Polycaprolactone-based scaffold plus BMP-2 in a sheep thoracic spine fusion model

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ABSTRACT: We report the application of a novel scaffold design in a sheep thoracic spine model for spine deformity correction. The combination of the calcium-phosphate coated polycaprolactone scaffolds with recombinant human bone morphogenic protein-2 are intended as a future bone graft substitute in ensuring the stability of bony intervertebral fusion. A solid free-form fabrication process based on melt extrusion has been utilized in the manufacturing of these scaffolds.

1 INTRODUCTION

1.1 Background

Anterior spinal surgery is a well- recognized and effective approach for adolescent idiopathic scoliosis correction (Dubousset 2001a, b; Lowenstein et al. 2007). The success of anterior scoliosis surgery depends on achieving a solid bony fusion between adjacent vertebrae after the intervertebral discs have been surgically cleared and the disc spaces filled with graft material. The golden standard for bone grafting in spinal fusion surgery is autograft (host graft material), however limited availability and donor site morbidity make synthetic alternatives to autograft desirable. Current research focuses on the development of synthetic scaffolds in combination with growth factors such as recombinant human bone morphogenic protein-2 (rhBMP-2) to achieve solid bony fusion following scoliosis surgery (Sandhu 2000a, b; Sawyer 2009, c; Abbah et al. 2009). To date there are no studies examining the use of such biodegradable implants in a sheep thoracic spine model.

1.2 Aim

The aim of the current project is to design and fabricate biodegradable polycaprolactone (PCL) scaffolds by using a solid free-form fabrication process based on melt extrusion. These scaffolds are coated with a biomimetic calcium phosphate (CaP) layer which actively promotes bone ingrowth and regeneration. Following functionalization of these scaffolds with rhBMP-2, surgical implantation is undertaken at one of the predefined thoracic spinal levels of either T5/6, T7/8 or T9/10.

2 MATERIALS AND METHODS

2.1 Anatomical considerations

Sheep (*ovis aries*) have been widely used in a large number of interbody spinal fusion models (Cunningham 1998a, b; Sandhu 2000, c; Sawyer 2009, d; Abbah et al. 2009). The physical size of the sheep spine is deemed sufficient to allow spinal surgery to be readily carried out and to allow for assessment of the success of the study using biomechanical testing, radiological and histological analysis. In addition, sheep are a large animal model which simulates an environment representative of an adolescent child, while securing optimal application and fixation techniques closely

corresponding to that in the human spine (Easley 2008). Sheep have also been established for use in both open chest spinal surgery as well as minimally invasive endoscopic assisted surgery (Cunningham 1998).

2.2 Scaffold design

The scaffold design has been conceptualized through cadaveric dissections. Solid acrylonitrile butadiene styrene (ABS) prototypes of the scaffold were first designed and manufactured in different thicknesses by fused deposition modeling (Dimension SST 768), and after trial implantations of the spinal thoracic levels of T5/6, T7/8 and T9/10, the optimal scaffold geometry was confirmed and a thickness of 2.5 mm was decided to be most appropriate for this animal Polycaprolactone-based scaffold plus BMP-2 in a sheep thoracic spine fusion model. The final scaffold design was based on a 0°-90° lay-down pattern plus scaffold contour to confer additional strength for surgical handling and implantation of the prepared disc space. The scaffolds (Figure 1.) were fabricated using PCL and a Dual_BioExtruder, a computer-controlled extrusion- based additive manufacturing device developed in-house at the Polytechnic University of Leiria, Portugal (Domingos et al. 2010). The semicircular shape conforms to the cleared anterior intervertebral disc space ensuring a low-profiled construct under compression.

2.3 Surgical procedure

In this in vivo sheep study, three thoracic intervertebral spaces (T5/6, T7/8, T9/10) in each animal receive either (i) PCL + CaP scaffold + rhBMP-2 (ii) PCL + CaP scaffold alone, or (iii) rib head autograft. The treated intervertebral disc spaces are stabilized with a 5.5mm titanium rod secured with two vertebral screws.

3 RESULTS

3.1 Micro CT analysis

The porosity of uncoated scaffolds measured 55.6±0.9% with an average pore size of 406±6 µm and average strut diameter of 328±6 µm. The calcium phosphate coating of the scaffolds conferred an increase in scaffold strut size (340±4 µm) which resulted in a decrease of pore size (386±5 µm) and porosity (49.9±2.0%). The µCT was also used to determine the cross-sectional surface area (103.3 mm^2) that was used for the compressive modulus and yield stress calculations. A representative 3D rendered image of a scanned scaffold is presented in Figure 1.

Figure 1. µCT 40 scan of a fabricated scaffold

3.2 Compressive testing of scaffolds

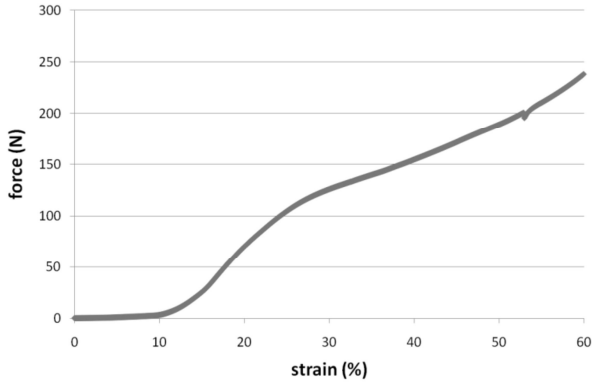

Figure 2. Representative force-strain diagram of compression test on uncoated scaffold.

From the compression tests, no influence of the coating procedure on the mechanical properties could be observed. Figure 2. depicts a representative force-strain diagram, showing an initial alignment phase (up to 10 %) followed by elastic deformation with a stiffness value of 9.4±1.3 N/% or an elastic modulus of 9.1±1.25 MPa. The scaffolds yielded at 25±1% strain and 117±14 N (1.14±0.13 MPa), after which permanent deformation progressively increases as the load is increased.

Macroscopic observation of compressed scaffolds revealed thinning of the construct by shearing between the deposited layers of material, while both the internal pore architecture and the overall scaffold shape and consistency were largely preserved. This observation is important as the preservation of the scaffold's overall shape once implanted surgically within the sheep's intervertebral disc space and placed under compression is a condition sine qua non.

3.3 Scanning electron microscopy (SEM)

Biomimetic properties of the calcium phosphate coating of the scaffolds actively promote bone regeneration. The coating of these scaffolds involve immersion in concentrated simulated body fluid (x10) and scaffold surface activation (Yang et al. 2008). Confirmation of the coating can be seen with SEM. Preliminary results show a heterogeneous coating whereby there is a clear difference between lightly coated outer layers of the scaffold contrasting with well-rounded coatings of central (inner) strut filaments.

3.4 Fusion assessments

To date, two pilot surgeries with implantation of earlier scaffold prototypes have been successfully undertaken. Triple bone fusion assessments (i.e. biomechanical testing, radiological imaging and histological analysis) of individual functional spinal units at the 6- month mark is currently underway. A functional spinal unit (FSU) consists of an instrumented intervertebral disc level as well as the adjoining top and bottom vertebrae. (See Figure 3.)

Figure 3. PCL-based scaffold in situ within a predefined intervertebral disc space. Internal fixation with a 5.5mm titanium rod and two vertebral screws stabilize the functional spinal unit. The 5.5mm titanium rod has been removed for illustrative purposes.

4 DISCUSSION

Bony fusion is essential for long-term stability of instrumented spinal segments in the setting of scoliosis deformity correction. Increasingly being studied are biologically active substances intended to extend, enhance, or even replace autologous graft. The use of rhBMP-2 as an osteoinductive implant component has been on the increase because of its proven potency in vivo (Burkus 2004).

The biomedical application of these PCL-based scaffolds aims at providing a structural support that promotes the repair and regeneration of tissues in combination of living cells and biologically active molecules. Bone formation is actively guided with the ensuing cell colonization, migration, growth and differentiation. This forms the basis of a viable tissue engineered construct (TEC) (Hutmacher 2007).

Scaffolds are required to provide sufficient initial mechanical strength and stiffness to substitute for the mechanical function of bone. This permits cell migration and population of the scaffold in vivo. In addition, the scaffold material should be adequately robust to resist deformation upon cell infiltration as well as wound contraction forces in vivo. An internal fixation construct stabilises the instrumented disc space thus reduces the mechanical role of the scaffold in situ. This maintains sufficient structural integrity critical to a stable biomechanical environment for vascularization and bone remodeling. This has been successfully demonstrated by Abbah et al. in an analogous porcine lumbar interbody fusion model whereby complete bony fusion was seen as early as 3 months with advanced bone remodeling at 6 months (Abbah et al. 2009).

A scaffold pore size of 406 +/- 6 microns with 100% pore interconnectivity fulfills the required minimum of 300 micron pore size recommended for sufficient vascularization of tissue-engineered graft (Karageorgiou et al. 2005). The scaffold architecture consists of a honeycomb configuration which is a regular two-dimensional array of polygonal pores, each defined by a wall shared between adjacent pores (Gibson et al. 1997). Structurally this consists of a 90 degree lay-down pattern which offers a hexagonal pore architectural pattern conferring desirable physiological and mechanical properties by way of combining high stiffness and strength with open vertical pore channels that facilitate bone ingrowth. Yeo et al. in an in vivo application of scaffolds fabricated to similar

dimensions matched closely the exhibited compressive strength and modulus of cancellous bone confirming its potential as a bone graft substitute (Yeo et al. 2008).

Calcium phosphate coating of these PCL scaffolds is intended to enhance its bioactivity hence promoting bone formation. The external scaffold geometry has been customized to allow for a lowprofiled construct confirming to the anatomical landmarks of the spine. This aims at a greater integration and guided spinal fusion. (Hutmacher et al 2000)

5 CONCLUSION

The combination of biologics and scaffold engineering represent a novel approach to promoting bony fusion in the setting of thoracic spine deformity correction. Application of computercontrolled extrusion- based additive manufacturing devices pave the way in customization of future spinal bone graft substitutes.

6 REFERENCES

Abbah, S.A. 2009. Biological performance of a polycaprolactone-based scaffold used as fusion cage device in a large animal model of spinal reconstructive surgery. Biomaterials 30(28): 5086-93.

Burkus, J.K. Bone morphogenetic proteins in anterior lumbar interbody fusion: old techniques and new technologies. Invited submission from the Joint section meeting on disorders of the spine and peripheral nerves. J Neurosurg Spine 1(3): 254-260.

Cunningham, B.W. 1998. Video-assisted thoracoscopic surgery versus open thoracotomy for anterior thoracic spinal fusion. A comparative radiographic, biomechanical and histological analysis in a sheep model. Spine 23(12): 1333-40.

Domingos, M. et al. 2010. Innovative developments in design and manufacturing - Advanced research in virtual and rapid prototyping, ed. Boca Raton: Crc Press- Taylor & F rancis Group : 67- 73.

Dubousset, J. 2001. Scoliosis and its pathophysiology: do we understand it? Spine 26(9): 1001

Easley, N.E. et al. 2008. Biomechanical and radiographic evaluation of an ovine model for the human lumbar spine. Proc Inst Mech Eng H 222 (6): 915-22.

Gibson, L.J. et al. 1997. Cellular Solids Structure and Properties. Cambridge University Press: New York.

Hutmacher, D.W. 2000. Scaffolds in tissue engineering bone and cartilage. Biomaterials 21(24): 2529-43.

Hutmacher, D.W. 2007. State of the art and future directions of scaffold- based bone engineering from a biomaterials perspective. J Tissue Eng Regen Med 1: 245-60.

Karageorgiou, V. et al. 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26: 5474-91.

Lowenstein, J.E. et. al. 2007. Coronal and sagittal plane correction in adolescent idiopathic scoliosis: a comparison between all pedicle screw versus hybrid thoracic hook lumbar screw constructs. Spine 32(4): 448-52.

Sandhu, H.S. 2000. Anterior lumbar interbody fusion with osteoinductive growth factors. Clin Orthop Relat Res 317: 254-60.

Sawyer, A.A. 2009. The stimulation of healing within a rat calvarial defect by mPCL-TCP/collagen scaffolds loaded with rhBMP-2. Biomaterials 30(13): 2479-88.

Yang, F. et al. 2008. Biomimetic calcium phosphate coating on electrospun poly (E-caprolactone) scaffolds for bone tissue engineering. Chem Eng J 137: 154-61.

Yeo, A. et al. 2008. The degradation profile of novel, bioresorbable PCL-TCP scaffolds: an in vitro and in vivo study. J Biomed Mater Res A 84: 208-18.