Biology, Engineering and Medicine

Research Article ISSN: 2399-9632

Antibacterial activity and composition of decellularized goat lung extracellular matrix for its tissue engineering applications

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Abstract

In this study, scaffold was fabricated from goat-lung of cadaver goat by decellularization method for its potential application in tissue engineering. The cellular components of goat-lung were removed by employing trypsin-SDS based method of decellularization. DNA quantification and DAPI staining verified the effective removal of native cells from the goat-lung. Verhoeff-Van Gieson (VVG) staining, H&E staining and Periodic-Acid Schiff (PAS) staining histologically assessed the composition of native and decellularized goat-lung matrix—demonstrating the presence of collagen, elastin and glycoproteins in the decellularized goat-lung matrix. Antibacterial activity of decellularized goat-lung matrix was evaluated against gram-negative (*Escherichiacoli*) and gram-positive (*Staphylococcusaureus*) bacteria, which shows the presence of naturally occurring bioactive peptides—exhibiting the antibacterial activity against *E. coli* for up to 9h and against *S. aureus* for up to 5h. The decellularized goat-lung matrix, as demonstrated by MTT assay, was found to be biocompatibile to L929 mouse fibroblasts, HepG2, human skin derived mesenchymal stem cells (hS-MSCs), osteoblasts and BMMSCs. All the above results confer the applicability of the decellularized goat-lung matrix as a potential scaffold for tissue engineering applications.

Introduction

Tissues are made up of cells, which are surrounded by extracellular matrix (ECM) consisting of various structural and functional proteins, e.g., collagen, fibronectin, laminin, proteoglycans and glycoproteins. In a tissue, the ECM of each cell is connected to the ECM of other cells—building three-dimensional (3D) cell-ECM architecture. If all the cells residing in a tissue are removed, a 3D architecture of ECM will be left behind, which could act as a scaffold for supporting new cells for tissue regeneration [1-5]. Various scientists have been trying to use the ECM (allogeneic or xenogeneic) network as scaffold for regenerating neo-tissues. Many organs, e.g. urinary bladder [6], small intestinal [7-11], heart valves [12-15], skin [16,17], liver [18], anterior crucial ligament bone [19], kidney [20], pericardium [21], blood vessels [22], nerves [23], skeletal muscle [24], tendon [25] and vocal cord [26,27] from bovine and porcine tissues, have been used for fabricating the ECM-based scaffold. But, the use of goat-lung has not been focused for tissue engineering applications. The development of scaffolds, from bovine and porcine tissues, possesses a risk of disease transmission from bovine and porcine tissues to humans [28-33], and their use in clinical applications might involve high risk of disease in recipient humans, e.g., Creutzfeldt-Jakob disease. On the other hand, goat tissue could be a better choice as they are least susceptible to viruses [34]. Besides, the prions (viruses) of goat scrapie have not yet been reported to cause any disease in humans [30,35-37] . Therefore, goat tissue could be a safer option, as there will be no/minimal risk to human health if they are used for fabricating scaffold for tissue engineering. Lung is highly porous and therefore the ECM from goat-lung, after cell removal, is supposed to provide a 3D highly porous architecture with a complex mixture of both structural and functional proteins [34,38]. Collagen is an important component in lung ECM for providing strength to the tissue and elastin fibers helps in natural recoiling of lung [39]. The assessment of ECM proteins (collagen, elastin and glycoproteins) present in the decellularized lung matrix is important as these proteins influence the growth and proliferation of cells in the tissue. Therefore, one of our aims is to investigate about the composition of decellularized goat-lung matrix for its applicability as scaffold for tissue engineering applications.

The components present in the ECM are subject to degradation during constructive tissue remodeling. The matrix, during degradation/proteolysis, releases various kinds of bioactive peptides/matricryptic molecules, which play significant role in cell recruitment and constructive restoration of the tissue [40-42]. Some of these bioactive peptides released during degradation have been reported to possess antibacterial properties that are essential for providing instantaneous protection in prevention of an implant-infection in the duration when the inflammatory cellular response and humoral immune response of the host become activated [40,43-56]. We hypothesize that such antibacterial activity exists within the goat-lung matrix. The antibacterial property of the goat-lung matrix has not been reported and therefore, we aim to investigate this important aspect, as this

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Key words: *antibacterial activity, decellularized scaffold, extracellular matrix, goat-lung, tissue engineering*

Received: January 07, 2017; **Accepted:** January 25, 2017; **Published:** January 28, 2017

property, if present, will make the decellularized goat-lung matrix a prime scaffold for tissue engineering applications. Our objective is to explore the cadaver goat-lung for tissue engineering applications, and in this work we aim to assess the ECM components and antibacterial property of the decellularized goat-lung matrix.

Materials and methods

Materials

Dulbecco's Phosphate Buffered Saline (PBS-/-, without Ca^{2+} and Mg^{2+}), Mueller Hinton (MH) broth, antibiotic antimycotic solution 100X liquid (endotoxin tested), collagenase enzyme, 3-(4,5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) (MTT), ethanol, RNase, DNase, dimethyl sulphoxide (DMSO) and agar powder (bacteriological grade) were purchased from Himedia Laboratories Pvt. Limited, Mumbai, India. 0.25% trypsin containing 0.02% ethylene-diamine-tetra-acetic acid (0.25% trypsin-EDTA) was obtained from Himedia, India. Ethylene diamine tetra acetic acid (dehydrate) dipotassium salt (EDTA) was obtained from Fischer Scientific. Tetracycline was obtained from Sigma, St. Louis, MO, U.S.A. Fetal bovine serum (FBS) was received from Hyclone, USA. T-75 flasks and Petri dishes (35mm), for cell culture, were obtained from Nunc, India. Double distilled water was prepared in our laboratory and the same was used during experiment.

Decellularization of goat-lung tissue

In order to decellularize the cadaver goat-lung tissue, it was first sectioned into $1cm^2$ dimensions, and then treated with 0.25% trypsin-EDTA in 1X PBS supplemented with 1% antibiotic–antimycotic solution to detach the cells from their ECM. Subsequent treatments were carried out by agitating the tissue in 0.1% SDS in 1X PBS at 37°C for 6–8h, followed by treating in a solution containing RNase (20µg/ml) and DNase (0.2mg/ml) for 24h. The decellularized goat-lung sections were then disinfected, by gently shaking in an aqueous solution of 0.1% (v/v) hydrogen peroxide (oxidizing agent) and 4% (v/v) ethanol, for 2h. The tissue sections were then extensively rinsed with 1X PBS to remove any residual oxidizing agent present in the tissue. The goat-lung matrix, after decellularization, was stored in 1X PBS at 4°C prior to further processing and seeding.

Verification of decellularization

The complete removal of cells, after decellularization, was verified quantitatively by estimating the DNA content of the native tissue and the decellularized tissue. The total genomic DNA of native and decellularized goat-lung tissue was isolated with the help of phenol-chloroform-isoamyl alcohol method. In brief, the weighed tissue samples (n=3) were at first minced with the scissors and degraded with 0.1% collagenase at 37°C. Subsequently samples were centrifuged at 14,000rpm and the supernatant was collected. The phenol-chloroform-isoamyl alcohol (25:24:1) were then added and centrifuged again. The supernatant was siphoned and precipitated by 100% ethanol at -85°C for 45 minutes followed by centrifugation at 14,000 rpm for 10minutes. The pellets were rehydrated in 1X Tris-EDTA buffer and quantified at 260nm, using spectrophotometer (Eppendorf Biophotometer, Hamburg, Germany). The removal of cells from the goat-lung tissue was also verified using DAPI (4' 6-diamidino-2-phenylindole) fluorescent staining. After the decellularization process, the goat-lung tissue section was stained with DAPI to observe any stained nucleus in the tissue. DAPI stained decellularized tissues were then incubated in the dark for 30min at room temperature, and visualized by an inverted microscope (Axiovert 25, Carl Zeiss, Germany). The images were captured with an attached camera linked to computer.

Histological assessment of decellularized goat-lung matrix

Tissues were fixed in neutral buffered formalin and then embedded in paraffin. The paraffin embedded native and decellularized goat-lung tissues were sectioned (5 µm in thickness). The effectiveness of trypsin-SDS based decellularization to remove cells from the native goat-lung tissue and the preservation of ECM components (collagen, elastin, glycoproteins) was evaluated by different staining methods. The tissue sections were stained using Hematoxylin and Eosin (H&E) staining, Verhoeff-Van Gieson (VVG) staining for assessment of collagen and elastin, and Periodic-Acid Schiff (PAS) staining for evaluation of glycoproteins present in the native and decellularized goat-lung tissue. The quantitative assessments of the stained decellularized sections were done by using Image J program (ImageJ 1.40g, Wayne Rasband, National Institute of Health, USA) as previously reported [57,58].

In vitro biodegradation of decellularized goat-lung matrix

The decellularized goat-lung tissue was weighed and placed in 10mL of 1X PBS (pH 7.4) having collagenase from *Clostridiumhistolyticum* (125 U/mg) and incubated in the shaking incubator at 37°C for 60h. At different time intervals, the decellularized tissues were removed from the shaker, lyophilized and weighed. The *in vitro* biodegradation of the goat-lung tissue (n=3) were calculated by using the following equation:

$$\text{Weight loss (\%)} = 100 \times [W_0 - W_t / W_0]$$

where, W_0 denotes the initial weight of the decellularized goat-lung tissue, and W_t denotes the weight of the degraded scaffold at different time intervals.

Assessment of antibacterial activity of decellularized goat-lung matrix

The antibacterial activity of decellularized goat-lung matrix was assessed against gram-negative *Escherichia coli* (*E. coli*, strain NCIM 2931) and gram-positive *Staphylococccus aureus* (*S. aureus*, strain MTCC 3160). MH broth and MH agar were prepared according to the manufacturer's instructions and incubated overnight at 37°C to confirm sterility. MH agar plates were streaked with *E. coli* and *S. aureus*, and isolated colonies (2–3 lag phase growth) were picked and transferred into vials containing 10mL of MH broth, and incubated at 37°C overnight in shaker. When the culture reached an optical density of 0.1 at 570nm, the cultures were harvested from MH broth, diluted to 105 CFU/mL, and 100µL of bacterial suspension were added to each well of a 96-well culture plate. On the other hand, 100 g of goat-lung matrix (90% moist) was suspended in 10 mL 1X PBS containing collagenase (2mg/mL). The matrix was incubated in shaker at 37°C for complete degradation until no visible pieces of the tissue remains in the tube. 25µL of degraded goat-lung matrix was added to both the bacterial suspension. Tetracycline (broad-spectrum antibiotic) was used as an antibiotic control for both *E. coli* and *S. aureus* bacterial cultures. The stock solution of tetracycline (10 mg/mL) was prepared in 50% methanol. MH broth (without matrix) was taken as negative control and *E. coli* and *S. aureus* bacterial suspension were taken as positive controls. The absorbance was measured at different predetermined time intervals at 570nm. In an effort to eliminate errors in the procedure, all assays were performed in triplicates.

Biocompatibility and cell viability study by MTT assay

MTT assay is a colorimetric test based on the conversion of MTT

tetrazolium salts into purple colored formazan products. Only viable cells with active metabolism are able to convert MTT into tetrazolium salt, therefore, the intensity of color formation is directly proportion to the number of viable cells. Various different types of cells: L929 mouse fibroblasts, HepG2, skin derived mesenchymal stem cells (hS-MSCs), osteoblasts and bone derived mesenchymal stem cells (BMMSCs) were maintained in DMEM with 10% FBS and 1% PenStrep solution in a 5% CO_2 incubator at 37°C (Binder, Germany). Xenogeneic goat-lung decellularized matrix sectioned into approximately 1mm^3 sizes, and 4-5 sections were plated in the 96-well plate to cover the entire surface with scaffolds, in triplicates. The scaffolds were then UV sterilized in the laminar airflow chamber for 30 min, and the cells were seeded. All the cells were seeded at the density of 1 x 10^3 cells/well directly over the scaffold, in triplicate, and incubated for 5min to maximize the adherence of cells only to scaffold and provide minimum chance to dribble into the culture plate. DMEM was then added into the wells containing decellularized scaffold with cells to make up the volume 200µl. After respective time period of 48h, 10µl of MTT solution (5mg/ml) and 90µl of DMEM were added in each well, and incubated for 4h. After incubation, purple colored formazan crystals were dissolved with 100µl DMSO and absorbance was taken at 490nm with the subtraction for plate absorbance at 650nm. The recorded absorbance value (i.e. O.D.) is considered as a measure of cell viability.

Statistical analysis

Antibacterial activity, biocompatibility and cell viability study of decellularized goat-lung matrix were obtained in triplicate samples. Data were expressed as the mean ± standard deviation. Using MINITAB statistical software (MINITAB release 13.32), statistical analysis (ANOVA test) was performed with $p<0.05$ considered as being statistically significant.

Results and discussion

Decellularization of goat-lung matrix and its verification

Cellular components, if present in the matrix, could elicit an immune response to host [59], therefore, an effective removal of cells from the matrix is important. To verify the effectiveness of the decellularization method and complete removal of cells from the goat-lung matrix, DNA quantification and DAPI staining had been performed (Figure 1). It was observed (Figure 1A) that the DNA content of the decellularized tissue was significantly less (34.2 ± 6.5 ng DNA/mg of dry weight decellularized tissue) as compared to the native tissues (875.3 ± 61.42ng DNA/mg of dry weight native tissue)—indicating effective removal of cells from the native tissue (Table 1). It has been reported that if the DNA content of a tissue is less than 50ng DNA/mg dry weight of ECM, then the adverse host immune response, after implantation [60], can be avoided. The residual DNA content of 34.2ng per mg of decellularized tissue indicates that it is below the threshold amount of 50ng DNA/mg tissue, and therefore, it should not induce any immunogenic response. The negative cell nuclei staining by DAPI stain (Figure 1B and Figure 1C) also demonstrated the efficient removal of cells from the goat-lung and supported the result of DNA quantification study. The native goat-lung tissue (control) containing cells showed positive nuclear staining by DAPI stain. These methods have been used repeatedly by several researchers for assessment of cell removal from the decellularized tissues [61-63]. DNA quantification and DAPI staining thus verified the efficient removal of cells from the goat-lung tissue demonstrating the applicability of decellularized goat-lung matrix as a scaffold.

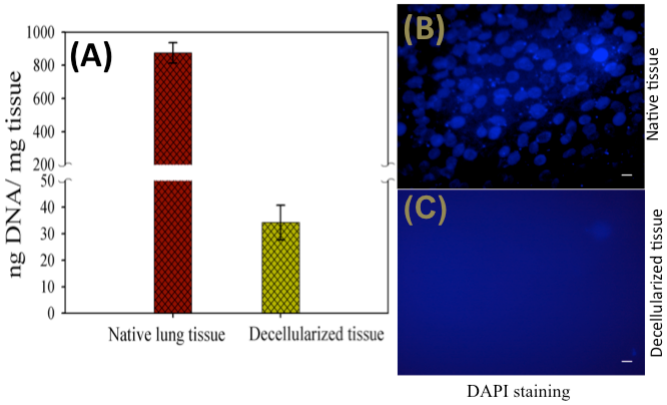

Figure 1. (A) DNA content of native and decellularized tissue indicating significant decrease in the DNA after decellularization, and (B) DAPI staining showing positive nuclear staining in the native tissue indicating the presence of cells, and (C) negative nuclear staining in the decellularized tissue indicating absence of cells. Sacle bars: 50 µm.

Histological assessment of decellularized goat-lung matrix

VVG staining of the native goat lung matrix indicated the presence of collagen fibers (red), elastin fibers (black) and nuclei (blue) (Figure 2A), however in the decellularized matrix, it showed the presence of collagen (red) and elastin fibers (black) only, with the absence of cell

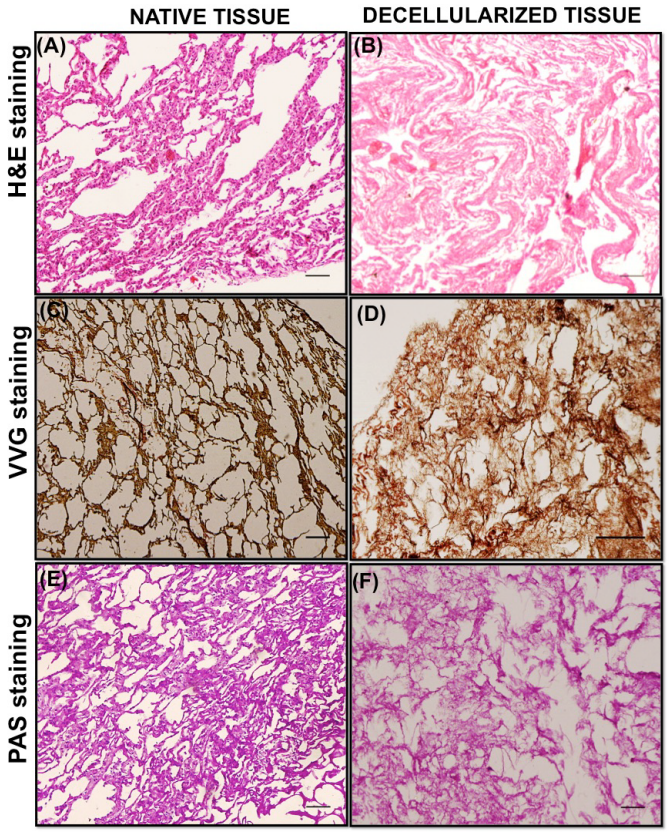

Figure 2. Representative staining images of native and decellularized goat-lung tissue. *A-B panels:* Hematoxylin and Eosin staining of native (A) and decellularized matrix (B) demonstrating the absence of cellular/nuclear components and the presence of collagenous structure in the decellularized matrix. *C-D panels:* VVG staining of native tissue (C) and decellularized matrix (D) showing the presence of collagen proteins stained red and elastin proteins stained black in the decellularized matrix. *E-F panels*: PAS staining of native (E) and decellularized matrix (F) demonstrated the presence of glycoproteins and proteoglycans in the decellularized matrix (arrows). Scale bars = 100 µm.

Table 1. Quantitative analysis of decellularization verification and ECM components in native goat-lung tissue (control) and decellularized goat-lung tissue.

Groups		Native tissue (Control)	Decellularized tissue	Remarks
Decellularization verification	DNA quantification	875.3 ± 61.42	34.2 ± 6.5	Significant cell removal from decellularized matrix
	DAPI staining	100	0	Absence of cells in decellularized matrix
Quantitative analysis of ECM components	H&E staining	100	90.10 ± 7.71	Presence of collagen and absence of cells in decellularized matrix
	VVG staining	100	92.23 ± 9.8	Presence of collagen and elastin in decellularized matrix
	PAS staining	100	88.29 ± 2.36	Presence of glycoproteins in decellularized matrix

DNA quantification is expressed as ng of DNA per mg of tissue sample. DAPI, H&E, VVG and PAS staining correspond to percentage (%) in reference to native goat-lung tissue, and are expressed as means ± standard deviations.

nuclei (Figure 2B). This indicates an effective elimination of cellular components during the decellularization process while maintaining the major ECM components (collagen and elastin) of the lung matrix. The presence of collagen fibers in the ECM of decellularized tissue was also demonstrated by H&E staining. The collagen fibers gets stained with eosin and appeared pink in color, which indicated the maintenance of collagenous matrix structure in the decellularized tissue (Figure 2D). The result of H&E staining also confirmed the presence of cells (nuclei stained blue) in the native tissue (Figure 2C) and absence of nuclear counterstaining in the decellularized tissue (Figure 2D). PAS staining helps in the evaluation of glycoproteins and present in the matrix [64]. The positive result of PAS staining (Figure 2E and 2F) verifies the existence of glycoproteins in the decellularized matrix.

The quantitative assessment of the collagen, elastin and glycoproteins in the decellularized tissue (Table 1) was demonstrated by Image J program. For each stained image, 5 small areas were randomly selected (n=5) and the staining intensity was calculated. VVG stained image and H&E stained image demonstrated good staining intensity, over 90% of the control (native tissue), for VVG stain (92.23 ± 9.8) and H&E stain (90.10 ± 7.71), which suggested the preservation of collagen and elastin proteins in the decellularized tissue, thus maintaining the 3D structural integrity of the goat-lung tissue. The assessment of PAS stained images(staining intensity of 88.29 ± 2.36) suggested the presence of glycoproteins with some loss during decellularization process. It was expected that some of the proteins would get dissolved and washed away during decellularization process as SDS tend to solubilize the ECM proteins [19,60,65]. Previous study by Oliveria *et al.* [58] has also performed similar histologic assessment using Image J program. Taken together, VVG, H&E and PAS staining confirmed that decellularized goat-lung matrix was devoid of cells and the ECM components (collagen, elastin and glycoproteins) were still retained in the matrix, after decellularization: these will help in maintaining the structural and functional properties of the ECM, which can be used as tissue engineering scaffold.

In vitro biodegradation of decellularized goat-lung matrix

Biodegradability is one of an important characteristic for tissue engineering scaffold. Following implantation, degradation of scaffold biomaterial is an essential step for constructive remodeling of the tissue [66]. As the major constituent of lung ECM comprises collagen proteins, therefore, collagenase enzyme was chosen to perform the biodegradation of decellularized lung matrix. The *in vitro* biodegradation of decellularized goat-lung matrix was evaluated by quantifying the weight loss after treating the matrix with 0.2% collagenase enzyme (Figure 3) at 37°C in 1X PBS solution. The results of *in vitro* biodegradation of decellularized goat-lung matrix showed that with the progression of days, the matrix gradually degrades, and after 60h, the matrix was completely degraded. This result confirms

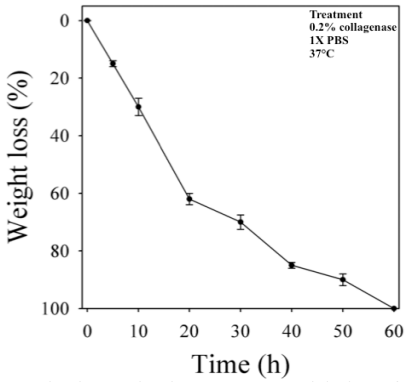

Figure 3. Representative image showing percentage weight loss of decellularized goat-lung matrix incubated in 1X PBS containing 0.2% collagenase at 37°C for 60 h. Data are expressed as means ± standard deviations.

that collagenases (endopeptidase) helped in degrading the triple helix region of the collagen molecule present in the ECM [67,68].

It has been reported earlier that the *in vitro* degradation of scaffold differs from *in vivo* degradation process as various matrix metalloproteinases [69] and macrophages [70,71] are responsible for degrading biological scaffold materials *in vivo*. The *in vitro* biodegradation of decellularized lung scaffold by 0.2% concentration of collagenase was used as an '*accelerated model*' of degradation and the collagenase activities *in vivo* are not expected to be as harsh as *in vitro* [72]. Moreover, the degradation rates of the scaffold can be controlled by varying the enzyme concentrations [73] or by using various crosslinking agents (carbodiimide, glutaraldehyde), which might affect the collagen structure of the scaffold and modify the cleavage sites for collagenase enzyme [60,74,75]. These results are an *in vitro* approximation, and the degradation characteristic provide an insight that the decellularized goat-lung matrix is highly biodegradable and could be used as potential biomaterial for tissue regeneration.

Antibacterial activity of decellularized goat-lung matrix

In vitro collagenase mediated degradation of decellularized goat-lung scaffold have been used as a model for demonstrating the antibacterial effects of degradation products. The results of antibacterial study (Figure 4) showed that the naturally occurring peptides obtained from the degradation of decellularized goat-lung matrix demonstrated activity against both Gram-positive (*E. coli*) and Gram-negative bacteria (*S. aureus*). The decellularized goat-lung matrix, after collagenase treatment, releases some bioactive peptides molecule, which significantly inhibited the growth of *E. coli* up to 9h (Figure 4A), and the growth of *S. aureus* (Figure 4B) up to 5h. After inhibiting the growth of *E. coli* and *S. aureus* for 9h and 5h

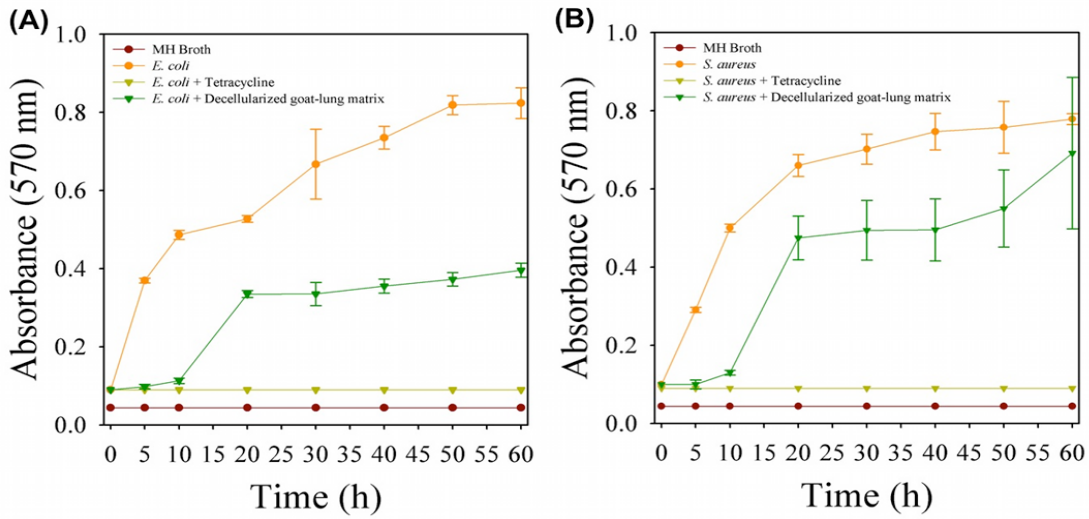

Figure 4. Antibacterial activity of decellularized goat-lung matrix digests on the growth of (A) gram-negative bacteria, *E. coli* and (B) gram-positive bacteria, *S. aureus*. Data are shown as means ± standard deviations with significance at $p<0.05$.

respectively, the antibacterial activity of goat-lung matrix components gets diminished and bacterial cells started increasing their growth and proliferation. This short term antibacterial effects of degradation products of decellularized goat-lung ECM is supposed to help in providing an immediate protection to the implanted tissue, until humoral immune response and host cell inflammatory response become activated. Similar type of activity has been demonstrated by the ECM scaffold components derived from small intestinal sub-mucosa (SIS) and urinary bladder sub-mucosa (UBS), which exhibit antibacterial activity against both Gram-negative and Gram-positive bacteria [76]. The antibacterial activity against *E. coli* and *S. aureus* has also been examined for porcine liver tissue and superficial layers of the porcine urinary bladder [77]. Although the exact mechanism of killing bacteria by action of peptides (produced during the degradation of the goat-lung ECM) is poorly understood [78], but it has been reported that either the antibacterial peptides may get directly associated with the bacterial cell membrane leading to lysis of the membrane or they get diffused into the bacterial cell cytoplasm and interferes with their protein synthesis [79]. The difference in antibacterial activity (Figure 4) of the peptides against *E. coli* and *S. aureus* might be due to difference in the defense mechanism of the bacteria. The intact decellularized goat-lung ECM (without collagenase digestion) did not possess any antibacterial activity, and similar findings have been reported in a previous study [80]. Thus, the result of antibacterial activity shows that decellularized goat-lung ECM digests have the potential to inhibit the growth of both Gram-positive and Gram-negative bacteria, thereby, proving its potential for tissue engineering applications. Therefore, if the decellularized goat-lung matrix is used as a tissue-engineering scaffold, then it will gradually degrade *in vivo* and release antibacterial peptides continuously- providing a sustained antibacterial effect in the host.

Biocompatibility and cell viability study by MTT assay

In order to assess whether xenogeneic decellularized goat-lung matrix would offer safe, non-cytotoxic and biocompatible scaffold for tissue regeneration, MTT assay was performed. We used various different types of cells (L929, HepG2, skin derived mesenchymal stem cells, osteoblasts and bone derived mesenchymal stem cells) to check the biocompatibility, viability and proliferation of all the cells over

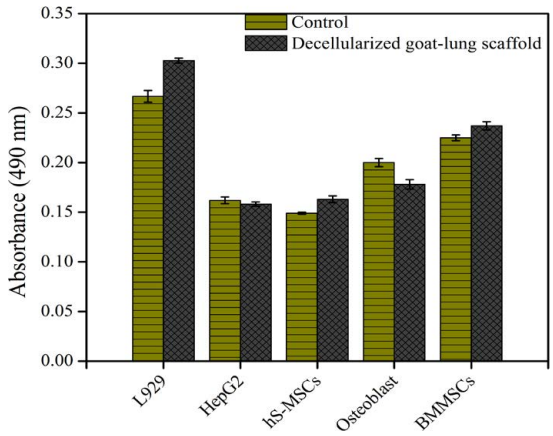

Figure 5. Biocompatibility and viability of L929, HepG2, skin derived mesenchymal stem cells (hS-MSCs), osteoblasts and bone derived mesenchymal stem cells (BMMSCs) over decellularized goat-lung matrix, assessed by MTT assay for 48h. Absorbance was measured in replicates of three and the calculated standard error of the mean plotted as error bars. Here, absorbance is directly proportional to cell viability.

decellularized goat-lung matrix. The result of MTT assay (Figure 5) showed that the decellularized goat-lung matrix was biocompatible and the cell viability was not affected after 48 h (2 days) of cell culture. All the cells were able to metabolize the MTT substrate, which indicates that the cell's mitochondria were functional over decellularized goat-lung matrix, and resulted in good cell viability and proliferation. Thus, MTT assay demonstrated that the decellularized goat-lung matrix, a xenogeneic tissue, constitutes a biocompatible biomaterial for cell culture, and could be used as a potential scaffold for tissue engineering applications.

Conclusion

Decellularized goat-lung matrix retained the structural 3D architecture after decellularization, and therefore it can be used as 3D scaffold for tissue engineering. The presence of collagen, elastin and glycoproteins in the decellularized goat-lung matrix, as demonstrated by VVG staining, H&E staining and PAS staining, provided the

structural support and bio-functional properties for cell growth and proliferation. The *in vitro* biodegradation of the decellularized matrix reflected the release the bioactive peptides, which exhibit antibacterial activity against *E. coli* and *S. aureus*. The viability of various cells (L929, HepG2, skin derived mesenchymal stem cells, osteoblasts and bone derived mesenchymal stem cells) over the xenogeneic decellularized goat-lung matrix revealed the biocompatibility and non-cytotoxic effects of the decellularized goat-lung matrix on the cellular metabolic activity. Thus, this study demonstrated the feasibility of using the decellularized goat-lung matrix as a potential scaffold for tissue engineering applications.

Acknowledgement

The authors appreciate the suggestions given by Dr. Amiq Gazdhar, Universität Bern, Bern and acknowledge him. The authors are also thankful to Council of Scientific and Industrial Research (Grant No. 27(0222)/10/EM R-II dated 31.05.10) and Ministry of Human Resource and Development for the financial assistance to carry out this research work.

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