Acta Biomaterialia 35 (2016) 330–340

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Combining microCT-based characterization with empirical modelling as a robust screening approach for the design of optimized CaP-containing scaffolds for progenitor cell-mediated bone formation

G. Kerckhofs [a,b,*], Y.C. Chai [a,b,1], F.P. Luyten [a,b], L. Geris [a,c,d]

[a] Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Onderwijs en Navorsing 1, Herestraat 49 – PB813, B-3000 Leuven, Belgium
[b] Tissue Engineering Laboratory, Skeletal Biology and Engineering Research Center, KU Leuven, Onderwijs en Navorsing 1, Herestraat 49 – PB813, B-3000 Leuven, Belgium
[c] Biomechanics Research Unit, Université de Liège, Chemin des Chevreuils 1 – BAT 52/3, B-4000 Liège, Belgium
[d] Division of Biomechanics and Engineering Design, KU Leuven, Celestijnenlaan 300C, B-3001 Heverlee, Belgium

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 22 February 2016
Accepted 26 February 2016
Available online 27 February 2016

Keywords:
Optimized calcium phosphate-based constructs
MicroCT characterization
Empirical modelling
Robust screening

ABSTRACT

Biomaterials are a key ingredient to the success of bone tissue engineering (TE), which focuses on the healing of bone defects by combining scaffolds with cells and/or growth factors. Due to the widely variable material characteristics and patient-specificities, however, current bone TE strategies still suffer from low repeatability and lack of robustness, which hamper clinical translation. Hence, optimal TE construct (i.e. cells and scaffold) characteristics are still under debate. This study aimed to reduce the material-specific variability for cell-based construct design, avoiding trial-and-error, by combining microCT characterization and empirical modelling as an innovative and robust screening approach. Via microCT characterization we have built a quantitative construct library of morphological and compositional properties of six CE approved CaP-based scaffolds (CopiOs®, BioOss™, Integra Mozaik™, chronOS Vivify, MBCP™ and ReproBone™), and of their bone forming capacity and *in vivo* scaffold degradation when combined with human periosteal derived cells (hPDCs). The empirical model, based on the construct library, allowed identification of the construct characteristics driving optimized bone formation, i.e. (a) the percentage of β-TCP and dibasic calcium phosphate, (b) the concavity of the CaP structure, (c) the average CaP structure thickness and (d) the seeded cell amount (taking into account the seeding efficiency). Additionally, the model allowed to quantitatively predict the bone forming response of different hPDC-CaP scaffold combinations, thus providing input for a more robust design of optimized constructs and avoiding trial-and error. This could improve and facilitate clinical translation.

Statement of Significance

Biomaterials that support regenerative processes are a key ingredient for successful bone tissue engineering (TE). However, the optimal scaffold structure is still under debate. In this study, we have provided a useful innovative approach for robust screening of potential biomaterials or constructs (i.e. scaffolds seeded with cells and/or growth factors) by combining microCT characterization with empirical modelling. This novel approach leads to a better insight in the scaffold parameters influencing progenitor cell-mediated bone formation. Additionally, it serves as input for more controlled and robust design of optimized CaP-containing bone TE scaffolds. Hence, this novel approach could improve and facilitate clinical translation.

1. Introduction

Biomaterials that support regenerative processes are a key ingredient for successful bone tissue engineering (TE). The latter is an interdisciplinary field of science focusing on the healing of bone defects typically by combining a biomaterial (scaffold) with

* Corresponding author at: Onderwijs en Navorsing 1, Herestraat 49 – PB813, B-3000 Leuven, Belgium.
E-mail addresses: greet.kerckhofs@med.kuleuven.be (G. Kerckhofs), yoke.chin.chai@vub.ac.be (Y.C. Chai), frank.luyten@uz.kuleuven.be (F.P. Luyten), liesbet.geris@ulg.ac.be (L. Geris).
[1] Present address: Dept. Basic (bio-) Medical Sciences, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussel, Belgium.

http://dx.doi.org/10.1016/j.actbio.2016.02.037
1742-7061/

cells and/or signalling molecules to support osteogenic (bone forming) processes [1]. The ideal scaffold serves as a 3D microenvironment that guides and stimulates bone tissue formation and is gradually replaced by newly formed bone (biodegradable) [1]. Other material characteristics that influence the bone forming capacity of scaffolds are the (i) biocompatibility (ii) high and interconnected porosity to enable cell migration and blood vessel ingrowth, (iii) mechanical integrity to support cell growth and tissue formation, (iv) chemical composition, (v) specific surface area, (vi) dissolution/degradation behaviour (e.g. to serve as a calcium (Ca) source [2]), (vii) surface macro- and microstructure [3,4] and (viii) surface chemistry for cell adhesion, growth, differentiation as well as protein/growth factor entrapment [5]. Most of these characteristics are coupled, making scaffold design, production, characterization and translation to clinical applications a challenging task [6,7]. Additionally, existing bone TE strategies still suffer from unpredictable and qualitatively inferior results, thus hampering clinical translation [8,9]. This low repeatability or lack of robustness is caused by widely variable material (due to inconsistency in the production process) and cell characteristics, the latter being also influenced by patient-specificities [8,10–14]. Hence, the optimal scaffold structure is still under debate.

As confirmed by our own findings [12], both material- and cell-specific variability should be reduced and trial-and-error should be avoided. Therefore, the aim of this study is to provide an innovative robust screening approach for TE constructs, avoiding trial-and-error, as assessed by successful bone formation *in vivo*. Using this approach, input for the design and fabrication of optimized TE constructs could be provided, aiming at an increase in repeatability and robustness. In our innovative screening approach, we have first built an elaborated construct (i.e. cell-scaffold combination) library based on microfocus computed tomography (microCT) analyses. MicroCT has been frequently reported in the literature as a relevant tool for the characterization of the 3D morphology of scaffolds [15–17], and bone formation within [18–22]. In this study, the construct library was created based on six different clinical-grade calcium phosphate (CaP)-based scaffolds, containing their 3D morphology, and bone forming capacity and *in vivo* material-dependent biodegradability when seeded with human periosteum-derived cells (hPDCs). This type of scaffolds has been reported to be successful in bone TE applications [23–25] and is known to induce bone formation on itself [26] and when combined with progenitor cells [12,20,27]. When combining the construct library with empirical modelling, our innovative screening approach allowed to (i) decipher the driving scaffold material properties for optimized bone formation when seeded with hPDCs and (ii) provide, in a robust manner, input for controlled design of scaffolds with optimized bone forming capacity. This could improve and facilitate clinical translation.

2. Materials and methods

2.1. Scaffold types

Six different commercially available and clinically approved CaP-based scaffold materials have been evaluated, namely CopiOs® (Zimmer Inc, Warsaw Indiana, USA), BioOss™ (Geistlich, Wolhusen, Switzerland), Integra Mozaik™ (Integra LifeSciences Corporation, Plainsboro, USA), chronOS Vivify (Synthes GmbH, Oberdorf, Switzerland), MBCP™ (Biomatlante, Vigneux de Bretagne, France) and ReproBone™ (Ceramysis, Sheffield, England). The first three are composed of a collagen matrix with CaP particles. The latter three only contain an interconnected CaP structure. The characteristics and composition of these materials, as provided by the supplier, can be found in Table 1. For each scaffold type, cubic scaffolds (~3 mm × 3 mm × 3 mm) were cut using a scalpel and a ruler. All preparations and handling of the scaffolds were accomplished in a sterile environment.

Table 1
Composition and pore characteristics of the different scaffold types, as provided by the suppliers.

Scaffold type	CaP type	Matrix type	Macro pore size	Micro pore size	Porosity
CopiOs®	dibasic calcium phosphate	Col-1 – bovine	5–1000 μm		93%
chronOS Vivify	100% β-TCP	No matrix	100–500 μm	<10 μm	70%
BioOss™	Bovine bone granules	Collagen type 1(Col-1) – porcine	200–600 μm	0.1–1 μm	83%
Integra Mozaik™	100% β-TCP	Col-1	Not available (N.A.)	N.A.	N.A.
MBCP™	60% HA + 40% β-TCP	No matrix	300–600 μm	<10 μm	70%
ReproBone™	60% HA + 40% β-TCP	No matrix	200–800 μm	1–10 μm	80%

2.2. In vivo evaluation

To evaluate the bone forming capacity of the different scaffold types, hPDCs were drop seeded with 30 μl of culture medium [DMEM-c; consisted of Dulbecco's Modified Eagle Medium (DMEM GlutaMax™) supplemented with 10% Fetal Bovine Serum, 1% antibiotics/antimycotics, and 5% sodium pyruvate] onto the scaffolds. The scaffolds only containing CaP were first prewetted by immersing them in phosphate buffered saline (PBS, Biowhittaker™) for 10 min. We used a cell pool of two male and two female donors with an age between 10 and 17 years old. The cells were cultured until passage P5. The cell seeding density was normalized to the open volume space of the scaffold types, which was determined from the microCT-based image analysis (see Section 3.1). The determination of the cell amount was based on results obtained in earlier experiments using NuOss scaffolds [12], where about 70,500 cells per mm^3 open scaffold volume were drop seeded onto the scaffolds. The theoretical normalized cell amount seeded per scaffold type is represented in Table 2. After 1 h of incubation at 37 °C, 5% CO_2 and 95% relative humidity, 5 ml of DMEM-c was added. The seeded scaffolds were incubated overnight to allow cell attachment. The remaining medium of the seeded scaffolds was collected and centrifuged. The pellet was then resuspended in RLT buffer (Qiagen, Venlo, The Netherlands) and stored at −80 °C for DNA quantification (Qubit system, Invitrogen, Belgium) to determine the cell seeding efficiency (CSE).

Subsequently, all the seeded scaffolds (n = 36, i.e. 6 per scaffold type) along with their non-seeded controls (n = 36, i.e. 6 per scaffold type) were randomly implanted subcutaneously in the shoulder area (n = 2) and the back (n = 2) at the cervical region of 8 weeks old NMRI-nu/nu mice. This anatomical site was chosen to evaluate the osteoinductive capacity of the constructs. In total, 18 mice were used. After 4 weeks (seeded: n = 3 per scaffold type; non-seeded: n = 3 per scaffold type) and 8 weeks (seeded: n = 3 per scaffold type; non-seeded: n = 3 per scaffold type) of implantation, the mice were sacrificed and the implants were collected. All handling was done according to the guidelines of the local ethical committee for Animal Research (KU Leuven). Each explant was then fixed in 4% paraformaldehyde for 2 h and stored in phosphate buffered saline (PBS, Biowhittaker™) at 4 °C until further analyses.