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***Research Article***

# EFFECT OF GAMMA RAY STERILISATION ON DIFFERENTLY DECELLULARISED OMENTUM BASED SCAFFOLDS

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**ABSTRACT: Terminal sterilisation of extracellular matrix scaffolds is an essential step in biomedical applications as well as ensuring product safety. Selection of appropriate sterilisation method is crucial in order to retain the structural and mechanical properties of the scaffold. The present study evaluated the effect of gamma ray sterilisation (25KGy) on biomechanical properties of differently decellularised omental scaffolds. Triton X- 100 and bile were used as decellularising agents and un-processed defattened omentum as control. Effect of irradiation on maximum force, tensile strength and Young's modulus before and after irradiation was evaluated using Texture Profile Analyser. Our results indicated that, gamma ray irradiation reduced the maximum force and tensile strength of differently processed omental scaffolds. Bile treatment was found to have more protective action on the structural component of the scaffolds than triton X-100. Thus, bile treated omental scaffold showed better biomechanical properties for maximum force and tensile strength than triton X- 100 after irradiation.**

**Key words:** Extracellular matrix, Gamma ray irradiation, Omental scaffold, Triton X-100, Bile, Texture profile analyser.

## INTRODUCTION

Scaffolds derived from extracellular matrix biomaterials are beneficial in a large number of applications in tissue engineering and regenerative medicine (Balakrishnan-nair *et al.* 2019). Increased demand for tissue derived scaffolds intensified the development of newer scaffolds for clinical translation (Balakrishnan-nair *et al.* 2018). These extracellular matrix (ECM) scaffolds are composed of proteins and polysaccharides which forms a 3-D structure, facilitating cell migration, proliferation and differentiation for constructive tissue regrowth and remodelling (Pawan *et al.* 2019). Allogeneic and xenogeneic tissues cannot be used directly as scaffolds because of adverse host reactions due to cellular remnants (Badylak *et al.* 2012). De-cellularisation is the process used to remove the cellular component, while retaining its ECM structure. Intensive research has been carried out for the development of ideal protocol using different de-cellularising agents for developing an ideal biomaterial (Li *et al.* 2018). Moreover, no effective de-cellularisation agent is so far available to preserve biomechanical properties of the scaffold. Chemical detergents have been used extensively for decellularisation, but in certain cases it will evoke adverse host response (Morris *et al.* 2016). Hence, there is a high need for developing natural de-cellularisation agents to limit this adverse host response.

After de-cellularisation, a successful sterilization method provides adequate mechanical properties, preservation of structure, and biocompatibility which has critical effect on the host response. Currently, methods employed for sterilisation include gamma irradiation (Fideler 1995), e-beam irradiation and treatment using ethylene trioxide (Muhammed *et al.* 2014), gas plasma, per-acetic acid and ethanol (Delgado *et al.* 2014). Gamma irradiation is the most common sterilization method for the commercial production of scaffolds (Kim *et al.* 2018). Sterilisation of human tissue derived scaffold using

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gamma-irradiation is well documented (Vernon *et al.* 2005). Gamma irradiation at a range of 5-25 (KGy) is used for the sterilisation of scaffolds derived from various tissue origins. Previous studies have shown the direct effect of gamma-irradiation on the mechanical properties of collagen-based scaffolds (Gouk *et al.* 2007). Here, we have investigated the effect of gamma ray irradiation on biomechanical properties of differently de-cellularised bovine omentum based scaffolds for the development of novel biomaterials to the veterinary and human patients.

## MATERIALS AND METHODS

### Scaffold preparation

Fresh bovine omenta were collected immediately after slaughter from Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy, Kerala. For de-fattening, manual and chemical methods (Chloroform: Methanol) were used. Chemical detergent, triton X- 100 and biological detergent, bile were used for de-cellularisation. Defattened omental scaffold was treated with at 1% triton X-100 solution for 48 hrs in a shaker incubator. Subsequently it was washed 4-5 times with 1% TBS and allowed to dry under lamina air flow. For bile treated de-cellularisation, defattened omentum was treated with 80% aqueous solution of bile for 48 hrs in a shaker incubator followed by washing with 1% TBS and dried under laminar air flow. Gamma ray irradiation at a rate of 25 KGy was employed for sterilisation. De-fattened un-processed omentum was used as control in this study. Treatment groups included un-processed bovine omentum (UBO), bile treated bovine omentum (BBO) and triton X- 100 treated bovine omentum (TBO) (Fig. 1a, 1b and 1c).

Six samples were randomly collected from differently processed omental scaffolds (UBO, BBO and TBO) before and after irradiation, and assessed their biomechanical properties. Texture profile analyser (Mitutoyo, Japan) was used for assessing biomechanical properties. Six strips of 1 × 5 $cm^2$ size were cut randomly from each differently processed omentum. The thickness of the strip material was measured at five different points using Vernier calliper and the mean thickness was calculated. At both ends of each strip, a paper strip was attached and placed between the clamps. All samples were loaded with a feed rate of 1 mm $min^{-1}$, until complete breakage. The same procedure was repeated for the material after irradiation also. Mechanical characteristics such as Young's modulus, maximum force and tensile strength were calculated using Trapezium software.

### Statistical analysis

The result was expressed as Mean ± SE. Statistical analysis was conducted using SPSS software version 24.0. The result was examined by one-way analysis of variance (One Way ANOVA), followed by Tukey's test at p value less than 0.01.

## RESULTS AND DISCUSSION

The differently de-cellularised omental scaffolds were processed and sterilised. The thickness of each material from each sample group (n=6) was measured using Vernier callipers and the mean thickness was calculated. The mean thickness of UBO, BBO and TBO was 0.07 mm, 0.05 mm and 0.06 mm, respectively.

### Maximum force

Before irradiation, maximum force for each material was analysed (Fig. 2 a). UBO, BBO and TBO showed a

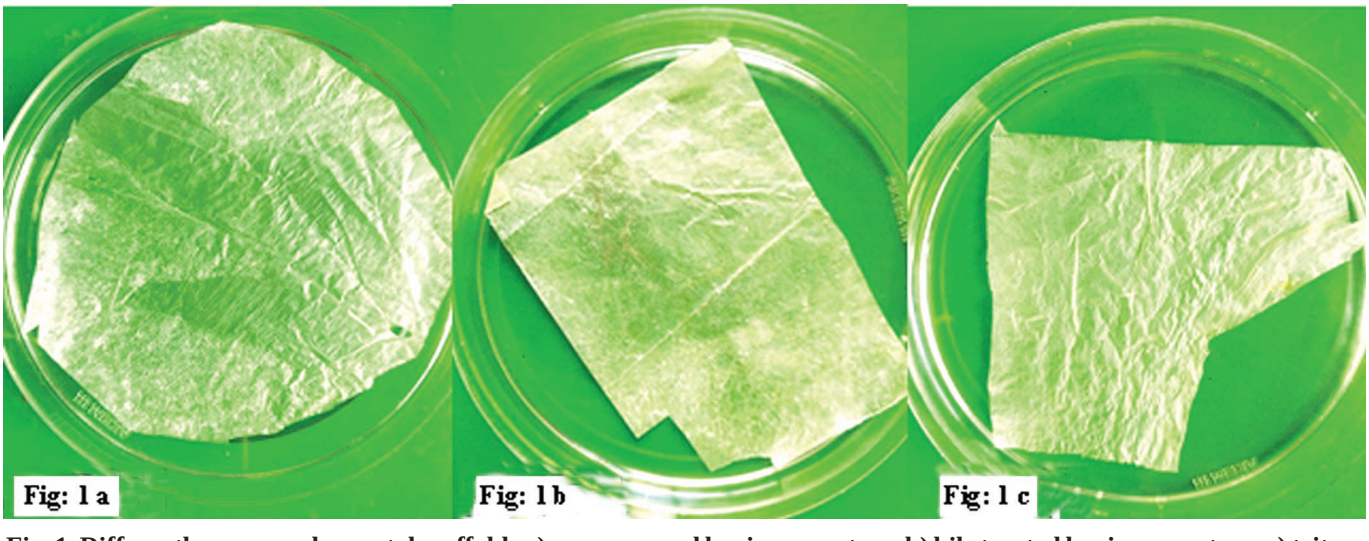


**Fig. 1. Differently processed omental scaffolds a) un-processed bovine omentum, b) bile treated bovine omentum, c) triton X-100 treated omentum.**

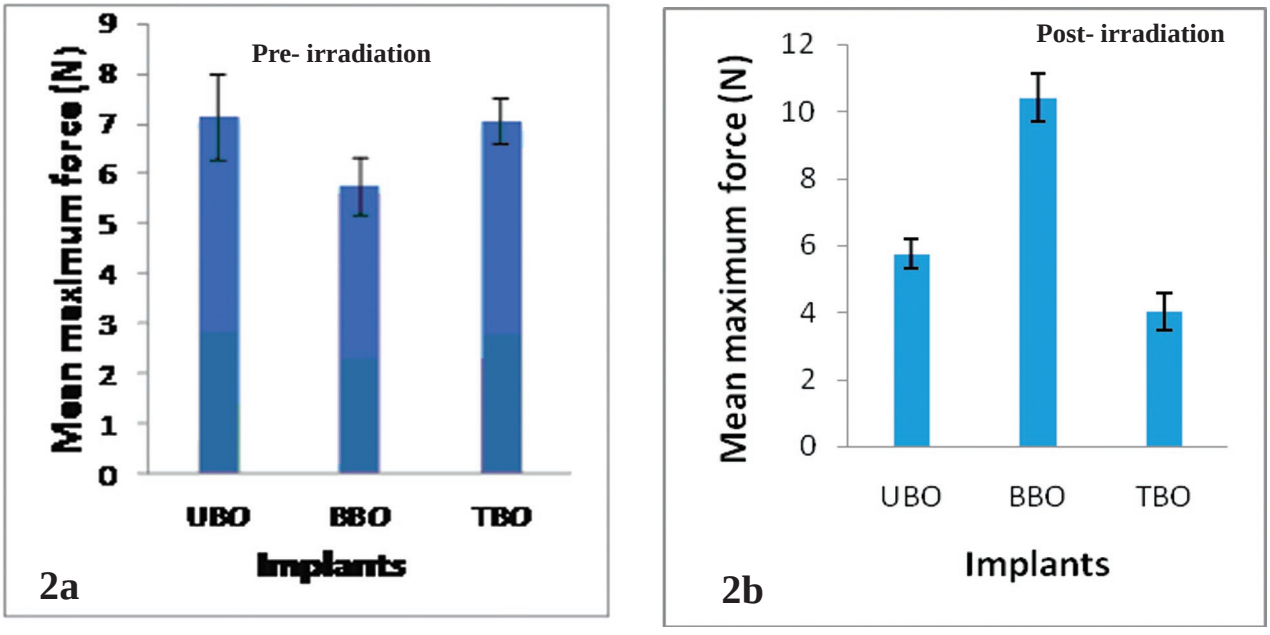


**Fig. 2. Comparison of maximum force of differently de-cellularised omental scaffolds before and after irradiation.** [UBO- Un-processed bovine omentum, BBO- Bile treated bovine omentum, TBO- Triton X-100 treated omentum. Value expressed as Mean ± SE. p value less than 0.01].

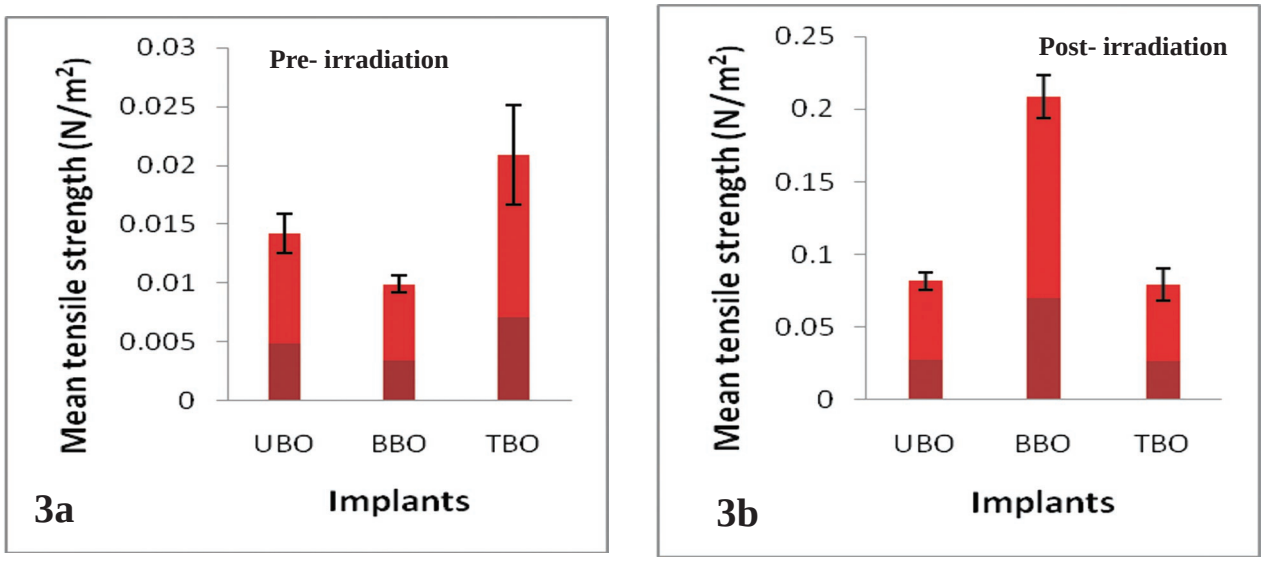


**Fig. 3. Comparison of tensile strength of differently decellularised omental scaffolds before and after irradiation.** [UBO- Un-processed bovine omentum, BBO- Bile treated bovine omentum, TBO- Triton X-100 treated omentum.Value expressed as Mean ± SE. p value less than 0.01].

mean maximum force of 7.1278 ± 0.857 N, 5.7405 ± 0.5657 N and 7.045 ± 0.4454 N respectively. The mean maximum force was high for UBO and TBO but there was no significant difference among the three groups. After irradiation, maximum force for UBO, BBO and TBO showed a mean maximum force of 5.739 ± 0.4322, 10.405 ± 0.7238 and 4.0317 ± 0.5595 respectively (Fig. 2b). Among the biomaterials, BBO showed the highest maximum force, which was significantly different compared to UBO and TBO.

**Tensile strength**

The mean tensile strength of differently de-cellularised scaffolds before irradiation are shown in Fig. 3 a. The tensile strength of UBO, BBO and TBO were 0.0142 ± 0.0017 N/ $m^2$, 0.0099 ± 0.0007 N/ $m^2$ and 0.0209 ±0.0042

N/ $m^2$ respectively. On statistical analysis, no significant difference was observed among the scaffold biomaterials in terms of tensile strength before irradiation. Mean tensile strength of differently de-cellularised omentum based scaffolds after irradiation are shown in Fig. 3b. The tensile strength of UBO, BBO and TBO are 0.0819 ± 0.0062 N/$m^2$, 0.2084 ± 0.0145 N/$m^2$, 0.0791 ± 0.011 N/$m^2$ respectively. There was significant difference in the tensile strength of BBO from that of UBO and TBO in terms of tensile strength after irradiation.

**Young's modulus**

Before irradiation, Young's modulus of UBO, BBO and TBO were 2.11 ± 0.269MPa, 1.21 ± 0.087MPa and 4.52 ± 0.55MPa respectively. On statistical analysis, there was no significant difference between UBO and BBO in terms of Young's modulus (Fig. 4a). However, Young's modulus of TBO was significantly different from UBO and BBO. After irradiation, Young's modulus of UBO, BBO and TBO were 3.0375 ± 0.2761MPa, 3.3193 ± 0.342MPa and 2.564 ± 0.2438MPa respectively. However, there was no significant difference observed between UBO, BBO and TBO in terms of Young's modulus (Fig. 4b).

Before biomaterial implantation and clinical applications, biological extracellular matrix scaffolds should be sterilized to ensure the product safety. Each step in the processing and preparation of scaffold has the potential to affect the mechanical and biodegradable properties of scaffolds (Hutmacher *et al*. 2014). Biomechanical properties of scaffolds have direct clinical applications and studies have shown that the sterilization process has got some effect on the mechanical properties of scaffolds (Dai *et al.* 2016). However, each study was different in respect to origin of tissues, processing methods and sterilization techniques.

Common sterilization methods for commercially available scaffolds include ethylene trioxide treatment, gamma irradiation and gas plasma, per-acetic acid and ethanol treatments. Sterilization with preservation of tissue matrix integrity is essential for the proper functioning of the scaffold. The present study was designed to evaluate the effect of gamma irradiation (25 KGy) on differently de-cellularised omentum based scaffolds. Maximum force, tensile strength and Young's modulus are the important parameters that were used to evaluate the biomechanical properties of biomaterials (Gouk *et al.* 2007). Maximum force denotes the load bearing capacity of the scaffold. Before irradiation, there was no change in the maximum force for differently processed omental scaffolds, but after irradiation, there was significant difference in the maximum force and it was more for bile treated omental scaffold. The mean value for maximum force in post irradiation was less compared to pre- irradiation except in BBO. It might be due to the effect of irradiation on the collagen and may have caused direct splitting of protein and polypeptide chain which further caused easy breakage (Goclawska *et al*. 2005, Nguyen *et al*. 2006). Higher value obtained for BBO might be due to the protective effect of bile on the cell membrane (Merritt *et al.* 2009).

Tensile strength represents the tissue capacity to

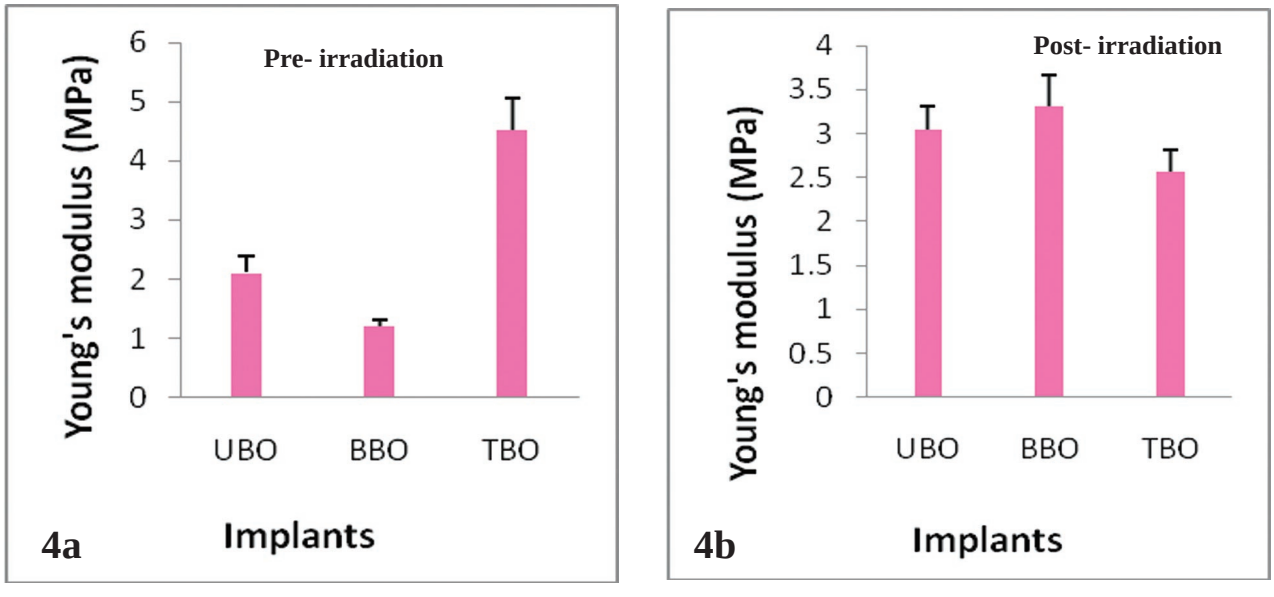


**Fig. 4. Comparison of Young's modulus of differently de-cellularised omental scaffolds before and after irradiation**. [UBO- Un-processed bovine omentum, BBO- Bile treated bovine omentum, TBO- Triton X-100 treated omentum. Value expressed as Mean ± SE. p value less than 0.01].

withstand load and to elongate. This parameter is well evaluated for the design of collagen rich scaffolds (Degarmo *et al*. 2003). There was no significant difference between treatment groups before irradiation. This may be due to the fact that, de-cellularisation methods might not have any effect on the tensile strength. Similar result was also reported by Purpura *et al.* (2018) on the tensile strength of human foreskin-based scaffold. But after irradiation, there was significant difference for tensile strength of BBO from TBO and UBO. BBO showed increased tensile strength than UBO and TBO. Deoxycholic acid is the proposed component in the bile which has detergent property. Suvaneeth *et al.* (2016), stated that deoxycholic acid treated bovine pericardium showed higher tensile strength than the enzymatic treated pericardium. Stiffness of the material is evaluated by Young's modulus. It is essential to evaluate the ability of the material to stretch under a load. TBO showed higher Young's modulus than BBO and TBO in pre-irradiation, but there was no significant difference observed among BBO, UBO and TBO in post-irradiation. These changes might have been primarily due to the degradation of collagen by gamma-irradiation, leading to a reduction in tensile strength of scaffold (Freytes *et al.* 2008).

While analyzing the biomechanical properties, gamma irradiation has been found to have some effect on the mechanical parameters without causing any adverse effects. Gamma-irradiation has many other superior qualities such as simplicity and effectiveness over other sterilisation techniques. Nonetheless, gamma ray sterilisation (15KGy) can get rid of *E. coli* and *Staphylococcus aureus* (Kim *et al*. 2018). Here, gamma-irradiation changes the structural and biomechanical properties which may affect the functionality of the scaffolds. On comparing different de-cellularisation agents, bile treated omental scaffolds showed significantly higher maximum force and tensile strength than un-processed and triton X-100 treated omental scaffolds. This may be due to the protective property of bile over triton X-100. Bile also ensures better preservation of biomechanical properties than triton X-100.

## CONCLUSION

We aimed to evaluate the effect of gamma ray irradiation on differently de-cellularised omentum based scaffolds. The results show that gamma irradiation affects the biomechanical properties such as maximum force, tensile strength and Young's modulus of omental scaffolds. Between the two de-cellularising agents used for the comparative study, bile has shown to have better protective effects on the biomechanical properties than triton X-100.

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## REFERENCES

Badylak SF, Turner NJ, Londono R, Keane TJ (2012) Consequence of in-effective decellularization of biologic scaffolds on the host response. Biomaterials 33: 1771-1781.

Balakrishnan-nair DK, Nair ND, Venugopal SK, Abraham MJ, Sunanda C *et al*. (2019) Herovici's staining: a useful differential staining method for evaluating collagen distribution in biomaterial-mediated hernia repair. J Vet Anim Sci 50(2): 154-158.

Balakrishnan-nair DK, Nair ND, Venugopal SK, Das VN, George S *et al.* (2018) An immunopathological evaluation of the porcine cholecyst matrix as a muscle repair graft in a male rat abdominal wall defect model. Toxicol Pathol. 46: 169-183.

Dai Z, Ronholm J, Tian Y, Sethi B, Cao X (2016) Sterilization techniques for biodegradable scaffolds in tissue engineering applications. J Tissue Eng 7: 204173141664881.

Degarmo EP, Black JT, Kosher RA (2003) Materials and process in manufacturing. Solutions manual. John Wiley & Sons, Inc.

Delgado LM, Pandit A, Zeugolis DI (2014) Influence of sterilisation methods on collagen-based devices stability and properties. Expert Rev Med Devices 11: 305-314.

Fideler BM, Vangsness JR, Lu CT, Orlando B, Moore T (1995) Gamma irradiation: effects on biomechanical properties of human bone-patellar tendon-bone allografts. Am J Sports Med 23: 643-646.

Freytes DO, Stoner RM, Badylak SF (2008) Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds. J Biomed Mater Res B Appl Biomater 84B: 408-414.

Goclawska A, Kaminski A, Uhrynowska-Tyszkiewicz I, Stachowicz W (2005) Irradiation as a safety procedure in tissue banking. Cell Tissue Bank 6: 201-219.

Gouk SS, Lim TM, Teoh SH, Sun WQ (2007) Alterations of human acellular tissue matrix by gamma irradiation: Histology,

biomechanical property, stability, *in vitro* cell repopulation, and remodelling. J Biomed Mater Res B Appl Biomater 84B: 205-217.

Hutmacher DW, Woodfield TBF, Dalton PD (2014) Scaffold design and fabrication, 2nd edn. Oxford: Academic Press, London.

Kim S, Jeong J, Lee S, Park J, Gwon H *et al*. (2018) Effective gamma-ray sterilization and characterization of conductive polypyrrole biomaterials. Sci Rep 8: 3721.

Li J, Tan J, Martino M M, Lui K O (2018) Regulatory T-cells: potential regulator of tissue repair and regeneration. Front Immunol 9: 55-59.

Merritt ME, Donaldson JR (2009) Effect of bile salts on the DNA and membrane integrity of enteric bacteria. J Medical Microbiol 58: 1533-1541.

Morris H, Chang J, Thermis R (2016) In adequate processing of decellularised dermal matrix reduces cell viability *in vitro* and increase apoptosis and acute inflammation *in vivo*. Biores Open Access 5(I): 177-187.

Muhammed J, Revi D, Rajan A, Geetha S, Anilkumar TV (2014) Biocompatibility and immunophenotypic characterization of a porcine cholecyst–derived scaffold implanted in rats. Toxicol Pathol 43: 536-545.

Nguyen H, Morgan DAF, Forwood MR (2006) Sterilization of allograft bone: effects of gamma irradiation on allograft biology and biomechanics. Cell Tissue Banking 8: 93-105.

Pawan KC, Hong Y, Zhang G (2019) Cardiac tissue-derived extracellular matrix scaffolds for myocardial repair: advantages and challenges. Regen Biomater 1-15.

Purpura VE, Bondioli, EJ, Cunningham, GD, Luca D, Capirossi E *et al*. (2018) The development of a decellularized extracellular matrix–based biomaterial scaffold derived from human foreskin for the purpose of foreskin reconstruction in circumcised males. J Tissue Eng 9: 1-11.

Suvaneeth P, Divakaran NN, Ramachandra UP, Narayanadas V, Abraham MJ *et al*. (2016) *In-vitro* characterization of differently processed decellularised bovine pericardium. J Livestock Sci 7: 13-18.

Vernon RB, Gooden MD, Lara SL, Wight TN (2005) Native fibrillar collagen membranes of micron-scale and submicron thicknesses for cell support and perfusion. Biomaterials. 26(11): 1117.

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