Adipose stem cells on a biodegradable polymer for spinal fusion

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2014

document version
Publisher's PDF, also known as Version of record

Summary

Autografted iliac crest bone is generally associated with donor-site morbidity. Moreover, since grafted bone and newly formed bone are difficult to distinguish on plain radiographs, new bone formation and/or bone remodelling are hard to determine during the fusion process *in-vivo*. We have therefore tried to find a suitable alternative. For this purpose, we evaluated a degradable and radiolucent polymeric material, (poly(L-lactide-co-caprolactone); PLCL) either or not supplemented with adipose derived stem cells *in-vitro* as well as *in-vivo*. We hypothesized that the use of these stem cells may enhance or accelerate bone formation *in-vivo*, by means of osteoinductivity . We used a caprine lumber interbody fusion animal model for the *in-vivo* analyses.

The general goal of this thesis was to evaluate the induction of bone formation using a degradable, polymeric scaffold material with and without the use of adipose-derived stem cells in a single surgical procedure. We formulated the following aims:

- To test the feasibility of a one-step surgical procedure to obtain and re-implant autologous adipose derived stem cells.
- To evaluate PLCL as a degradable and radiolucent cage filler material.
- To evaluate the addition of freshly isolated adipose stem cells to the PLCL cage filler as a possible adjunct in spinal fusion.

In **Chapter 2**, we give a brief and general overview concerning the use of biodegradable polymers in bone tissue engineering.. The nomenclature used in polymer science is described together with the issues of biocompatibility and biofunctionality of polymers. The pivotal importance of the latter two subjects, when using degradable polymers in vivo, are emphasized. Furthermore, several polymer material properties are mentioned which should be described in biomedical papers to achieve transparency among research groups.

In **Chapter 3**, we speculated that the type of sterilization method used may influence the cellular stem cell responses by altering the surface characteristics of the PLCL material. Therefore, we cultured adipose stem cells onto PLCL sheets, sterilized using either ethylene-oxide (EO), argon glow-discharge (aGD), or electron-beam (E-beam). Significant differences in surface roughness (EO>aGD>E-beam), contact angles (EO>E-beam>aGD) and surface energies (aGD>E-beam>EO) were observed. Increased cell attachment and proliferation rates were observed with decreasing contact angles. Osteogenic differentiation of the stem cells cultured on EO sterilized PLCL outperformed the other groups, as indicated by a significantly higher alkaline phosphatase activity. Since this difference in differentiation

potential may enhance the bone formation process when using PLCL in bone tissue engineering, we employed EO as the PLCL sterilization technique for our experiments

The aim of **Chapter 4** is to describe the various osteogenic differentiation protocols for adipose derived stem cells currently applied within our laboratory. The development and implementation of protocols is always subject to ongoing optimizations, and may vary even within the same laboratory to some degree between researchers. Hopefully, exchanging differentiation protocols between research groups will facilitate the development of consensus protocols, which in turn will allow better comparison of data sets generated by different research groups.

With the knowledge gathered from chapter 3 that cultured adipose stem cells would differentiate towards the osteogenic lineage on PLCL, we tested the feasibility of the one-step surgical procedure with freshly isolated stem cells *in-vitro*. Therefore, in **Chapter 5** we evaluated two radiolucent and biodegradable polymeric scaffolds for cartilage and bone tissue engineering purposes, respectively: a natural porous Collagen I/III scaffold and the PLCL scaffold. Freshly isolated stem cells present in the so-called stromal vascular fraction (SVF) were seeded on the polymeric scaffolds using the one-step surgical procedure. Interestingly, cell attachment was rapid (~10 min) when seeding the heterogeneous SVF cells onto both scaffolds. At least the stem cells adhered to the scaffolds, since the non-adhered cell fraction contained a 10-fold lower amount of stem cells as determioned with colony-forming unit assays. We confirmed that the attached cell population was capable of proliferation and differentiation both towards the chondrogenic and the osteogenic lineage on both scaffold types. The one-step surgical procedure using PLCL as a scaffold and SVF as a stem cell source for bone tissue engineering was shown to be feasible *in-vitro*.

We then proceeded to an *in-vivo* large animal lumbar interbody fusion study. This caprine model without instrumentation has been used before in our group and for this thesis, the cages were made of radiolucent and inert polyetheretherketone (PEEK). In the pilot study described in **Chapter 6**, both SVF (using the one step surgical procedure) and cultured adipose-derived stem cells (ASCs) were seeded onto the PLCL scaffold and inserted as an cage filler in the goat lumbar spine. The primary outcome parameter of this study was the biocompatibility and biofunctionality of PLCL either or not seeded with freshly isolated or cultured adipose stem cells. After 1 month follow-up, no systemic and/or local adverse reactions were observed, indicating the biocompatibility and biofunctionality of the components used. In addition, compared to the autologous bone group, we found an increased number of blood vessels for the PLCL group and an increased vascular diameter for the SVF group, indicating favourable vasculogenesis and thus prognosticating accelerated bone formation.

Consequently, this study was followed by a larger caprine lumbar interbody fusion study with an identical set-up, to test if PLCL either or not seeded with SVF or ASCs could serve as a radiolucent cage filler replacement of autologous bone graft (**Chapter 7)**. Importantly, this study showed the feasibility of the OSP in a pre-clinical setting. Neither the PLCL scaffold nor the stem celld did evoke any local or systemic adverse responses in the goat at any time-point (3 and 6 months). This indicates the feasibility and safety of employing these components in-vivo with longer follow-up times. In addition, the PLCL cage filler material did allow visualization for *in-vivo* bone formation using plain radiography. Although we did obtain some cases of solid fusion in all experimental groups after 6 months, results were too variable, making it difficult to draw firm conclusions on the PLCL as a cage filler and the possible enhancement of the stem cell therapy in spinal fusion.

Chapter 8 describes the biomechanical assessment of explanted caprine spines, during different radiological stages of lumbar interbody fusion, from the goat study described in chapter 7. Biomechanical analyses showed significant reduction in range of motion in the operated segments with moderate bone ingrowth in flexion/extension and with only limited bone ingrowth in lateral bending, compared to the post-surgical situation. This study showed that vertebral stability occured well before solid artrodesis was seen on plain radiography. Finally, **Chapter 9**, the general discussion, describes the results of all experiments performed, placing them in a broader context en discusses the limitations of these studies and indicating possible future experiments.

In conclusion, the feasibility of a one-step surgical procedure to obtain and re-implant autologous adipose derived stem cells was shown for the first time in a bone tissue engineering application. Furthermore, the polymeric and radiolucent cage filler did allow visualisation of in-vivo bone formation using simple radiography. Finally, PLCL seems inferior compared to ABG as a cage filler material, and the addition of stem cells (freshly isolated and cultured) did not clearly enhance or accelerate the spinal fusion process in these initial studies. However, these studies identified some of the limitations encountered in this thesis which can be optimized in future studies.