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Tailoring the subchondral bone phase of a multi-layered osteochondral construct to support bone healing and a cartilage analog

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ABSTRACT

Focal chondral and osteochondral defects create significant pain and disability for working-aged adults. Current osteochondral repair grafts are limited in availability and often fail due to insufficient osseous support and integration. Thus, a need exists for an off-the-shelf osteochondral construct with the propensity to overcome these shortcomings. Herein, a scalable process was used to develop a multi-layered osteochondral graft with a subchondral bone (ScB) phase tailored to support bone healing and integration. Multiple ScB formulations and fabrication techniques were screened via degradation, bioactivity, and unconfined compression testing. An optimized ScB construct was selected and its cytotoxicity assessed. Additionally, a cartilage analog was secured to the optimized ScB construct via a calcified cartilage layer, and the resulting osteochondral construct was characterized via interfacial shear and dynamic mechanical testing. The optimized ScB construct did not significantly alter local pH during degradation, exhibited measurable bioactivity *in vitro*, and had significantly greater compressive mechanical strength compared to other constructs. The attachment strength of the cartilage analog was significantly greater by an increase in compressive dynamic mechanical properties. Furthermore, this ScB construct was found to be cytocompatible with human bone marrow-derived mesenchymal stromal cells. Taken together, this optimized ScB material forms the robust foundation of a novel, off-the-shelf osteochondral construct to be used in defect repair.

Statement of Significance

The quality of life for millions of individuals worldwide is detrimentally affected by focal chondral or osteochondral defects. Current off-the-shelf biomaterial constructs often fail to repair these defects due to insufficient osseous support and integration. Herein, we used a scalable process to fabricate and optimize a novel boney construct. This optimized boney construct demonstrated biochemical, physical, and mechanical properties tailored to promote bone healing. Furthermore, a novel cartilage analog was successfully attached to the boney construct, forming a multi-layered osteochondral construct.

1. Introduction

Focal chondral and osteochondral defects often result from joint wear and tear, traumatic injury, or metabolic disorders [1]. Between 2004 and 2011, approximately 200,000,000 incidences of focal chondral or osteochondral defects occurred in adults in the United States, with 2,000,000 incidences requiring surgical intervention. If left untreated, these defects result in persistent joint pain that limits the ability to perform daily, work, and recreational activities [1,2]. Long-term, untreated defects can lead to further degenerative changes in the joint. This poses an especially challenging clinical problem because focal chondral defects are unable to heal intrinsically due to the absence of vasculature and the presence of a relatively immobile and quiescent resident cell population. However, a variety of surgical approaches can be implemented in an attempt to repair the damaged cartilage tissue.

Osteoarticular transplantation (OAT) is one of the most commonly utilized surgical methods for chondral and osteochondral focal defect repair. A graft is generated through the removal of an osteochondral tissue plug from a non-weight-bearing region of the knee (autograft) or donor tissue (allograft) and is subsequently press-fit into the existing focal defect. While this

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technique uses healthy patient or donor bone and cartilage in an attempt to preserve joint physiology, it suffers from major drawbacks including: donor site morbidity, limited donor tissue availability, donor graft rejection, and bone/cartilage depth mismatch [3]. Thus, off-the-shelf osteochondral constructs have been developed and investigated as a potential method of overcoming these limitations and improving patient outcomes. Although there are currently a wide variety of commercially available osteochondral constructs, they fail to provide long-term repair attributed to a lack of cartilage integration [4]. Thus, the development of a multilayered osteochondral construct has been primarily focused on the overlaying cartilage layer and less effort has been devoted to the underlying subchondral bone.

However, clinical failure of osteochondral repair constructs is also caused by insufficient osseous support [5,6]. This lack of support is attributed either to a lack of mechanical integrity of the boney layer or a failure to integrate with adjacent host bone [5,6]. In order to address this failure, osteochondral constructs should incorporate a subchondral bone layer that has sufficient mechanical strength and other biochemical properties tailored towards supporting boney integration and healing while demonstrating the ability to securely attach an overlaying cartilage analog. Thus, the primary aim of this study was to develop a subchondral bone (ScB) construct with biochemical, biophysical, and bioactive characteristics targeted towards supporting ScB integration and promoting bone healing. This was accomplished by creating ScB constructs using a combination of different formulations and fabrication techniques and evaluating their impact on critical aspects that govern bone formation. The objectives of the work herein were to 1) assess the impact of different construct formulations on ScB construct degradation parameters including mass loss, change in local environmental pH, bioactivity, and compressive mechanical properties, 2) select a ScB formulation and further tailor it to improve material homogeneity and compressive mechanical properties, and 3) combine the ScB with a cartilage analog to form a multi-layered osteochondral construct. These multilayered osteochondral constructs were further evaluated for ScB impact on dynamic mechanical properties and the interfacial shear strength of cartilage analog. Finally, cytocompatibility of the ScB constructs were assessed using human bone marrow derived mesenchymal stromal cells (hMSC-BMs) to determine attachment and cytocompatibility.

2. Materials and methods

2.1. Fabrication of Non-Compacted subchondral bone (ScB) constructs

ScB constructs were fabricated using a casting method. Varying amounts of 75:25 lactide to glycolide poly (D, L-lactide-*co*-glycolide) (PLGA) (Mw: 128.5 kDa, Mn: 66.0 kDa, molecular weight distribution: 1.95) polymer were dissolved in 15 mL of acetone (Table 1). Granulated hydroxyapatite (HAp) (diameter: 1000–1700 μm, Ca/P ration: 1.67, average pore size: 197 μm, porosity: 77%, specific surface area: 0.96 m^2/g, x-ray diffraction pattern can be found in Appendix A) and bioactive glass 13–93 powder (BG1393) (diameter: 25–150 μm) were thoroughly mixed into the PLGA solution. The mixture was cast into 50 mm diameter perfluoroalkoxy alkane (PFA) dishes, dried for 72 h at room temperature, followed by 48 h at 60 °C. Cylindrical constructs were subsequently created with a 10 mm stainless steel punch and stored under desiccation prior to further analyses (Fig. 1).

Table 1
ScB implant component concentrations.

	PLGA1	PLGA2	PLGA3
PLGA [g]	15	10	5
HAp [g]	12	12	12
BG1393 [g]	9	9	9

2.2. Fabrication of compacted ScB constructs

Compacted ScB constructs (PLGA1C) were fabricated with the PLGA1 formulation using the same technique as described above (Section 2.1). Prior to drying, casted construct mixtures were subjected to a defined compressive load of 5lbs in order to compact the matrix.

2.3. Fabrication of compacted and annealed ScB constructs

Compacted constructs (PLGA1C) were fabricated as described above (Section 2.2). Cylindrical PLGA1C constructs were then annealed in an oven at 115 °C for 30, 60, 120, or 240 min. PLGA1C constructs that were annealed for 240 min were designated as PLGA1CA.

2.4. Modified simulated body fluid

Modified simulated body fluid (mSBF) was prepared as described by Oyane *et al.* and adjusted to a starting pH of 7.50 [7]. All mSBF was made on Day 0 of the respective degradation study in order to maximize solution stability.

2.5. Non-Compacted ScB construct degradation study

PLGA1, PLGA2, and PLGA3 ScB constructs were weighed prior to being bathed in 35 mL of mSBF at 37 °C for up to 28 days. Bathing solution pH was recorded every two days for the study duration. At 0, 7, 14, and 28-day time points, constructs (n = 3/time point/formulation) were dried and weighed. Mass loss was calculated as:

$$\frac{\text{Mass Loss (Day 0)} - \text{Mass Loss (time point)}}{\text{Mass Loss (Day 0)}} \times 100$$

Constructs were stored under desiccation and their respective bathing solutions frozen at −80 °C.

2.6. Compacted and compacted/annealed ScB construct degradation follow-up study

Constructs were weighed prior to being bathed in 35 mL of mSBF at 37 °C for up to 14 days. During the initial degradation study (Section 2.5), mSBF pH and ion concentrations drifted after 21 days. Thus, this study duration was shortened to 14 days. Bathing solution pH was recorded every two days for the study duration. At 0, 7, and 14-day time points, constructs (n = 3/time point/formulation) were dried, weighed, and mass loss was calculated as described above. Constructs were stored under desiccation and their respective bathing solutions frozen at -80 °C. To confirm that any change in pH did not impact bioactivity, an additional negative control group (PLGA pellets only) were included (Appendix B).

2.7. ScB construct biphasic morphological analysis

Constructs were imaged using a Bruker SkyScan micro computed tomography (μCT) system at a resolution of 18 μm. Non-compacted constructs (n = 3/formulation/time point) were imaged subsequent to degradation at Days 0, 7, 14, and 28 while compacted and compacted/annealed constructs (n = 3/formulation/time point) were imaged subsequent to degradation at Days 0, 7, and 14 to monitor the effect of degradation on the biphasic morphology. Sample reconstruction was consistently performed over a defined cylindrical volume of interest (VOI) based around

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