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The effect of *Scrophularia striata* on cell attachment and biocompatibility of decellularized bovine pericardia

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Abstract Since using tissue transplantation has faced limitations all over the world, regenerative medicine has introduced decellularized tissues as natural scaffolds and researchers are trying to improve their efficiency and function. In this study, to increase cell attachment and ultimately cell proliferation on decellularized bovine pericardia, *scrophularia striata* extract was used. *Scrophularia striata* is an Iranian traditional medicinal plant. For this aim after decellularization of bovine pericardium and analysis of its morphology, it was incubated in *scrophularia striata* solution. Next, isolated human adipose-derived mesenchymal stem cells were cultured on the tissue. Finally, MTT assay, nitric oxide assay, and scanning electron microscopy observation were performed. MTT showed an increase in cell survival after treating the tissue with the plant extract after 48 h in a dose dependent manner significantly. The survival of cells in 0.5%, 2.5%, and 5% groups was about 5, 10 and 15 folds higher in comparison to control groups, respectively. Additionally, nitric oxide secretion in 2.5% and 5% samples was three and five folds higher than that in control group, respectively. Moreover, SEM observation indicated an impressive and dose-dependent effect of using *Scrophularia striata on tissue biocompatibility*. The results of this study showed that using *Scrophularia striata* increased cell viability and cell attachment on decellularized pericardia which could pave the way for the use of natural extracts of medicinal plants to reduce unwanted effects and make desired changes in decellularized tissues.

Keywords *Scrophularia striata* · Decellularization · Bovine pericardia · Cell attachment

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Introduction

Inadequate availability of organs has recently become a serious problem in regenerative medicine and many researchers are trying to find a way to overcome it; as

far as regards to the claim of the United Network for Organ Sharing (UNOS) in America, less than 33% of candidates receive a transplant. In addition to applying autotransplantation and xenotransplantation, tissue engineering has introduced a method which is using decellularized human or animal organs and tissues and then recellularize acellular organ and tissue to reestablish function (Wang et al. 2020).

Decellularized scaffolds

Decellularized tissues, like bovine pericardia, are natural scaffolds for tissue engineering which contain preserved extracellular matrix (ECM) (Mirsadraee et al. 2006; Rijal 2017; Heuschkel et al. 2019). These natural scaffolds imitate the ECM perfectly and widely used in many clinical applications for the reconstruction of damaged tissues. These scaffolds also have recently been used in 3D (3-dimensional) and whole organ engineering (Mirsadraee et al. 2006; Heuschkel et al. 2019). It widely used for reconstruction of valvular heart defects and post-infarction septal defects, mitral valve annulus failures, and obstruction of outflow.

Different types of scaffolds

Veritas Collagen Matrix®, Tutopatch®, Peri-Guard®, Collagen Matrix®, Integra® are the examples of commercialized pericardia scaffolds (Swetha et al. 2010; Badylak et al. 2011; Keane et al. 2015; Rijal 2017). In addition, non-cardiac usages of the pericardium in tracheoplasty surgeries and treatment of vaginal and abdominal wall defects have been reported. Still, there is a need to improve methods of decellularization to retain natural structure and function of decellularized ECM as well as modification of its natural properties to be more stable and biocompatible scaffold with anti-inflammatory and antibacterial effects as well as promoting the reconstruction abilities.

Modifiaction of scaffold surface to facilitate the cell adhesion

Modification of decellularized tissues with different agents reported as an appropriate method to improve the needed characteristics of these scaffolds. For example, the laser was used for surface modification of the decellularized extracellular cartilage matrix for cartilage tissue engineering successfully (Goldberg-Bockhorn et al. 2018). In another study decellularized cornea developed by organic acid treatment for improved corneal regeneration (Lin et al. 2019).

In regenerative medicine, most cells must be attached to a bed in order to proliferate and function. So, attachment is a vital factor which are taken into consideration by researchers (Saltzman et al. 1991; Lih et al. 2016). So, increasing cell attachment and biocompatibility of decellularized pericardia tissue can be considered as a particularly appealing goal in tissue regeneration.

Fibronectin and laminin are some molecules that have been used in cell attachment (Saltzman et al. 1991). Sometimes coating the bed for increasing its hydrophilicity is a solution to improve and have suitable cell attachment and growth (Lih et al. 2016). In some researches, cryogel is considered as an active analogue of the natural ECM which provides an appropriate condition for attachment and proliferation of cells (Akilbekova et al. 2018). Elebring et al. decellularized a porcine pancreas and recellularized acellular scaffolds with human fetal pancreatic stem cells. After 14 days, proliferation and attachment of cells were observed which shows their success in pancreas decellularization (Elebring et al. 2017). In other study, a rotating bioreactor was used for human umbilical vein endothelial cells seeding onto the decellularized artery to determine whether the decellularized Porcine carotid artery is conducive for cell attachment or not. Ultimately, they conclude that decellularized Porcine carotid artery improves cell adhesion which demonstrating its potential use as a small-diameter vascular graft (Ho 2018).

Scrophularia striata is a medicinal plant which belongs to the genus Scrophularia and widely use in Iranian folk medicine for infectious diseases and inflammatory (Kerdar et al. 2018). Nowadays, aqueous extraction of *Scrophularia striata* has shown biological activities including antimicrobial, antioxidant, anti-inflammatory, anticancer, antifungal, antibacterial, anti-asthmatic, anti-parasitic, neuroprotective anxiolytic, and anti-depressant in different studies (El-Naggar and Beal 1980; Boros and Stermitz 1990; Del Carmen Recio et al. 1994; Oh 2009; Haddadi et al. 2019). Naserzadeh et al. showed that by using this plant, the antibacterial activity of gold nanoparticles was increased due to the presence of

polyphenol compound in aqueous extraction (Naserzadeh et al. 2019). Nepitrin, Quercetin, acteoside and cinnamic acid are the main compounds identified in *Scrophularia striata* extract. Moreover, n-hexane, caryophyllene oxide, spathulenol, α-cadinol and docosane were the main compounds identified in the essential oil of *Scrophularia striata*. Antimicrobial activity of *Scrophularia striata* was proved by Ethanolic, aqueous, methanolic and ethyl acetate extractions from this plant (Haddadi et al. 2019).

In this study we evaluate the effect of the *scrophularia striata* extract on cell attachment and biocompatibility of decellularized bovine pericardia. At first, extraction of *scrophularia striata* and decellularization of bovine pericardia were prepared. Next, decellularized tissue was incubated in *scrophularia striata* solution. Isolated human adipose-derived mesenchymal stem cells were cultured on tissues and analysis were done.

Materials and methods

Plant isolation and extraction

Scrophularia Striata plant in spring in Ilam Province (Zagros) collected and dried in the shade. The plant approved by experts of Shahid Chamran University of Ahvaz. To extract the effective material from the plant, 200 g of powdered plant incubated in 1000 cc 70% alcohol for 3 days. Then, the extract was filtered and vacuum dried as far as possible. After that, solution with concentrations of 2%, 5%, and 10% weight—volume prepared.

The bovine pericardia

The Dashtyari breed's male bovine pericardia (two-year-old) was collected after the animals were sacrificed (Iran-Shahrekord slaughterhouse). The adipose tissue was removed and the samples placed in a bottle containing 1000 cc phosphate-buffered saline (PBS). Then, it was transferred to laboratory.

Decellularization method

All samples were placed in roller bottles (Hybridization Incubator GFL-7610) and incubated by SDS 1% (Biochem CAS: 151-21-3) for 48 h at 40 °C. The detergent was changed every 12 h. The decellularized tissues were washed at 4 °C for 12 h twice with distilled water. Then, the samples were washed three times for 8 h with PBS (Alizadeh et al. 2019).

Histological study

Hematoxylin and eosin staining (H&E) used for the morphological assessment of pericardium after decellularization. All the samples were fixed in paraformaldehyde 4% (Merck CAS 30525–89-4) and after that, the tissue processing was performed and samples were blocked in paraffin and have been cut in 5 µm thickness by microtome (Leitz 1512) and stained with hematoxylin and eosin (Rezakhani et al. 2020).

Remaining DNA assay (Hoechst staining)

The Hoechst 33258 staining (Sigma Aldrich. USA) was used for DNA evaluation before and after tissue decellularization. The stain was prepared for stock working dilution 1:5000 (1 mg/ml concentration). All samples were processed with a tissue processor and blocked with paraffin. Afterward, 5–7 µm slices were cut by microtome (Leitz 1512). Then, deparaffinization in 60 °C incubator PBS was used for washing and stained for 30 s by working diluted stain. Finally, DAKO fluorescent microscope was used to image recording (Li et al. 2018).

Treatment of the decellularized pericardium with *Scrophularia Striata* extract

The decellularized tissues after washing and sterilization prior to use were incubated an hour in solution with concentrations of 1%, 2.5%, and 5% weight – volume of *Scrophularia Striata*.

Isolation and culture of human adipose-derived mesenchymal stem cells (Ad-hMSCs)

The human adipose tissue of the inguinal section has been harvested from 15 hernias patients. The tissues were transferred to the laboratory,washed in PBS for three times,cut to the 3 × 3 mm and digested with collagenase I in 37 °C. Then, it was neutralized with DMEM (Bioidea) + 10%FBS (Gibco). The cell suspension was centrifuged at 1200 rpm for 5 min and the supernatant was discarded. Afterwards the cells

were transferred to the culture flask and incubated with DMEM + 10%FBS + 1% Pen-strep medium in culture incubator (Memert). After 3 passages, cells were used for the MTT assay (Alizadeh et al. 2021).

MTT assay

The MTT test was based on tetrazolium salt which was broken down by living cells with mitochondrial succinate dehydrogenase. This enzyme breaks down the tetrazolium and converts it to insoluble purple crystals. During the test, 900 µl of the cell culture medium (DMEM (Sigma) with 10% FBS (Gibco) + 1% (penicillin–streptomycin) were added to a 24-well plate and 10^5 stem cells (Ad-MSCs) of human dispersed in it. Then, it was incubated for 24 h in 37 °C with 5% CO2 incubator (Memert). Next, the samples (decellularized bovine pericardia) were cut into 5 × 5 mm and placed inside the wells. The test was performed 48 h. 5 mg/ml MTT solution was prepared and ultimately, 100 µl MTT solution was added to each well and incubated for 4 h at 37° C (the final concentration of MTT in each well should be 0.5 mg/ml). Then, the content of the wells was removed and 200 µl DMSO was added to each well. After 30 min, the content transferred to a 96-well plate and 570 to 630 nm ELISA Reader beam was used to absorbance (Stat fax-2100, USA). The cell survival percentage was calculated by using its formula for each concentration (Koopman et al. 1994; Darzynkiewicz and Traganos 1998; Alizadeh et al. 2020).

Nitric Oxide (NO) assay

NO was measured by the Griess staining method. In the time span of 48 h, the supernatant of the mesenchymal stem cells which was exposed to treated decellularized bovine pericardia, was collected. 400 µl of supernatant were deproteinized by adding 6 mg of zinc sulfate. The vials were centrifuged at 4 °C and 12,000 rpm for 12 min. 100 µl of the supernatant of the de-proteinized samples were added to the wells of the 96-well plate. Then,100 µl of Vanadium chloride, 50 µl of sulfanilamide, and 50 µl of N-(1-Naphthyl) ethylene diamine dihydrochloride (NEDD) were added to each well; the plate was incubated for 30 min at 37 °C. Next, 100 µl of standard sodium nitrate solution, with concentrations of 0, 6.25, 12.5 25, 50, 100, and 200 mM, were prepared. Afterwards Vanadium chloride, sulfanilamide, and NEDD were added to the standard wells similar to the approach used for the samples. The standards and the samples were read at 540 and 630 nm wavelengths by an ELISA reader (Stat Fax 2100, USA) (Ayala et al. 2018; Rezakhani et al. 2017).

SEM examination

To assay cell attachment, 48 h after culturing of mesenchymal stem cells on treated decellularized pericardia, the SEM examination was performed. All of the samples were fixed in paraformaldehyde 4% and freeze-dried and next, examined on surface and cross-section.

Statistical analysis of data

With regard to the various doses, the one-way ANOVA test and the Toky post-hoc test was used, and the data were statistically analyzed by the GraphPad Prism software (Version 8). Mean differences with a $P \leq 0.05$ were considered to be statistically significant. Each assay was performed 3 times and the mean of the resulted data was analyzed.

Results

Histology of decellularized bovine pericardia (H&E staining)

H&E staining showed that the natural bovine pericardia as a connective tissue is the reach of collagen fiber bundles. Connective tissue and fibroblast cells were intact (Fig. 1A). In the decellularized tissue, the cells completely removed and the collagen bundles remained intact (Fig. 1B).

Remaining DNA assay (Hoechst staining)

For detecting of DNA debris in decellularized tissue, the Hoechst staining was performed. Both native and decellularized samples were stained and examined (Fig. 2A and B). The Native sample was glowing blue, which means that all cells and the cell nucleus were intact. In the decellularized tissue, the field of microscope was so dark and cell nucleus and DNA debris were removed.

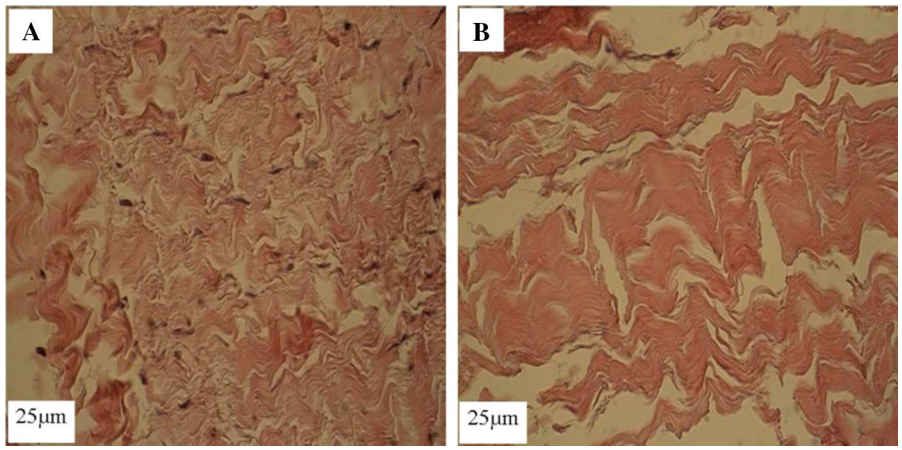

Fig. 1 The H&E staining of decellularized bovine pericardium. **A**: native tissue, **B**: decellularized tissue

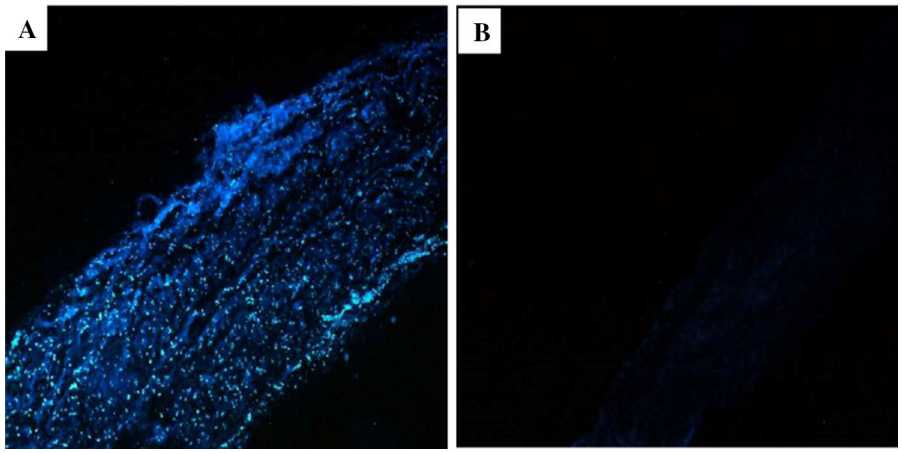

Fig. 2 The Hoechst staining of decellularized bovine pericardia. **A**: native tissue, **B**: decellularized tissue

Effect of *Scrophularia striata* on survival of Ad-hMSCs (MTT assay)

A comparison of the mean survival of Ad-hMSCs by MTT assay after 48 h showed an increase in cell survival after treating the tissue with the extract. With increment in concentration, cell survival significantly was increased ($p \geq 0.001$). The survival of cells in 0.5%, 2.5%, and 5% groups was about 5, 10 and 15 folds higher in comparison to control groups by the significance of $p \geq 0.01$, $P \geq 0.001$, and $p \geq 0.001$ respectively. There was such a significant difference between the treated groups. So that the viability of 2.5% sample was closely two folds higher than 0.5 group ($p \geq 0.001$). In 5% group, the viability was almost three times higher than the viability of 0.5% group ($p \geq 0.001$). The p-value of 2.5% and 5% groups was 0.001 and the viability in the 5% group was 1.5 folds higher than 2.5% group ($p \geq 0.001$) (Fig. 3).

Effect of *Scrophularia striata* on NO secretion

The results showed that NO secretion increased in cells which were cultured in treated decellularized bovine pericardium with *Scrophularia striata* in a dose-dependent manner significantly ($p \geq 0.001$).

There was no significant change in NO secretion between the control and 0.5% treated samples, but in other two treated samples (2.5% and 5%) the NO secretion was increased significantly ($p \geq 0.001$). NO secretion in 2.5% and 5% samples was three and five folds higher than the NO secretion in control group,

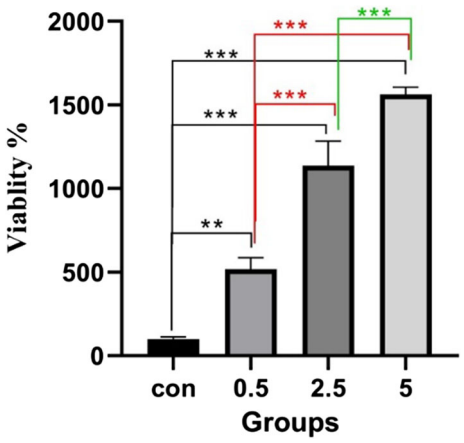

Fig. 3 MTT assay for Ad-hMSCs after culturing in decellularized bovine pericardia

respectively. NO secretion in 2.5 and 5% treated samples was 1.5 and 2.5 times higher than 0.5% samples. NO secretion in 5% treated samples was 1.5 folds higher than 2.5% treated samples ($p \geq 0.001$) (Fig. 4).

SEM examination

The SEM examination was performed to evaluate the attachment of the cells to the decellularized pericardium. The results showed that in decellularized pericardium treated with *scrophularia striata* extract, the cell attachment was increased in a dose-dependent manner. Also, morphology and size of the cells were different. In treated samples, the cells were more elongated (Fig. 5A). In 0.5% samples, the cells were compacted and attached to each other and the cell pedicles were not seen clearly (Fig. 5B). In 2.5% samples, the attached cells were elongated and cell pedicels were visible. In 5% samples, SEM showed that the cells completely were attached to the scaffold, cell pedicels were clearly visible and each cell covered a greater part of the scaffold.

Discussion

In this study our aim was to use *Scrophularia striata* extract to improve biocompatibility of decellularized bovine pericardium. The biocompatibility is a critical characteristic of engineered biomaterials for designing an optimized novel biomeshes for clinical applications. In principle, the biological properties of implantable scaffold should be matched to the target tissues and able to promote cell proliferation and attachment (Badylak and Gilbert 2008; Williams 2008). Reconstruction and regeneration of damaged tissues can be considered as a key purpose of using biologic scaffolds in tissue engineering (O'Brien 2011). In fact, the grafted biomaterials must accelerate new blood vessels formation through the promotion of new ECM components production (Liang et al. 2004).

Bovine pericardia tissue has been broadly applied as a biomedical tool to restore cardiac valves and congenial disease treatment because of many desirable features including fibroserous structure which is made- up of collagen bundles and elastic fibers (Bielli et al. 2018). So, the decellularized bovine pericardium seems to be favorable in regenerative medicine approaches. The successful decellularization method can remove nuclear substances and cell debris to avoid immune rejection as well as the lowest level of ECM nature and structural damage that provides cell growth improvement (Wolf et al. 2014). Although, the efficiency of decellurization methods depends on tissues' origin and materials and procedures which were used. The chemical decellularization protocol is using some detergents such as SDS which is widely used. In addition to collagenous and non-collagenous proteins damage that is caused by SDS, the remaining detergents decrease biocompatibility of the decellularized tissues. For this reason, some modifications in decellularization methods as well as the treatment with some materials such as medicinal plants extract for the treatment of decellularized scaffold after chemical decellularization could be efficient in increasing biocompatibility (Badylak et al. 2011).

Scrophularia striata is an Iranian herbal medicinal plant known as "Tashaneh Dari" from the Scrophulariaceae family. Many investigations have been reported the beneficial pharmacologic effects of striata including anti-inflammatory properties, cell proliferation promotion, and wound healing progress (Azadmehr et al. 2009; Monsef-Esfahani et al. 2010; Ghashghaii et al. 2017). Our results of this study suggested that the improvement of biocompatibility of decellularized bovine pericardia due to different concentrations of *Scrophularia striata* extracts. Here, decellularization process was achieved successfully by SDS 1% solution. As shown in the final histological evaluation (Figs. 1 and 2), the intact collagen bundles

without fibroblast cells present in H&E and Hoechst images that confirmed the efficacy of the decellularization method. Our results were as similar as Heuschkel MA et al. study who reported complete decellularization using SDS1% (Heuschkel et al. 2019). In addition, Ad-hMSCs viability and NO secretion increased after treatment of decellularized tissue with different concentrations of herbal extracts (Figs. 4, 5). The MTT and NO secretion data supports the concepts of treated tissues biocompatibility elevation. The assay results showed that 5% weight—volume of *Scrophularia Striata* increased Ad-MSCs cell proliferation and NO production was much more than 1% and 2.5% weight—volume herbal extract (Gardin et al. 2015; Li et al. 2018; Heuschkel et al. 2019). The effect of presence this plant on cell viability and proliferation was investigated by Haddadi et al. They showed that

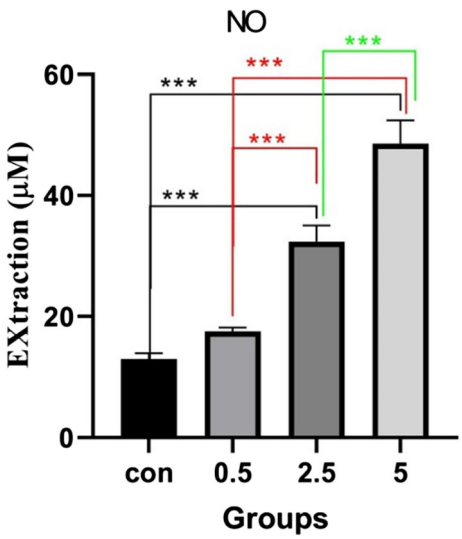

Fig. 4 The NO secretion from Ad-hMSCs cells after culturing in treated decellularized bovine pericardium

because of using *Scrophularia striata*, by passing the time, the number of live cells and their growth will increase. Additionally, the higher the concentration of the plant (up to a limited value), the higher the cell viability and life span (Haddadi et al. 2019).

Moreover, the ability of cell for attachment increased after treatment with 1%, 2.5%, and 5% weight—volume of *Scrophularia Striata* resulted in the approperiate morphology and attachment of cells on the treated decellularized bovine pericardia, as shown in SEM data (Fig. 5). This finding has been proven by Haddadi et al. in 2019. SEM data showed that higher concentration of *Scrophularia Striata,* were associated with a higher cell attachment and migration (Haddadi et al. 2019).

Here, we introduced a novel decellularization procedure to enhance the biocompatibility of decellularized bovine pericardia by *Scrophularia striata* extraction. In the regard, this biocompatible natural scaffold will be able to increase regeneration related to the elevation of cell engraftment, proliferation, and attachment after applying in skin or heart disorders.

Conclusion

The results of this study indicated that *Scrophularia striata* in a dose-dependent manner increased significantly cell viability and cell attachment in decellularized bovine pericardia using SDS. In conclusion, treating or washing SDS decellularized bovine pericardia with *Scrophularia striata* extract can increases the biocompatibility of this scaffold. These findings could pave the way for the use of natural extracts of medicinal plants to reduce unwanted effects and make desired changes in decellularized tissues.

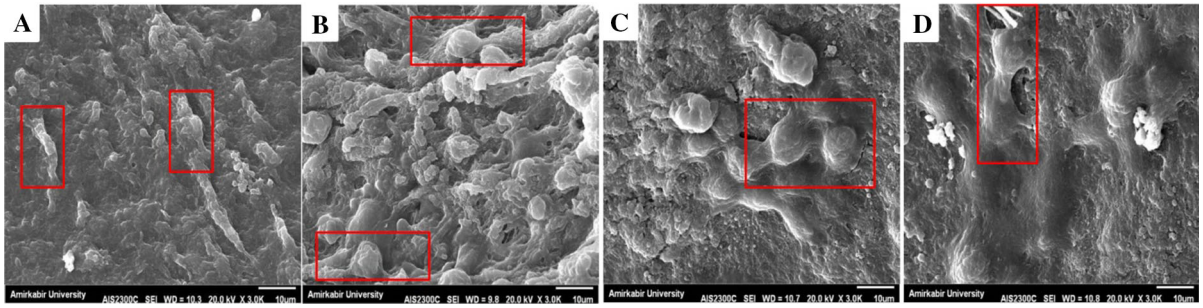

Fig. 5 SEM examination, control (**a**), 0.5% (**b**), 2.5% (**c**) and 5% (**d**). The attached cells marked using red lines

Authors' contributions All the authors contributed in experiments and data analyzing, and writing the manuscript.

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Availability of data and material Data will be available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate All the authors are in agreement to participate.

Consent for publication All the authors agree for publication

References

Akilbekova D, Shaimerdenova M, Adilov S, Berillo D (2018) Biocompatible scaffolds based on natural polymers for regenerative medicine. Int J Biol Macromol 114:324–333

Alizadeh A, Rezakhani L, Shoa MA, Ghasemi S (2020) Frequency of CD44 positive cells in MKN45 cell line after treatment with docetaxel in two and three-dimensional cell cultures. Tissue Cell 63:101324

Alizadeh M, Rezakhani L, Khodaei M, Soleimannejad M, Alizadeh A (2021) Evaluating the effects of vacuum on the microstructure and biocompatibility of bovine decellularized pericardium. J Tissue Eng Regen Med 15:116–128

Alizadeh M, Rezakhani L, Soleimannejad M, Sharifi E, Anjomshoa M, Alizadeh A (2019) Evaluation of vacuum washing in the removal of SDS from decellularized bovine pericardium: method and device description. Heliyon 5:e02253

Ayala P, Dai E, Hawes M et al (2018) Evaluation of a bioengineered construct for tissue engineering applications. J Biomed Mater Res Part B Appl Biomater. https://doi.org/10.1002/jbm.b.34042

Azadmehr A, Afshari A, Baradaran B et al (2009) Suppression of nitric oxide production in activated murine peritoneal macrophages in vitro and ex vivo by Scrophularia striata ethanolic extract. J Ethnopharmacol. https://doi.org/10.1016/j.jep.2009.03.042

Badylak SF, Gilbert TW (2008) Immune response to biologic scaffold materials. Semin Immunol 20(2):109–16. https://doi.org/10.1016/j.smim.2007.11.003

Badylak SF, Taylor D, Uygun K (2011) Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. https://doi.org/10.1146/annurev-bioeng-071910-124743

Bielli A, Bernardini R, Varvaras D et al (2018) Characterization of a new decellularized bovine pericardial biological mesh: Structural and mechanical properties. J Mech Behav Biomed Mater. https://doi.org/10.1016/j.jmbbm.2017.12.003

Boros CA, Stermitz FR (1990) Iridoids. An updated review. part. J Nat Prod. https://doi.org/10.1021/np50071a001

Darzynkiewicz Z, Traganos F(1998) Measurement of apoptosis. Adv Biochem Eng Biotechnol 62:33–73. https://doi.org/10.1007/BFb0102305

Del Carmen RM, Giner RM, Manez S, Rios JL (1994) Structural considerations on the iridoids as anti-inflammatory agents. Planta Med. https://doi.org/10.1055/s-2006-959465

El-Naggar LJ, Beal JL (1980) Iridoids. a review. J Nat Prod. https://doi.org/10.1021/np50012a001

Elebring E, Kuna VK, Kvarnström N, Sumitran-Holgersson S (2017) Cold-perfusion decellularization of whole-organ porcine pancreas supports human fetal pancreatic cell attachment and expression of endocrine and exocrine markers. J Tissue Eng 8:2041731417738145

Gardin C, Ricci S, Ferroni L et al (2015) Decellularization and delipidation protocols of bovine bone and pericardium for bone grafting and guided bone regeneration procedures. PLoS ONE. https://doi.org/10.1371/journal.pone.0132344

Ghashghaii A, Hashemnia M, Nikousefat Z et al (2017) Wound healing potential of methanolic extract of Scrophularia striata in rats. Pharm Sci. https://doi.org/10.15171/PS.2017.38

Goldberg-Bockhorn E, Schwarz S, Subedi R et al (2018) Laser surface modification of decellularized extracellular cartilage matrix for cartilage tissue engineering. Lasers Med Sci. https://doi.org/10.1007/s10103-017-2402-8

Haddadi R, Tamri P, Jooni FJ (2019) In vitro wound healing activity of Scrophularia striata hydroalcoholic extract. South African J Bot 121:505–509

Heuschkel MA, Leitolis A, Roderjan JG et al (2019) In vitro evaluation of bovine pericardium after a soft decellularization approach for use in tissue engineering. Xenotransplantation. https://doi.org/10.1111/xen.12464

Keane TJ, Swinehart IT, Badylak SF (2015) Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods 84:25–34. https://doi.org/10.1016/j.ymeth.2015.03.005

Kerdar T, Moradkhani S, Dastan D (2018) Phytochemical and biological studies of scrophularia striata from Ilam. Jundishapur J Nat Pharm Prod. https://doi.org/10.5812/jjnpp.62705

Koopman G, Reutelingsperger CPM, Kuijten GAM et al (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. https://doi.org/10.1182/blood.v84.5.1415.bloodjournal8451415

Li N, Li Y, Gong D et al (2018) Efficient decellularization for bovine pericardium with extracellular matrix preservation and good biocompatibility. Interact Cardiovasc Thorac Surg. https://doi.org/10.1093/icvts/ivx416

Liang HC, Chang Y, Hsu CK et al (2004) Effects of crosslinking degree of an acellular biological tissue on its tissue regeneration pattern. Biomaterials. https://doi.org/10.1016/j.biomaterials.2003.09.109

Lih E, Park KW, Chun SY et al (2016) Biomimetic porous PLGA scaffolds incorporating decellularized extracellular matrix for kidney tissue regeneration. ACS Appl Mater Interfaces 8:21145–21154

Lin HJ, Wang TJ, Li TW et al (2019) Development of decellularized cornea by organic acid treatment for corneal regeneration. Tissue Eng Part A. https://doi.org/10.1089/ten.tea.2018.0162

Mirsadraee S, Wilcox HE, Korossis SA et al (2006) Development and characterization of an acellular human pericardial matrix for tissue engineering. Tissue Eng 12:763–773

Monsef-Esfahani HR, Hajiaghaee R, Shahverdi AR et al (2010) Flavonoids, cinnamic acid and phenyl propanoid from aerial parts of Scrophularia striata. Pharm Biol. https://doi.org/10.3109/13880200903133829

Naserzadeh Y, Mahmoudi N, Pakina E et al (2019) Parameters affecting the biosynthesis of gold nanoparticles using the aquatic extract of Scrophularia striata and their antibacterial properties. J Nanoanal 6:105–114

O'Brien F (2011) Biomaterials & scaffolds for tissue engineering. Materials Today 14: 88–95. https://doi.org/10.1016/S1369-7021(11)70058-X

Oh CH (2009) Monitoring of residual pesticides in herbal drug materials of Korea and China. Bull Environ Contam Toxicol. https://doi.org/10.1007/s00128-009-9649-0

Rezakhani, Leila and Alizadeh, Morteza and Alizadeh, Akram (2021) *A three dimensional in vivo model of breast cancer using a thermosensitive chitosan-based hydrogel and 4 T1 cell line in Balb/c.* Journal of Biomedical Materials Research - Part A, 109 (7). pp. 1275–1285. ISSN 15493296

Rezakhani L, Khazaei MR, Ghanbari A, Khazaei M (2017) Crab shell extract induces prostate cancer cell line (LNcap) apoptosis and decreases nitric oxide secretion. Cell J (yakhteh) 19:231

Saltzman WM, Parsons-Wingerter P, Leong KW, Lin S (1991) Fibroblast and hepatocyte behavior on synthetic polymer surfaces. J Biomed Mater Res 25:741–759

Swetha M, Sahithi K, Moorthi A, Srinivasan N, Ramasamy K, Selvamurugan N (2010) Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering. Int J Biol Macromol 47(1):1–4. https://doi.org/10.1016/j.ijbiomac.2010.03.015

Taylor DA, Sampaio LC, Ferdous Z, Gobin AS, Taite LJ (2018) Decellularized matrices in regenerative medicine. Acta Biomater 1(74):74–89. https://doi.org/10.1016/j.actbio.2018.04.044

Wang Y, Dominko T, Weathers PJ (2020) Using decellularized grafted leaves as tissue engineering scaffolds for mammalian cells. Vitr Cell Dev Biol. https://doi.org/10.1007/s11627-020-10077-w

Williams DF (2008) On the mechanisms of biocompatibility. Biomaterials. https://doi.org/10.1016/j.biomaterials.2008.04.023

Wolf MT, Dearth CL, Ranallo CA et al (2014) Macrophage polarization in response to ECM coated polypropylene mesh. Biomaterials. https://doi.org/10.1016/j.biomaterials.2014.04.115

Xu Y, Yan M, Gong Y, Chen L, Zhao F, Zhang Z (2014) Response of endothelial cells to decellularized extracellular matrix deposited by bone marrow mesenchymal stem cells. Int J Clin Exp Med 7(12):4997–5003