The *in vivo* Compatibility, Integration and Regeneration of a Decellularised Porcine Bone Scaffold for Musculoskeletal Tissue Repair and Regeneration

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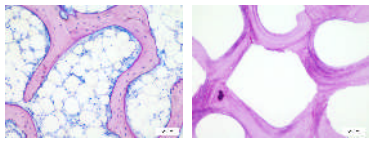
Introduction

- Acellular natural tissue scaffolds for musculoskeletal regeneration (eg.. ACL repair) show great promise.
- Achieving adequate fixation of these scaffolds into the joint is challenging.
- Incorporation of the bony attachment sites of acellular musculoskeletal scaffolds may improve fixation.
- It is thought this will provide superior scaffold integration and regeneration.
- **Aim:** To investigate the capacity of decellularised porcine bone to osteointegrate and regenerate when implanted *in situ* in an ovine condyle.



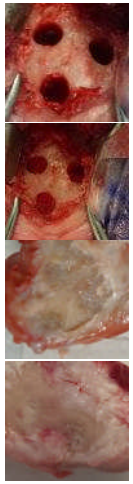
Native Decellularised

Figure 1. Toluidine blue/basic fuchsin stained resin sections of native and decellularised porcine bone (scale bars = 100 µm).

Results

Decellularisation: Decellularised porcine bone contained no cell nuclei [Fig 1]. DNA content of decellularised bone was 20.2 ± 8.5 ng.mg^{-1} dry weight [native porcine bone 546.5 ± 216.8 ng.mg^{-1}].

Compatibility: No contact or extract cytotoxicity was observed with BHK or L929 cells. No adverse host response to the decellularised porcine bone when implanted in mice at 4 or 12 weeks.



Empty 6 mm Ø defect in an ovine femoral condyle

T=0 implanted acellular bone scaffold

4 weeks post implantation

12 weeks post implantation

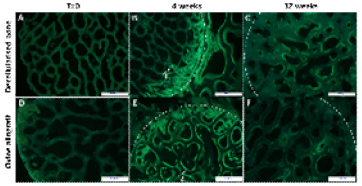


Figure 2. Histological assessment of new bone deposition. Fluorescent labelling of mineralised bone deposition between 15 and 5 days prior to sacrifice. Dotted lines indicate the graft-host bone margin, arrows indicate osteoconduction. Scale bar = 1000 µm.

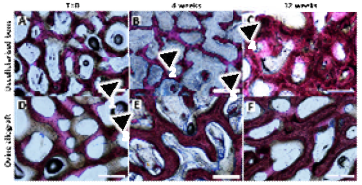


Figure 3. Histological assessment of tissue remodelling. Modified paragon stained sections of explanted tissues. Arrow 1 = lymphocytic infiltrate, 2 = fibro-mesenchymal infiltrate, 3 = necrotic bone marrow degeneration, 4 = new bone marrow formation, 5 = residual lymphocytic infiltrate. Scale bar = 500 µm.

	4 week implantation		12 week implantation	
	Ovine allograft bone	Decellularised porcine bone	Ovine allograft bone	Decellularised porcine bone
Osteoblastic cells	2.7 ± 0.5	1.3 ± 0.5	2.0 ± 0.0	1.5 ± 0.5
Bone neoformation	2.5 ± 0.5	2.5 ± 0.5	3.0 ± 0.0	3.0 ± 0.0
Bone remodelling	1.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Material degradation	0.3 ± 0.5	1.3 ± 0.5	0.0 ± 0.0	1.0 ± 0.0
Osteointegration	0.5 ± 0.0	3.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
Osteoconduction	2.5 ± 0.5	1.5 ± 0.5	4.0 ± 0.0	3.0 ± 0.0
Osteotransduction	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Bone density	2.5 ± 0.5	1.5 ± 0.5	3.0 ± 0.0	3.0 ± 0.0
Bone mineralisation	3.7 ± 0.5	1.5 ± 0.5	4.0 ± 0.0	3.2 ± 0.4

Table 1. Semi-quantitative histopathological analysis of the bone healing response to allograft and decellularised porcine bone implants. Healing response parameters were graded 0-4 (0 = absent, 1 = slight, 2 = moderate, 3 = marked and 4 = severe).

	4 week implantation		12 week implantation	
	Ovine allograft bone	Decellularised porcine bone	Ovine allograft bone	Decellularised porcine bone
Polymorphonuclear cells	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lymphocytes	1.2 ± 0.7	2.5 ± 0.7	2.0 ± 0.0	1.5 ± 0.5
Plasma cells	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Macrophages	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
Giant cells/ osteoclast cells	1.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Necrosis	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fibrous encapsulation	0.5 ± 0.5	1.0 ± 0.4	0.5 ± 0.5	0.5 ± 0.7
Neovascularisation	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Fatty infiltrate/ bone marrow	0.8 ± 0.7	2.5 ± 0.5	2.0 ± 0.0	2.8 ± 0.4
Fibrinous exudate (fibrin)	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fibroplasia	1.0 ± 1.4	3.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5
Osteolysis	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.4
Tissue degeneration	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0

Table 2. Semi-quantitative histopathological analysis of the host response to allograft and decellularised porcine bone implants.

Osteointegration & regeneration: Decellularised porcine bone had integrated after 4 weeks [Fig 2]. An initial lymphocytic response was seen at 4 weeks which slowed new bone deposition, this had resided at 12 weeks.

Discussion

- Porcine bone was fully decellularised and the resultant scaffold was cytocompatible and biocompatible.
- The decellularised porcine bone fully integrated and regenerated in the ovine condylar model, with the level of integration comparable to that of allograft bone.
- Although an initial lymphocytic response to the acellular xenogeneic bone was observed, this was greatly reduced after 12 weeks.

Conclusion

- These studies supported the hypothesis that inclusion of bony attachment sites in bone-soft tissue-bone acellular scaffolds will enable improved fixation and integration into the joint.
- The successful *in vivo* integration and regeneration of decellularised porcine bone indicates it may have clinical use as a bone substitute biomaterial.
- Further work is required to understand the biomechanical function of the acellular porcine scaffold prior to and following regeneration, to provide insight into the potential clinical applications of this biomaterial.

Materials and Methods

- **Decellularisation:** Porcine bone plugs from the distal femur (6 mm diameter, 10 mm long; n=6) were decellularised using a process incorporating water flossing, followed by acetone treatment and washing in low concentration sodium dodecyl sulphate (SDS) with protease inhibitors, sonication was used throughout [1, 2]. Decellularisation was assessed by resin (toluidine blue/basic fuchsin, DAPI) and frozen section (sudan black) histology. DNA was extracted from tissues using Qiagen kits and quantified by nano-spectrophotometry.
- **Compatibility:** Contact and extract cytotoxicity tests were carried out using BHK and L929 cells, cell viability was determined by quantification of cellular ATP. Biocompatibility of the acellular bone was assessed by subcutaneous implantation in mice (4 and 12 weeks, n=3 native and n=3 decellularised porcine bone). Explanted tissues were assessed by paraffin wax histology (H&E).
- **Osteointegration and regeneration:** Decellularised porcine and control allograft ovine bone plugs (6 mm diameter, 10 mm long; n=6) were implanted into the medio-distal femoral condyle of skeletally mature sheep for 4 and 12 weeks. At 15 and 5 days before termination, sheep received oxytetracycline injections to fluorescently label newly formed bone. Explants were assessed by resin histology (modified paragon, epifluorescence) and semi-quantitative pathological assessment of the inflammatory and healing responses to the grafts was performed.

References

1. *Stapleton ,T., et al. Tissue Eng. Part A 14, 505 (2008)*
2. *Ingham, E., Jones, G., Fermor, H., Hasan, J., & Fisher, J. Composite bone implants; a decellularised implant material. 2013. UK Patent No. PCT/GB2013/052312.*

Financial Disclosure

E Ingham and J Fisher are academic founders of Tissue Regenix and are shareholders and advisers to Tissue Regenix Group PLC