Semana de Engenharia 2010
Guimarães, 11 a 15 de Outubro

THE INFLUENCE OF SILK FIBROIN 3D SCAFFOLD COMPOSITION FOR IN VITRO BONE TISSUE ENGINEERING

Cristina Correia[1,2,3] , Sarindr Bhumiratana[3], Yan Leping[1,2], Ana L. Oliveira[1,2], Jeffrey M. Gimble[4], David L. Kaplan[5], Rui A. Sousa[1,2], Gordana Vunjak-Novakovic[3], Rui L. Reis[1,2]

[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal
[2]IBB – Institute for Biotechnology and Bioengineering, PT Associated Laboratory, Guimarães, Portugal
[3]Department of Biomedical Engineering, Columbia University, New York, USA
[4]Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana
[5]Department of Biomedical Engineering, Tufts University, Medford, MA, USA
E-mail: ccorreia@dep.uminho.pt

KEYWORDS
Bone tissue engineering, Silk fibroin, Human adipose stem cells

ABSTRACT
Scaffold composition, configuration and resulting properties critically affects tissue development. In this study, we evaluated the influence of silk fibroin concentration (6 or 17%) and correspondent processing method (aqueous or HFIP-derived) and three-dimensional scaffold structure (lamellar or porous, with distinct pore size) on bone tissue formation by osteogenic differentiation of human adipose tissue derived stem cells (hASC). We observed that very similar bone tissue was formed in all silk fibroin scaffold groups, evaluated by alkaline phosphatase activity, calcium production, collagen type I deposition and scaffold bone volume fraction.

INTRODUCTION

Bone congenital defects, diseases and/or injuries leads to the clinical need to regenerate functional bone tissue. Adipose tissue derived stem cells (ASC) may be used as an adequate stem cell source to form bone tissue once these are obtained through a simple surgical procedure, a subsequent uncomplicated enzyme-based isolation procedure, and exhibit high osteogenic differentiation potential (Schaffler and Buchler 2007). On the other side, silk fibroin scaffolds have high potential as support for cell growth and bone tissue development. Silk fibroin is easily obtained from processing *bombix mori* cocoons and exhibit strength, flexibility and compression properties that result in mechanical integrity adequate for osseoreintegration. These are highly biocompatible and degrade slowly as a function of proteolytic degradation and matrix mechanical fatigue (Meinel et al. 2003). With silk fibroin, it is possible to generate custom-designed scaffold architectures with controlled porosity, pore size and mechanical properties (Altman et al. 2002, 2003; Kim et al. 2005). This work aimed to evaluate the influence of silk fibroin concentration; correspondent processing method and three-dimensional scaffold structure on bone tissue formation by human adipose tissue derived stem cells (hASC).

MATERIAL AND METHODS

hASCs were isolated from liposuction aspirates of subcutaneous adipose tissue, donated with written consent by a healthy 44-year-old female, with body mass index (BMI) of 24.98, undergoing elective liposurgery. hASC were expanded to p3 in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin–streptomycin (1%), and 1 ng/mL basic fibroblast growth factor (bFGF).
Silk fibroin from silkworm (*Bombix mori*) cocoons was extracted with 0.2M sodium carbonate solution, rinsed in distilled water, dissolved in a 9.3 M lithium bromide solution and dialyzed for 48h against distilled water in dialysis membrane.

Table 1. Scaffold properties. LC-LP:Low concentration – Low pore size, LC-HP: Low concentration – High pore size, LC-LP: Low concentration – Lamellar, HC-HP: High concentration – High pore size

		% Silk	Porosity	Pore size
LC-LP	Aqueous-based	6%	94%	250-500μm
LC-HP		6%	94%	500-1000μm
LC-Lam		6%	94%	50-200μm
HC-HP	HFIP-based	17%	83%	400-600μm
Bone	-	-	70-90%	250-400μm

Porous aqueous-derived silk fibroin scaffolds were prepared by adding granular NaCl (particle size 250-500 or 500-1000 μm) silk fibroin aqueous solutions (6 wt%) in disk-shaped containers. After 24h at room temperature (RT), the container was immersed in water and the NaCl extracted for 2 days.
Lamellar scaffolds were prepared by freeze-drying the aqueous silk solution in a cylindrical mold for 2 days and subsequently autoclaving for 20 min.
HFIP silk solution was prepared by dissolving the silk fibroin produced after lyophilizing the silk fibroin aqueous solution. Porous scaffolds were prepared by

adding granular NaCl (particle size; 400–600 μm) to silk fibroin in HFIP (17 wt%). The solvent was evaporated at RT for 3 days and then treated in methanol for 30min, followed by immersion in water for 2 days to remove the NaCl.

Decellularized trabecular bone scaffolds were cored from the carpotecarpal joints of calves, washed in water and further washed for 1 h in PBS with 0.1% EDTA at RT, followed by sequential washes in hypotonic buffer (10 mM Tris and 0.1% EDTA) overnight at 48^{o} C, in detergent (10 mM Tris and 0.5% SDS) for 24 h at RT, and in enzyme solution (100 U/mL DNAse, 1 U/mL RNAse, and 10 mM Tris) for 6 h at 37^{o} C. Scaffolds were then rinsed in PBS, freeze-dried, and cut into cylindrical plugs.

Five groups of scaffolds, 4mm diameter, 2mm height, (Table 1) were seeded with 0.5x10^{6} hASCs/scaffold and cultured in osteogenic media (low glucose DMEM, 1% Pen/Strep, 10 mM sodium-β-glycerophosphate, 10μM HEPES, 100 nM dexamethasone, 50 μg/ml ascorbic acid-2-phosphate, 10% FBS,) for 7 weeks.

RESULTS

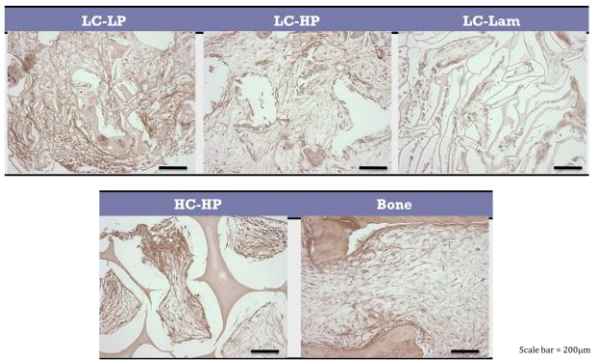

Figure 1: Representative images of collagen type I expression detected by immunohistochemistry

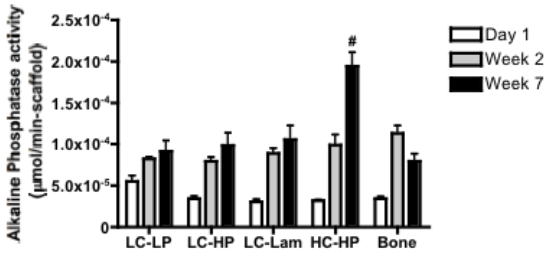

Figure 2: Alkaline Phosphatase activity normalized by scaffold. n=4, #p<0.001 to all groups

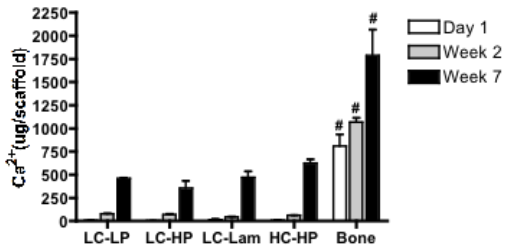

Figure 3: Calcium content normalized by scaffold. n=3, #p<0.001 to all groups

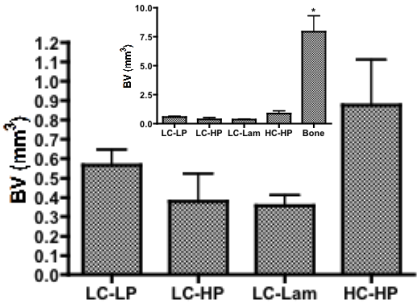

Figure 4: Bone volume (BV) of scaffolds measured by microCT at week 7. n=3

CONCLUSIONS

6% water-based silk scaffolds pore size:

Generally, 250-500μm pore size demonstrated slightly higher bone formation markers such as calcium deposition, ALP activity and collagen type I secretion in comparison to 500-1000μm. The same trend was observed for bone volume fraction.

6% water-based silk scaffolds structure:

Analyzing calcium deposition and ALP activity on total scaffold, no major differences were observed between both porous and lamellar structure, as well for collagen type I secretion.

6% water-based and HFIP 17% silk scaffolds:

HFIP 17% silk scaffolds globally promoted higher bone formation demonstrated by increased calcium deposition, ALP activity and collagen type I secretion when compared to all aqueous-based 6% silk scaffolds.

REFERENCES

Altman, G.H., Horan, R.L., et al. 2002. "Silk matrix for tissue engineered anterior cruciate ligaments". *Biomaterials* 23(20): 4131–4141.

Altman, G.H., Diaz, F., et al. 2003. "Silk-based biomaterials" *Biomaterials* 24(3): 401–416.

Meinel, L., Zoidis, E., et al. 2003. "Localized insulin-like growth factor I delivery to enhance new bone formation". *Bone* 33(4): 660–672.

Schaffler, A. and C. Buchler. 2007. "Concise review: adipose tissue-derived stromal cells - basic and clinical implications for novel cell-based therapies." *Stem Cells* 25(4): 818-827.

ACKNOWLEDGMENTS

Portuguese Foundation for Science and Technology for PhD grant SFRH/BD/42316/2007.

CRISTINA CORREIA was born in Miami, USA. She studied in University of Minho where she graduated in Applied Biology in 2007. In the same year she started developing her PhD research at 3B's Research Group, University of Minho. She is presently at the Laboratory for Stem Cells and Tissue Engineering, Columbia University, USA studying osteogenic differentiation of human adipose tissue derived stem cells in precise biomechanical systems. Her e-mail address is ccorreia@dep.uminho.pt**.**