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Analysis of the *in vitro* degradation and the *in vivo* tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components – Guidance of the inflammatory response as basis for osteochondral regeneration

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ABSTRACT

The aim of the present study was the *in vitro* and *in vivo* analysis of a bi-layered 3D-printed scaffold combining a PLA layer and a biphasic PLA/bioglass G5 layer for regeneration of osteochondral defects *in vivo* Focus of the *in vitro* analysis was on the (molecular) weight loss and the morphological and mechanical variations after immersion in SBF. The *in vivo* study focused on analysis of the tissue reactions and differences in the implant bed vascularization using an established subcutaneous implantation model in CD-1 mice and established histological and histomorphometrical methods.

Both scaffold parts kept their structural integrity, while changes in morphology were observed, especially for the PLA/G5 scaffold. Mechanical properties decreased with progressive degradation, while the PLA/G5 scaffolds presented higher compressive modulus than PLA scaffolds. The tissue reaction to PLA included low numbers of BMGCs and minimal vascularization of its implant beds, while the addition of G5 lead to higher numbers of BMGCs and a higher implant bed vascularization. Analysis revealed that the use of a bi-layered scaffold shows the ability to observe distinct *in vivo* response despite the physical proximity of PLA and PLA/G5 layers.

Altogether, the results showed that the addition of G5 enables to reduce scaffold weight loss and to increase mechanical strength. Furthermore, the addition of G5 lead to a higher vascularization of the implant bed required as basis for bone tissue regeneration mediated by higher numbers of BMGCs, while within the PLA parts a significantly lower vascularization was found optimally for chondral regeneration. Thus, this data show that the analyzed bi-layered scaffold may serve as an ideal basis for the regeneration of osteochondral tissue defects. Additionally, the results show that it might be able to reduce the number

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of experimental animals required as it may be possible to analyze the tissue response to more than one implant in one experimental animal.

1. Introduction

The regeneration of osteochondral tissue defects after traumata or resections is a major clinical challenge. In this context, different healing mechanisms for these both tissues – although both tissues are in close proximity such as in case of joints – have been described [1]. The main differences in the regeneration processes of bone and articular cartilage is the need for vascularization [1–3]. In case of bone tissue, a high vascularization is a basic factor for its regeneration as it permits the transport of nutrients, *e.g.* oxygen, to the defect sides [3]. It has been shown that a high expression of vascular endothelial growth factor (VEGF) and a related high implant bed vascularization but also direct effects of this molecule enable to increase the bone healing process [2,3]. In contrast, the regeneration of articular cartilage is not dependent on a high blood support as it is a relatively avascular tissue including a hypoxic milieu [4].

In the last decades, many different substitute materials for both bone and cartilage repair were developed that should allow simultaneous regeneration of both tissues while becoming resorbed over time. In case of bone substitutes, one aim of these new materials was even to increase the implant bed vascularization and, thus, their regenerative potential. Interestingly, the incorporation of VEGF into different bone substitutes has been tested but this combination has not been established as a real clinical alternative until know based on different reasons such as the high costs, the very short half-life of this molecule and the lack of knowledge about the (individual) concentration of this growth factor [2,5]. Furthermore, different concepts combining bone substitutes with different cell types such as osteoblasts or their precursor cells, i.e., for example different stem cells, and also with endothelial cells (in mono- and co-cultures) have already been tested and showed impressive results but different limitations exist that does not allow successful transmission of these concepts into the clinical routine [2,6–10].

Strategies for articular cartilage regeneration most often include the addition of different cell types such as chondrocytes or different precursor or stem cells but have also not reached a clinically applicable level [11–13]. Interestingly, it has been shown that blocking of the VEGF pathway supports chondrogenesis [14]. However, also this regeneration concept is also far away from its translation into the clinic.

As a consequence, there is a need for an “optimal” biomaterial applied as basis for successful osteochondral regeneration. Theoretically, this material should provide two components that induce different niches for the simultaneous regeneration of both tissues. One component should provide “bioactive” or inductive properties for establishment of a high scaffold vascularization for bone growth, while the other component should simultaneously induce a reduced vascularization milieu needed for cartilage repair.

In this context, it has been demonstrated that resorbable materials most often induce a tissue reaction cascade called “foreign body response to biomaterials”, which is an inflammatory cellular response whose severity is dependent on the physicochemical properties of a biomaterial [15–20]. This cascade includes different cell types not only involved in material degradation but also contributing to implant bed vascularization by expression of factors such as VEGF [15–20]. Especially macrophages, which have been identified as key components of this tissue reaction cascade, and also biomaterial-associated multinucleated giant cells (BMGCs) have been shown to be potent sources of this angiogenic factor and contribute also in the process of tissue regeneration by expression of anti-inflammatory molecules [15,18,21–23]. Thus, from this point of view, it should be possible to develop more suitable biomaterials for simultaneous bone and cartilage regeneration by modulating the inflammatory tissue response to different parts of such a biomaterial, which includes orchestrating the material-induced vascularization processes based on macrophages and BMGCs, and finally its tissue regenerative abilities.

Additionally, manufacturing methods such as 3D printing have introduced new possibilities for tissue regeneration using scaffolds individually tailored to suit the morphology of tissue defects [24]. The use of 3D printing techniques allows the fabrication of scaffolds in a controllable way with a precise spatial deposition of material components [25]. In this context, polylactic acid (PLA) has been shown to be favorable for scaffold fabrication via 3D printing as the use of this polymer allows for the rapid engineering required in clinical fields like traumatology [25]. Furthermore, it is known that PLA does not induce a high level of bioactivity as tissue responses with a low level of inflammation and also low vascularization rates have been described [26,27]. Thus, a PLA scaffold alone is proposed to be a suitable biomaterial for cartilage repair. In contrast, PLA-based materials most often become combined with other compounds to increase the level of bioactivity and its regenerative potential for bone tissue regeneration [28]. Among the synthetic bone substitute materials based on calcium phosphates (CaP), calcium phosphate-based glasses, in particular the one known as G5 (P_2O_5 - CaO - Na_2O - TiO_2), has been shown to contribute significantly to the vascularization of tissues both *in vitro* and *in vivo* by induction of angiogenesis [29–31]. Thus, it is expected that the angiogenic effect of G5 will support bone tissue regeneration [29,30]. Indeed, the combination of G5 glass with PLA to fabricate a biphasic PLA/G5 scaffold has proven to be a favorable composite bone substitute material based on previous study results by Charles-Harris and colleagues [32]. Furthermore, it has been revealed that the addition of bioglass has also impact on the tissue response to such kind of biphasic scaffold as a higher level of inflammation including BMGCs [33].

Altogether, it should be possible to develop a bi-layered scaffold for promoting both bone and cartilage repair by induction of two different tissue response pattern within one scaffold for guidance of the implant bed vascularization. However, no more profound knowledge of the tissue reactions to those kinds of scaffolds exists until now, this being a pre-requisite for improving their tissue compatibility and regenerative potential.

Accordingly, the aims of the present study are as follows: 1) The development of novel bi-layered scaffolds composed of a polymeric layer (PLA) and a biphasic layer (PLA/G5 glass), 2) the evaluation of the *in vitro* degradation of the scaffolds and 3) the analysis of *in vivo* tissue responses, with special focus on implant bed vascularization and the occurrence of BMGCs, using an established subcutaneous implantation model as well as specialized histological and

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