential Applications, *Mar. Drugs*, 12(12), 5881-5901 (2014).
11. F.F. Felician, C. Xia, W. Qi, H. Xu, Collagen from Marine Biological Sources and Medical Applications, *Chem. Biodivers.*, 15(5), e1700557 (2018).
12. M. Akita, T. Kono, K. Lloyd, T. Mitsui, K. Morioka, K. Adachi, Biochemical Study of Type I Collagen Purified from Skin of Warm Sea Teleost Mahi Mahi (*Coryphaena hippurus*), with a Focus on Thermal and Physical Stability, *J. Food Biochem.*, 43(11), e13013 (2019).
13. H. Hong, H. Fan, M. Chalamaiiah, J. Wu, Preparation of Low-Molecular-Weight Collagen Hydrolysates (Peptides): Current Progress, Challenges, and Future Perspectives, *Food Chem.*, 301, 125222 (2019).
14. P. Singh, S. Benjakul, S. Maqsood, H. Kishimura, Isolation and Characterisation of Collagen Extracted from the Skin of Striped Catfish (*Pangasianodon hypophthalmus*), *Food Chem.*, 124(1), 97-105 (2011).
15. F. Yu, C. Zong, S. Jin, J. Zheng, N. Chen, J. Huang, et al., Optimization of Extraction Conditions and Characterization of Pepsin-Solubilised Collagen from Skin of Giant Croaker (*Nibea japonica*), *Mar. Drugs*, 16(1), 29 (2018).
16. A. Sionkowska, K. Adamiak, K. Musia, M. Gadowska, Collagen Based Materials in Cosmetic Applications: A Review, *Materials*, 13(19), 4217 (2020).
17. S. Saghaazadeh, C. Rinoldi, M. Schot, S.S. Kashaf, F. Sharifi, E. Jalilian, et al., Drug Delivery Systems and Materials for Wound Healing Applications, *Adv. Drug Deliv. Rev.*, 127, 138-166 (2018).
18. E. Hanani, H.D. Ardiyani, H.A. Sulistya, H. Melisa, A. Fawzy, The Potential Therapeutic Role of Flavonoids and Mangiferin in Mango Peel for Burn Wound Healing: A Comprehensive Literature Review, *Int. J. Med. Sci. Clin. Res. Stud.*, 4(6), 1132-1139 (2024).
19. V.F. Sardi, A. Astika, I.M. Jalius, F. Ismed, Quantification of Mangiferin from the Bioactive Fraction of Mango Leaves (*Mangifera indica* L.) and Evaluation of Wound-Healing Potential, *Jordan J. Pharm. Sci.*, 16(3), 595-606 (2023).
20. R.K. Bulugonda, K.A. Kumar, D. Gangappa, H. Beeda, G.H. Philip, D. Muralidhara Rao, et al., Mangiferin from *Pueraria tuberosa* Reduces Inflammation via Inactivation of NLRP3 Inflammasome, *Sci. Rep.*, 7(1), 42683 (2017).
21. R. Jayasuriya, K. Ganesan, K.M. Ramkumar, Mangiferin Represses Inflammation in Macrophages Under a Hyperglycemic Environment Through Nrf2 Signaling, *Int. J. Mol. Sci.*, 25(20), 11197 (2024).
22. J. Boateng, O. Catanzano, Advanced Therapeutic Dressings for Effective Wound Healing—A Review, *J. Pharm. Sci.*, 104(11), 3653-3680 (2015).
23. D. Liu, G. Wei, T. Li, J. Hu, N. Lu, J.M. Regenstein, et al., Effects of Alkaline Pretreatments and Acid Extraction Conditions on the Acid-Soluble Collagen from Grass Carp (*Ctenopharyngodon idella*) Skin, *Food Chem.*, 172, 836-843 (2015).
24. S. Sinthusamran, S. Benjakul, H. Kishimura, Characteristics and Gel Properties of Gelatin from Skin of Seabass (*Lates calcarifer*) as Influenced by Extraction Conditions, *Food Chem.*, 152, 276-284 (2014).
25. Z. Liu, N. Li, W. Li, S. Xu, L. Yao, J. Xiao, Molecular Weight-Controllable Oral Yak Skin Collagen for Enhanced Healing of Photoaged Skin, *Collagen Leather*, 7(1), 24 (2025).
26. A.A. Abbas, K.A. Shakir, M.K. Walsh, Functional Properties of Collagen Extracted from Catfish (*Silurus triostegus*) Waste, *Foods*, 11(5), 633 (2022).
27. K. Nilsuwan, U. Patil, C. Tu, B. Zhang, S. Benjakul, Salmon Skin Acid-Soluble Collagen Produced by a Simplified Recovery Process: Yield, Compositions, and Molecular Characteristics, *Fishes*, 7(6), 330 (2022).
28. N.K. Vate, P. Strachowski, I. Undeland, M. Abdollahi, Structural and Functional Properties of Collagen Isolated from Lumpfish and Starfish Using Isoelectric Precipitation vs Salting Out, *Food Chem. X*, 18, 100646 (2023).
29. M.L. Speranza, G. Valentini, A Simple Procedure for the Purification of Neutral Salt Soluble Type I Collagen from Skin, *Ital. J. Biochem.*, 35(1), 42-48 (1986).
30. I. Roy, M.N. Gupta, Freeze-Drying of Proteins: Some Emerging Concerns, *Biotechnol. Appl. Biochem.*, 39(2), 165-177 (2004).
31. T. Riaz, R. Zeeshan, F. Zarif, K. Ilyas, N. Muhammad, S.Z. Safi, et al., FTIR Analysis of Natural and Synthetic Collagen, *Appl. Spectrosc. Rev.*, 53(9), 703-746 (2018).
32. R.C.G. Coelho, A.L.P. Marques, S.M. Oliveira, G.S. Diogo, R.P. Pirraco, J. Moreira-Silva, et al., Extraction and Characterization of Collagen from Antarctic and Sub-Antarctic Squid and Its Potential Application in Hybrid Scaffolds for Tissue Engineering, *Mater. Sci. Eng. C*, 78, 787-795 (2017).

33. J. Zhang, R. Duan, C. Ye, K. Konno, Isolation and Characterization of Collagens from Scale of Silver Carp (*Hypophthalmichthys molitrix*), *J. Food Biochem.*, 34(6), 1343-1354 (2010).
34. K.E. Drzewiecki, D.R. Grisham, A.S. Parmar, V. Nanda, D.I. Shreiber, Circular Dichroism Spectroscopy of Collagen Fibrillogenesis: A New Use for an Old Technique, *Biophys. J.*, 111(11), 2377-2386 (2016).
35. L. Wang, X. An, F. Yang, Z. Xin, L. Zhao, Q. Hu, Isolation and Characterisation of Collagens from the Skin, Scale and Bone of Deep-Sea Redfish (*Sebastes mentella*), *Food Chem.*, 108(2), 616-623 (2008).
36. X. Hou, L. Zhang, Z. Zhou, X. Luo, T. Wang, X. Zhao, et al., Calcium Phosphate-Based Biomaterials for Bone Repair, *J. Funct. Biomater.*, 13(4), 187 (2022).
37. F.J. O'Brien, Biomaterials & Scaffolds for Tissue Engineering, *Mater. Today*, 14(3), 88-95 (2011).
38. W. Song, J. Xu, L. Gao, Q. Zhang, J. Tong, L. Ren, Preparation of Freeze-Dried Porous Chitosan Microspheres for the Removal of Hexavalent Chromium, *Appl. Sci.*, 11(9), 4217 (2021).
39. E.R. Fischer, B.T. Hansen, V. Nair, F.H. Hoyt, D.W. Dorward, Scanning Electron Microscopy, *Curr. Protoc. Microbiol.*, 25(1), Unit2B.2 (2012).
40. I. Siddique, Exploring Functional Groups and Molecular Structures: A Comprehensive Analysis Using FTIR Spectroscopy, *Chem. Res. J.*, 9(2), 70-76 (2024).
41. S. Zhu, Q. Yuan, M. Yang, J. You, T. Yin, Z. Gu, et al., A Quantitative Comparable Study on Multi-Hierarchy Conformation of Acid and Pepsin-Solubilized Collagens from the Skin of Grass Carp (*Ctenopharyngodon idella*), *Mater. Sci. Eng. C*, 96, 446-457 (2019).
42. E.P. Milan, M.Á.V. Rodrigues, V.C.A. Martins, A.M.G. Plepis, T. Fuhrmann-Lieker, M.M. Horn, Mineralization of Phosphorylated Fish Skin Collagen/Mangosteen Scaffolds as Potential Materials for Bone Tissue Regeneration, *Molecules*, 26(10), 2899 (2021).
43. J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Evaluation of a Tetrazolium-Based Semiautomated Colorimetric Assay: Assessment of Chemosensitivity Testing, *Cancer Res.*, 47(4), 936-942 (1987).
44. J. Van Meerloo, G.J.L. Kaspers, J. Cloos, Cell Sensitivity Assays: The MTT Assay, *Methods Mol. Biol.*, 731, 237-245 (2011).
45. R. Khan, M.U. Aslam Khan, G.M. Stojanović, A. Javed, S. Haider, S.I. Abd Razak, Fabrication of Bilayer Nanofibrous-Hydrogel Scaffold from Bacterial Cellulose, PVA, and Gelatin as Advanced Dressing for Wound Healing and Soft Tissue Engineering, *ACS Omega*, 9(6), 6527-6536 (2024).
46. Q.L. Loh, C. Choong, Three-Dimensional Scaffolds for Tissue Engineering Applications: Role of Porosity and Pore Size, *Tissue Eng. Part B Rev.*, 19(6), 485-502 (2013).
47. V. Karageorgiou, D. Kaplan, Porosity of 3D Biomaterial Scaffolds and Osteogenesis, *Biomaterials*, 26(27), 5474-5488 (2005).
48. M. Imran, T. Aslam Gondal, M. Atif, M. Shahbaz, M.S. Qadir, T.A. Malik, et al., Mangiferin: A Natural Miracle Bioactive Compound Against Hypercholesterolemia and Cardiovascular Diseases, *Lipids Health Dis.*, 16, 1-17 (2017).
49. P.B. Pal, K. Sinha, P.C. Sil, Mangiferin Attenuates Oxidative Stress-Induced Cell Death and Rejuvenates Cell Survival Through the Activation of PI3K/Akt Pathway, *Toxicol. Appl. Pharmacol.*, 275(2), 160-172 (2014).
50. J. Rouwkema, N.C. Rivron, C.A. van Blitterswijk, Vascularization in Tissue Engineering, *Trends Biotechnol.*, 26(8), 434-441 (2008).
51. I. Bruèauskaitė, D. Bironaitė, E. Bagdonas, E. Bernotienė, Scaffolds and Cells for Tissue Regeneration: Different Strategies in Engineering: A Review, *Cytotechnology*, 68, 355-369 (2016).