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Molecular Evaluation of the Effect of Using Chitosan Nanopolymers on Loaded Marine Fish Acellular Dermal Matrices in Reconstruction of Induced Ventral Hernias in Bucks

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ABSTRACT

The aim of present study was to compare between the effectiveness of marine fish acellular dermal matrices (ADM) and Chitosan Nanopolymers (ChNPs) loaded on fish ADM in the reconstruction of large abdominal wall hernias in Iraqi bucks. Molecular evaluations were conducted using Real Time-quantification Polymerase Chain Reaction (RT-qPCR) to measure the levels of basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF) genes during the healing process. Ventro-lateral abdominal wall hernias (6x6cm) were induced in 18 bucks. Thirty days post-induction of hernia, the bucks were divided into two groups: Group A, control group treated with fish ADM and group B, ChNPs group which was treated with fish ADM loaded with ChNPs. Evaluations were performed at 2, 8, and16 weeks post-treatment. Molecular analysis revealed significantly higher b-FGF and VEGF gene expression in the ChNPs group compared to control group, particularly at week 16th week post-treatment.

KEYWORD: Hernia, Bucks, Growth factors, Biological implants, Fish skin, Chitosan Nanopolymers.

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INTRODUCTION

Hernia is a condition characterized by the protrusion of the contents of a body cavity through a normal or abnormal opening in the wall of that cavity, either lying beneath the intact skin or occupying another adjacent body cavity [1]. Herniorrhaphy is the most accepted treatment for hernias, although tight suturing to close the defect can lead to wound dehiscence, recurrent hernias, and non-healing wounds [2]. The closure of large hernias generally requires the use of synthetic or biologic prosthetic mesh [3,4]. Biomaterials offer distinct advantages over synthetic meshes due to their ability to

become vascularized, remodel into autologous tissue, and resist infection [5]. These biological materials are derived from various tissues such as dermis, small intestine submucosa, and pericardium from species like humans, pigs, and cows [6]. Recently, fish ADM has been utilized in numerous clinical applications due to its physicochemical properties and amino acid composition 8 [7,8]. Fish ADM has been used effectively for the reconstruction of large abdominal wall hernias in goats, accelerating and enhancing the healing process without adverse immune responses [9]. Researchers continuously seek new materials for biological applications, particularly in medicine, to develop novel therapeutic strategies, among these materials, nanoparticles (NPs) have garnered significant attention [10-12]. Chitosan (Ch) is a polysaccharide acquired by partial deacetylation of chitin. It is a type of safe, non-toxic and non-antigenic natural cationic polysaccharide [13,14]. ChNPs have become of excessive interest for nanomedicine, biomedical engineering and development of new therapeutic drug release systems with improved bioavailability, increased specificity and sensitivity, and reduced pharmacological toxicity [15,16]. Additionally, due to its well bio-adhesive, antibacterial and hemostatic properties, it can be used as a dressing for open wound treatment [17,18]. Studies found that ChNPs promotes wound healing by promoting the migration of inflammatory cells and fibroblasts to the wound site and the deposition of collagen. Furthermore, Ch has hemostatic properties by mediating the aggregation of red blood cells [19,20]. Due to the absence of studies about the use of ChNPs Loaded on fish ADM on the reconstruction of large abdominal wall hernias, the current study is designed to evaluate the effect of this material through molecular evaluation of the tissue level of b-FGF and VEG genes.

MATERIALS AND METHODS

In the present study 18 clinically healthy local adult bucks were used, ages ranged among (1-2) years and weighing (25-30 kg). The animals were kept for acclimatization and observation at the animal farm, College of Veterinary Medicine/ University of Baghdad, for two weeks before the study began. Below the effect of sedation by intramuscular injection of 2% Xylazine hydrochloride (Woerden, Holland) at dose of (0.2 mg/kg B.W) and local anesthesia by local infiltration of 2% lidocaine hydrochloride (Canelones, Uruguay) by a dose of 4 mg/Kg B.W., ventral hernias (6 cm) in diameter were performed in right abdominal wall of all experimental animals in the study. The animals were randomly divided into two groups (9 Bucks/ group), 30 days post-operative of hernia inducing.

Preparation of ChNPs

Chitosan Nanopolymers Preparing through Chemical Method, dissolving 1.5 g of chitosan in 150 ml of deionized distilled water, the mixture was agitated for 30 minutes with a magnetic stirrer. The pH was brought to 12 using NaOH (1N), and the mixture was left at room temperature on the rotor for 60 minutes with a magnetic stirrer. Next, the pH was gotten down to 4 using HCL (1N), and the mixture was left on the rotor for 60 minutes at room temperature by a magnetic stirrer. Finally, the pH was adjusted to 7 with NaOH (1N), and left on the rotor for 60 minutes at room temperature by a magnetic stirrer. The solution was distributed into tubes and centrifuged for 20 minutes at 8000 rpm. Then threw away the supernatant and the precipitate was transferred to a Petri dish in the incubator and left for overnight at 37ºC to obtain Chitosan Nanopolymers dried powder [15].

Loading Method

The fish ADM ware loaded with the nanoparticles by means of the casting technique. The nanoparticles were dissolved by deionizing distilled water by using magnetic stirrer. Using a Pasteur pipette, 300 uL of the solution were dropped onto each 1 cm^2 of fish ADM, and dried at room temperature for 2 hours [21]. Each scaffolds were containing (1 wt%) of nanoparticles.

Treatment of Hernias

The hernias in group A (control group) were treated with Sublay implantation of decellularized marine fish ADM sheet. While, in group B (ChNPs group) the hernias were treated with Sublay implantation of decellularized marine fish ADM sheet loaded with ChNPs. The margins were interposed between the parietal peritoneum and the muscular layers of the abdominal wall. The sheet was sutured to the abdominal muscle layer, 2cm far from margin of hernia ring by sublay method using interrupted U shape suture pattern with knots on outer side by using No.1 synthetic non absorbable suture materials (polypropylene; Brussels-Belgium). The access skin and subcutaneous tissue were sharply removed and then apposite by silk No.1 in interrupted horizontal mattress suture pattern. The animals of each group were divided into three subgroups (three animals / subgroup) to make molecular evaluation.

Molecular Evaluation

A biopsy of the native tissue weighing one gram was taken from the implanted area in each animal of the study at 2, 8 and 16 week's post-treatment (three animals/ period) and single biopsy of normal abdominal muscle tissue was taken from the same animal before operation which resemble as a control group. The biopsies were kept in liquid nitrogen (- 196 ºC), this biopsy was used for detection of both b-FGF and VEGF gene expression according to the protocol described by Zhang et al. [22]. Analysis and calculation of gene expression levels of one or more genes depend on RNA /mRNA concentration after conversion it to cDNA.

Total RNA extraction: using Easy-spin™ (DNA free) total RNA extraction Kit

Preparation of primers

The primers (which were first lyophilized) were dissolved in free ddH_2O to reach a final concentration of 100 pM/µl. This was done in accordance with the primer synthesizer company's instructions, and the stock solution was kept at -20 °C. To be utilized as a work primer, a concentration of 10 pM/µl was generated from the stock primers.

Primers of gene expression used in this study

Table 1: Shows the details of primers that used in the study.

Organism	Target gene	Primer name	5' - 3'	PCR Product	Accession Number	Reference
Capra hircus	FGF2	F	AGTGTGTGCAAACCGTTACCTTGC	172	XM_018061205.1	**[23]**
		R	ATACTGCCCAGTTCGTTTCAGTGC			
Capra hircus	VEGF	F	AACCTGACATGAAGGAAGAGGGAG	150	XM_018038496.1	
		R	CGGTGATTTAGCAGCAAGAGAA			
Capra hircus	GAPDH	F	TGTTTGTGATGGGCGTGAACCA	154	XR_0019118676.1	
		R	ATGGCGTGGACACAGTGGTCATAA			

1-Step RT-qPCR System Protocol covers the real-time qPCR (gene expression test) process. (1) Program the real-time instrument for standard or fast mode one-step RT-qPCR (Table 3). (2) Thaw the components of the GoTaq® 1-Step RT-qPCR System, RNA templates, and primer pairs on ice, at room temperature, or at 37°C. Thoroughly mix each thawed component immediately. If using a vortex mixer, mix at low speed to minimize aeration. Keep thawed reagents on ice. (3) Prepare the RNA samples (mRNA [500 fg–100 ng]) in water or another qPCR-compatible diluent. (4) Combine the reaction components (refer to Table 2) in a non-stick, sterile tube on ice. Mix gently after each addition. Carefully pipet the reaction volumes into the plate while keeping it on ice. (5) Transfer the plate from ice to the pre-programmed instrument and start the run immediately. (6) Once the run is complete, collect and analyze the data.

Table 2: Preparation the solutions for Real-Time PCR

Components	Concentration	Volume (20µl)
GoTaqTM qPCR master mix, 2X	1X	10 µl
Forward primer	10 µM/µl	2µl
Reverse primer	10 µM/µl	2 µl
GoScriptTM RT mix for 1-step RT-qPCR	1X	0.4 µl
ddH2O	-	3.6 µl
RNA template	250 ng	2µl

Table 3: Conditions for Real-Time PCR (In accordance with the GoTaq® 1-Step RT-qPCR System instructions)

Stage	Ta (°C)	Time	Cycles
Reverse transcription	42	15 min	1
RT inactivation/Hot-start activation	95	10 min	1X
Denaturation	95	10 sec.	40X
Annealing/data collection	60	30 sec.	
Extension	72	30 sec.	
Dissociation	72	2 min	1X

RESULT

In current study, the estimation of levels of b-FGF and VEGF genes revealed significant differences in mean values of these growth factors (GFs) among the different periods of each treatment group and at the same period between the treatment groups themselves. (Table 4) shows that the mean values of b-FGF gene at two weeks in control group was (0.28 ± 0.016) and in ChNPs group was (0.76 ± 0.034). It was significantly higher in ChNPs group than in control group. At eight weeks the mean values of b-FGF gene

was in control group (0.90 ± 0.037) and in ChNPs group was (0.93 ± 0.032), It was higher in ChNPs group although, not significant. As well as, the mean values of b-FGF gene at 16th weeks was in control group (2.02 ± 0.074) and in ChNPs group was (2.34 ± 0.1). At this period, it was higher in ChNPs group without presence of significant difference (P<0.05) between it and control group.

The same table appeared there were differences in mean values of these GFs among the different periods of each treatment groups. in control group at two weeks was (0.28 ± 0.016), then increased at eight weeks (0.90 ± 0.037) with presence of significant difference between these two periods, and it continuous increased at week 16th (2.02 ± 0.074) post-treatment with presence of a significant differences (P<0.05) with the values at two weeks and eight weeks. Whereas, in ChNPs group, the mean values of level of b-FGF gene at two weeks was (0.76 ± 0.034), then, increased at eight weeks (0.93 ± 0.032) with presence of significant difference between these two periods and it increased at 16th weeks (2.34 ± 0.1) post-treatment with presence of significant differences (P<0.05) between this periods and other tow periods.

Table 4: The means ± SE values of b-FGF in control group and ChNPs group at different periods of study post-treatment.

Group	2-week post treatment	8-week post treatment	16-week post treatment
Group A	C 0.28 ± 0.016 b	B 0.90 ± 0.037	A 2.02 ± 0.074
Group B	C 0.76 ± 0.034 a	B 0.93 ± 0.032	A 2.34 ± 0.1
LSD	0.683		

Different capital letters mean significant differences (P<0.05) within group.

Different small letters mean significant differences (P<0.05) among groups.

Table 5 shows The mean values of level of VEGF gene at two weeks was in control group (2.69 ± 0.17) and in ChNPs group was (5.34 ± 0.18). At this period, it was higher in ChNPs group with the presence of a significant differences (P<0.05) between it and control group. At eight weeks the mean values of level of VEGF gene was in control group (4.77 ± 0.33) and in ChNPs group was (7.11 ± 0.14). At this period, it was higher in ChNPs group with presence of significant difference between this group and control group. The mean values of level of VEGF gene at 16th weeks was in control group (6.04 ± 0.20) and in ChNPs group was (8.45 ± 0.29). At this period, it was significantly higher in ChNPs group than in control group.

The same table showed that the mean values of level of VEGF gene in control group at two weeks was (2.69 ± 0.17), then increased significantly at eight weeks to (4.77 ± 0.33), while, it increased at 16th weeks (6.04 ± 0.20) post-treatment with presence of significant differences (P<0.05) with the values at two and eight weeks. Furthermore, in ChNPs group, the mean value of level of VEGF gene at two weeks was (5.34 ± 0.18), then it increased at eight weeks (7.11 ± 0.14) and increase again at 16th weeks (8.45 ± 0.29) post-treatment with presence of significant

differences between the three periods with each other.

Table 5: The means ± SE values of VEGF in control group and ChNPs group at different periods of study post-treatment.

Periods Groups	2-weeks post treatment	8-weeks post treatment	16-weeks post treatment
Group A	**C** 2.69 ± 0.17**c**	**B** 4.77 ± 0.33**b**	**A** 6.04 ± 0.20**c**
Group B	**C** 5.34 ± 0.18**b**	**B** 7.11 ± 0.14**a**	**A** 8.45 ± 0.29**a**
LSD	0.72		

Different capital letters mean significant differences (P<0.05) within group.

Different small letters mean significant differences (P<0.05) among groups.

DISCUSSION

The current study highlights dynamic changes in b-FGF and VEGF gene expression throughout the healing process, with significant variations among the treatment groups. In ChNPs group, the highest increase in b-FGF levels, indicating enhanced fibroblast activity and ECM, which are crucial for scaffold integration and tissue regeneration. Conversely, ChNPs group exhibited the highest levels of VEGF, suggesting a stronger angiogenic response, essential for supplying nutrients and oxygen necessary for tissue repair and regeneration. These findings underscore the differential impact of the treatments on growth factor regulation and tissue healing. These findings are consistent with previous studies indicating that the regulation of GFs like b-FGF and VEGF is critical for effective tissue regeneration and healing [24]. Additionally, previous studies have highlighted that GFs such as b-FGF are vital for coordinating biological processes required for tissue regeneration, including wound healing [25]. Al-Ebadi and Al-Bayati [26] emphasized that the levels of FGF and VEGF in BP and UBM implants vary and are released after implantation. This releasing occurs due to the degradation of the bioimplants, driven by the infiltration of inflammatory cells like monocytes and macrophages. These cells bind to ECM proteins, initiating phagocytosis and subsequently breaking down ECM fragments through proteolytic and collagenase enzymes produced by the inflammatory cells [26]. Reing et al. [27] referred that GFs are retained in the biomaterials, especially (b-FGF, VEGF, and Transforming Growth Factor Beta 1 (TGF-β1), and liberate them during the gradual degradation of biomaterials [27].

Fish ADM has demonstrated significant potential in enhancing wound healing due to its unique composition, which includes structural and biochemical components crucial for tissue regeneration. These components include glycosaminoglycans (GAGs), proteoglycans, fibronectin, collagen, and omega-3 fatty acids. Fibronectin and collagen in fish ADM promote cell adhesion and proliferation, facilitating the activity of fibroblasts and endothelial cells essential for

synthesizing and releasing GFs [28,29,30]. GAGs, proteoglycans, and fibronectin in the ECM provide structural support and biochemical signals for cell adhesion, proliferation, and differentiation, which are vital for upregulating GFs during wound healing [31,32,33]. Collagen, primarily types I and III, supports cellular activities and enhances the mechanical properties of the matrix, aiding the migration and proliferation of fibroblasts and endothelial cells necessary for FGF and VEGF production [34,35]. Omega-3 fatty acids in fish ADM enhance angiogenesis by upregulating VEGF expression, providing necessary nutrients and oxygen to regenerating tissues, thus supporting effective wound healing [31,23].

While, in ChNPs group, further enhancing wound healing due to the incorporation of ChNPs to the scaffolds which provide a controlled and sustained release of GFs, maintaining their bioactivity and stability over time. This ensures a continuous supply of GFs at the wound site, promoting angiogenesis and tissue regeneration. The controlled release mechanism of ChNPs makes them highly effective in delivering GFs like FGF and VEGF, which are critical for wound healing and tissue repair. ChNPs stimulate the activity of various cell types, including fibroblasts and endothelial cells, which are crucial for the production and release of FGF and VEGF. This stimulation supports the proliferation and migration of these cells, enhancing tissue repair processes [36,37]. As well as, Mawazi et al. [38] found that the inherent biocompatibility and bioactivity of chitosan nanoparticles significantly enhance the effectiveness of scaffolds used in tissue engineering. Chitosan supports crucial processes such as cell adhesion, proliferation, and differentiation, which are vital for the upregulation of GFs like FGF and VEGF. These interactions are essential for successful tissue regeneration and healing [38].

In the present study, the elevated levels of b-FGF and VEGF genes in the hernia site tissues at two weeks post-treatment across both groups, with ChNPs group showing high significantly of b-FGF and VEGF levels, can be attributed to the inflammatory response and the healing process. This process involves the infiltration of inflammatory cells that release b-FGF and VEGF. Similar results were found by Gumaa and AL-Bayati [39] who observed elevated b-FGF and VEGF levels in hernia site tissues at the 2nd week post-treatment using freshwater and marine fish acellular dermal matrices in bucks. Biomaterials have the capability to modulate the various stages of the healing response by shifting the process from inflammation to constructive remodeling and functional tissue restoration [39]. This modulation supports the transition from an initial inflammatory phase to a phase where constructive tissue remodeling and functional recovery are promoted [40,41]. Mahdi and AL-Bayati [42] mentioned that surgical

wounds are characterized by a rapid early angiogenesis and repair, that is mediated in part by b-FGF, which can be selectively released by cellular injury, in addition to, the tissues and platelet are stores the b-FGF and act to deliver it in the site of injury for help initiate wound repair [42]. The gradual increase in b-FGF levels in tendon wound healing from 5 to 30 days post-operation due to the presence of inflammatory cells during this healing phase. He also noted that the use of biological materials in wound healing leads to an earlier increase in b-FGF levels at the wound site. This effect is attributed to the ability of biological materials to attract inflammatory cells, which in turn secrete GFs at the injury site [43,44].

Newly formed granulation tissue requires a vascular supply to meet its metabolic needs, initiating angiogenesis at the injury site. Cell disruption and hypoxia, key features of tissue injury, strongly induce angiogenesis factors. FGF released from disrupted cells, have potent angiogenic effects [45]. Hypoxia stimulates macrophages to produce VEGF, which lead to the formation of new endothelial cells and capillaries at wound edges. Additionally, nitric oxide (NO) promotes VEGF production and aids granulation tissue formation by triggering endothelial cell migration, proliferation, and differentiation [46,47]. Karaman et al. [48] noted that the activity of VEGF increases significantly after the inflammatory phase, particularly during the proliferative and remodeling phases of wound healing [48].

In the current study, b-FGF and VEGF levels continued to increase in both group at eighth and 16th weeks post-treatment. Group B consistently exhibited significantly higher b-FGF and VEGF levels. This suggests that the degradation of the implant continued to increase at these time points, leading to the release of substantial amounts of b-FGF and VEGF during this period of the study. These results align with the findings of Al-Ebadi and Al-Bayati, who noted that GFs are retained in biomaterials and are gradually released during the degradation of these materials post-implantation. This process is further facilitated by infiltrating inflammatory cells, which bind to implant proteins and stimulate phagocytosis. The resulting proteolysis and collagenase activity from these inflammatory cells lead to the breakdown of implant fragments, thereby releasing additional GFs [26]. The GFs which released during implant degradation, exert their biologic effects as they are dissociated from their binding proteins and activated, where the process of implant degradation and releasing of GFs continue until the implant is completely degraded [49,50].

CONCLUSION

Molecular analysis revealed significantly higher b-FGF and VEGF gene expression in ChNPs group when compared to control

group, particularly at week 16th post-treatment.

REFERENCES

1- Das BC, Nath BK, Pallab MS, Mannan A, Biswas D. Successful management of ventral abdominal hernia in goat: a case report. International J Natural Sci 2012; 2.2: 60-62. DOI: https://doi.org/10.3329/ijns.v2i2.11387

2- Köckerling F, Simons MP. Current concepts of inguinal hernia repair. Visceral Med 2018; 34.2: 145-150. https://doi.org/10.1159/000487285

3- AL-Asadi RN, Hummadi SK. Ultrasonographic Evaluation of Hernioplasty of Experimentally Induced Large Ventro-lateral Hernia in Bucks. Iraqi J Vet Med 2011; 35.2: 105-112. https://doi.org/10.30539/iraqijvm.v35i2.582

4- Köckerling F, Scheuerlein H, Schug-Pass C. Treatment of large incisional hernias in sandwich technique-a review of the literature. Frontiers Surg 2018; 5: 37.https://doi.org/10.3389/fsurg.2018.00037

5- Badylak SF, Gilbert TW. Thomas W. Immune response to biologic scaffold materials. In: Seminars Immunol. Academic Press 2008; p. 109-116. https://doi.org/10.1016/j.smim.2007.11.003

6- King KS, Albino FP, Bhanot P. Biologic mesh for abdominal wall reconstruction. Chronic Wound Care Manag Res 2014; 57-65. https://doi/full/10.2147/CWCMR.S58816

7- Hu Z, Yang P, Zhou C, Li S, Hong P. Marine collagen peptides from the skin of Nile Tilapia (Oreochromis niloticus): Characterization and wound healing evaluation. Marine drugs 2017; 15.4: 102. https://doi.org/10.3390/md15040102

8- Rathore C, Maiti SK. Preparation and characterization of acellular matrix from fish swim bladder for tissue engineering applications. J Biomed Mater Res Part A 2017; 105(9), 2539–2549. https://doi.org/10.1002/jbm.a.36113

9- Gumaa BH, AL-Bayati AH. Ultrasonographic Evaluation Of Efficacy Of A Cellular Marine And Freshwater Fish Skin Matrixes In The Reconstruction Of Ventro-Lateral Hernias In Bucks. Biochem Cellul Arch 2021; 21.1. Docid: Https://Connectjournals.Com/03896.2021.21.603

10- Moeini-Nodeh S, Rahimifard M, Baeeri M, Abdollahi M. Functional improvement in rats' pancreatic islets using magnesium oxide nanoparticles through antiapoptotic and antioxidant pathways. Biol Trace Elem Res 2017; 175: 146-155.https://doi.article/10.1007/s12011-016-0754-8

11- Salih SI, Al-Falahi NH, Saliem AH, Abedsalih AN. Effectiveness of platelet-rich fibrin matrix treated with silver nanoparticles in fracture healing in rabbit model. Vet World 2018; 11.7: 944.https://doi:10.14202/vetworld.2018.944-952

12- Malik ZJ, Eesa MJ. Effect of magnesium oxide nanoparticles, hydroxyapatite and hydrogel on regeneration of transverse fracture of distal radius: Macroscopically and histologically study in rabbit model. Internation J Health Sci 2022; II: 5094-5106. https://doi.org/10.53730/ijhs.v6nS2.6206.

13- Pillai CK, Paul W, Sharma CP. Chitin and chitosan polymers: Chemistry,

solubility and fiber formation. Progress in Polymer Sci 2009; 34.7: 641-678. https://doi.org/10.1016/j.progpolymsci.2009.04.00

14- Liang J, Yan H, Puligundla P, Gao X, Zhou Y, Wan X. Applications of chitosan nanoparticles to enhance absorption and bioavailability of tea polyphenols: A review. Food Hydrocol 2017; 69: 286-292. https://doi.org/10.1016/j.foodhyd.2017.01.041

15- Ghadi A, Mahjoub S, Tabandeh F, Talebnia F. Synthesis and optimization of chitosan nanoparticles: Potential applications in nanomedicine and biomedical engineering. Caspian J Inter Med 2014; 5.3: 156. https://doi.10.22088/cjim.5.3.156.

16- Soorbaghi FP, Isanejad M, Salatin S, Ghorbani M, Jafari S, Derakhshankhah H. Bioaerogels: Synthesis approaches, cellular uptake, and the biomedical applications. Biomed Pharm 2019; 111: 964-975. https://doi.org/10.1016/j.biopha.2019.01.014

17- Patrulea V, Ostafe V, Borchard G, Jordan O. Chitosan as a starting material for wound healing applications. European J Pharmaceut Biopharmaceut 2015; 97: 417-426. https://doi.org/10.1016/j.ejpb.2015.08.004

18- Shariatinia Z, Jalali AM. Chitosan-based hydrogels: Preparation, properties and applications. Internation J Biol Macromolecul 2018; 115: 194-220. https://doi.org/10.1016/j.ijbiomac.2018.04.034

19- Ong SY, Wu J, Moochhala SM, Tan MH, Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials, 2008, 29.32: 4323-4332. https://doi.org/10.1016/j.biomaterials.2008.07.034

20- Loo HL, Goh B H, Lee L H, Chuah LH. Application of chitosan-based nanoparticles in skin wound healing. Asian J Pharmaceut Sci 2022; 17.3: 299-332. https://doi.org/10.1016/j.ajps.2022.04.001

21- Fernández-Gutiérrez M, Pérez-Köhler B, Benito-Martínez S, García-Moreno F, Pascual G, García-Fernández L, Bellón J. M.Development of biocomposite polymeric systems loaded with antibacterial nanoparticles for the coating of polypropylene biomaterials. Polymers 2020; 12.8: 1829. **https://doi.org/10.3390/polym12081829.**

22- Zhang Y, Zhang X-D, Liu X, Li Y-S, Ding JP, Zhang XR Zhang YH. Reference gene screening for analyzing gene expression across goat tissue. Asian-Australasian J Anima Sci 2013; 26.12: 1665. https://doi:10.5713/ajas.2013.13199

23- Magamage MPS, Sathagopam S, Avula K, Velmurugan S. Kisspeptin regulates the development of caprine primordial follicles in vitro. J Anim Reprod and Biotechnol 2021; 36.1: 51-58. https://doi.org/10.12750/JARB.36.1.51

24- Paskal W, Gotowiec M, Stachura A, Kopka M, Włodarski P. VEGF and other gene therapies improve flap survival—a systematic review and meta-analysis of preclinical studies. Internation J Mol Sci 2024; 25.5: 2622. https://doi.org/10.3390/ijms25052622

25- Xiang J, Zhou L, Xie Y, Zhu Y, Xiao L, Chen Y, Guo L. Mesh-like electrospun membrane loaded with atorvastatin facilitates cutaneous

wound healing by promoting the paracrine function of mesenchymal stem cells. Stem Cell Res Therap 2022; 13.1: 190. https://doi.org/10.1186/s13287-022-02865-5

26- Al-Ebadi AK, Al-Bayati AH. Effect of Acellular Bovine Pericardium and Urinary Bladder Submucosa Matrixes in Reconstruction of Ventro-Lateral Hernias in Bucks; Molecular Evaluation. The Iraqi J Vet Med 2019; 43.1: 67-74. https://doi.org/10.30539/iraqijvm.v43i1.474

27- Reing JE, Brown BN, Daly KA, Freund JM, Gilbert TW, Hsiong SX, Badylak SF. The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. Biomaterials 2010; 31.33: 8626-8633. https://doi.org/10.1016/j.biomaterials.2010.07.083

28- Al-Bayati AH, Al-Tememe HA, Al-Mudallal NH. Role of acellular bovine urinary bladder submucosa on skin wound healing in Iraqi goats. Iraqi J Vet Med 2016; 40.1: 53-60. https://doi.org/10.30539/iraqijvm.v40i1.138

29- Mohammad FA, Al-Ebadi AK. Macroscopically and histopathological study of Using Fenestrated and Non-Fenestrated Catfish Acellular Dermal Matrix on Healing Ventro-Lateral Hernia in Bucks. J Survey Fisher Sci 2023; 10.3S: 844-859. https://doi.org/10.17762/sfs.v10i3S.91 .

30- Gholipourmalekabadi M, Seifalian AM, Urbanska AM, Omrani MD, Hardy JG, Madjd Z, Samadikuchaksaraei A. 3D protein-based bilayer artificial skin for the guided scarless healing of third-degree burn wounds in vivo. Biomacromolecules 2018; 19.7: 2409-2422. https://doi.org/10.1021/acs.biomac.7b01807

31- Badois N, Bauer P, Cheron M, Hoffmann C, Nicodeme M, Choussy O, Lesnik M, Poitrine FC, Fromantin I. Acellular fish skin matrix on thin-skin graft donor sites: a preliminary study. J Wound Care 2019; 28.9: 624-628. https://doi.org/10.12968/jowc.2019.28.9.624

32- Alam K, Jeffery SL. Acellular fish skin grafts for management of split thickness donor sites and partial thickness burns: a case series. Military Med 2019; 184.Supplement_1: 16-20. https://doi.org/10.1093/milmed/usy280

33- Kirsner RS, Margolis DJ, Baldursson BT, Petursdottir K, Davidsson, OB, Weir D, Lantis JC. Fish skin grafts compared to human amnion/chorion membrane allografts: a double-blind, prospective, randomized clinical trial of acute wound healing. Wound Rep Regener 2020; 28.1: 75-80. https://doi.org/10.1111/wrr.12761

34- Chen Y, Liu X, Zheng X, Huang X, Dan W, Li Z, Wang Y. Advances on the modification and biomedical applications of acellular dermal matrices. J Leather Sci Engineer 2022; 4.1: 19. https://doi.org/10.1186/s42825-022-00057-2

35- Mauer ES, Maxwell EA, Cocca CJ, Ganjei J, Spector D. Acellular fish skin grafts for the management of wounds in dogs and cats: 17 cases (2019–2021). American J Vet Res 2022; 83.2: 188-192. https://doi.org/10.2460/ajvr.21.09.0140

36- Kovacevic I, Hoffmeister M, Oess S. Fibroblast Growth Factor Signaling in Vascular Development. Endoth Signal

Develop Dis 2015; 93-114. https://doi.org/10.1007/978-1-4939-2907-8

37- Sharifi-Rad J, Quispe C, Butnariu M, Rotariu LS, Sytar O, Sestito S, Calina D. Chitosan nanoparticles as a promising tool in nanomedicine with particular emphasis on oncological treatment. Can Cell Internat 2021; 21(1), 318. https://doi.org/10.1186/s12935-021-02025-4.

38- Mawazi SM, Kumar M, Ahmad N, Ge Y, Mahmood S. Recent applications of chitosan and its derivatives in antibacterial, anticancer, wound healing, and tissue engineering fields. Polymers, 2024; 16(10), 1351. https://doi.org/10.3390/polym16101351

39- Gumaa BH, AL-Bayati AH Molecular evaluation of efficacy of freshwater and marine acellular fish skin matrixes in reconstruction of ventro-lateral hernia in bucks. Indian J Foren Med Toxicol 2021; 15(4), 880-886. https://connectjournals.com/03896.2021.21.603

40- Londono R, Badylak SF. Biologic scaffolds for regenerative medicine: mechanisms of in vivo remodeling. Annals of Biomed Engineer 2015; 43, 577-592. https://doi.org/10.1007/s10439-014-1206-6.

41- Zahiri M, Khanmohammadi M, Goodarzi A, Ababzadeh S, Farahani MS, Mohandesnezhad S, Ai J. Encapsulation of curcumin loaded chitosan nanoparticle within poly (ε-caprolactone) and gelatin fiber mat for wound healing and layered dermal reconstitution. Internat J Biol Macromolecul 2020; 153, 1241-1250. https://doi.org/10.1016/j.ijbiomac.2019.10.255

42- Mahdi AK, AL-Bayati AH. Evaluation of two biological matrices for repairing of ventral hernia in bucks. Iraqi J Vet Med 2018; 42(2), 21-32. https://doi.org/10.30539/iraqijvm.v42i2.282

43- Franklin ME, Trevino JM, Portillo G, Vela I, Glass JL, Gonzalez, J. The use of porcine small intestinal submucosa as a prosthetic material for laparoscopic hernia repair in infected and potentially contaminated fields: long-term follow-up. Surgic Endoscop 2008; 22(9):1941-1946. https://doi.10.1007/s00464-008-0037-3.

44- Hammoodi OT, SALIH SI. Ultra-Sonographic Evaluation and Growth Factors Measurement of Using Tendon Derived Hydrogel and Hyaluronic Acid in Tendon Repair in Rams. Al-Anbar J Vet Sci, 2019; 12.2. https://doi.org/10.37940/AJVS02019.12.2.

45- Okabe K, Hayashi R, Aramaki-Hattori N, Sakamoto Y, Kishi K. Wound treatment using growth factors. Moder Plast Surg 2013, 3.3: 108-112. https://doi:10.4236/mps.2013.33022

46- Stephan B, Olivera S, Michael SG, Harold B, Marjana TC. Growth factors and cytokines in wound healing. Woun Rep Regen 2008; 16:585-601. https://doi.org/10.1111/j.1524-475X.2008.00410.x

47- Stone R, Saathoff EC, Larson DA, Wall JT, Wienandt NA, Magnusson S, Kjartansson H, Natesan S, Christy RJ. Accelerated Wound Closure of Deep Partial Thickness Burns with Acellular Fish Skin Graft. Internat Molecul Sci 2021; 22(4):1590. https://doi.org/10.3390/ijms22041590

48- Karaman S, Leppänen VM, Alitalo K. Vascular endothelial growth factor signaling in development and disease. Development 2018; 145(14),

dev151019. https://doi.org/10.1242/dev.151019

49- Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007; 28(25), 3587-3593. https://doi.org/10.1016/j.biomaterials.2007.04.043

50- Ayele T, Zuki A, Noorjahan BMA. Noordin MM. A comparative study of lyophilized bovine pericardium and tunica parietalis vaginalis for repair of large abdominal wall defects in a rabbit model. Afr J Biol 2011; 10(44):8942-8949. https://doi.10.5897/AJB10.1920.