Quantitative bioimaging of glycosamoglycans in cartilage tissue-engineering using contrast enhanced computed tomography

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DOI
10.1016/j.joca.2019.02.458

Publication date
2019

Document Version
Final published version

Published in
Osteoarthritis and Cartilage

Citation (APA)
Garcia, J., Longoni, A., Grinstaff, M. W., Toyras, J., Weinans, H., Creemers, L. B., & Pouran, B. (2019). Quantitative bioimaging of glycosamoglycans in cartilage tissue-engineering using contrast enhanced computed tomography. *Osteoarthritis and Cartilage*, *27*(Supplement 1), S433-S433. https://doi.org/10.1016/j.joca.2019.02.458

growth and activity but despite that, they do not represent the natural environment for chondrocyte metabolism, collagen production and cartilage regeneration. A combination collagen hydrogel and PHA scaffold that addresses this issue would be highly desirable.
Methods: *Polymer production and preparation* PHAs were produced by *Pseudomonas mendocina* CH50 in batch fermentation conditions using a 15 L bioreactor with an operating fermenter volume of 10 L. with glucose as the sole carbon source under nitrogen limiting conditions. PHA film and scaffolds were either prepared using the solvent casting method incorporating large NaCl crystals (which were later dissolved out) to form a porous structure or with a 3D melt printing method. *Collagen gel preparation and seeding:* Type I collagen constructs were prepared with chondrocytes by mixing type I collagen polymer suspension with 10×modified Eagle's medium (MEM) and 1M NaOH solution was added dropwise, to neutralise the acidic gel mixture. This gel solution was then mixed with a cell suspension of C-20/A4 cells in Dulbecco's MEM (DMEM)-based medium containing 1% foetal calf serum (FCS) . 1.2 ml constructs were cast in 24 well plates either on their own or with the addition of solvent cast PHA polymers or 3D printed PHA polymers. Gels were then allowed to solidify by incubation at 37 °C for 5-10 minutes, overlaid with DMEM medium (1% FCS) and cultured for 1 or 7 days, at 37 °C and 5% CO_2. *Compression Studies:* Gels were subjected to compression, at 0,6KN/m2 for 5 minutes in a custom built apparatus. The displacement of gels was measured over time.
Results: The compression testing data demonstrates that type I collagen gels, seeded with C-20/A4 chondrocytes (control gels), exhibit changes in their mechanical properties over time, with an increased resistance and smaller displacement at Day 7 compare to Day 1. The addition of solvent cast and 3D printed scaffolds increase the mechanical resistance of the construct when compared to the populated control gels (collagen matix only) at DAY 1 and is even more evident at DAY 7. 3D printed scaffolds also show higher stiffness of the construct compared to the control and solvent cast polymer samples. Cytotoxicity testing performed with Alamar blue staining, on chondrocyte populations grown on PHA films, indicates that there are no cytotoxic effects of the polymer on cell viability at any of time points analysed over 7 days. Trypan Blue staining, after 5 minutes compression test, reveals no significant difference in viability in any of the samples containing PHA polymer (solvent cast or 3D printed) compare to control samples.
Conclusions: In conclusion, these studies suggest that a composite collagen gel-PHA construct provides a supportive environment for the maintenance of chondrocyte activity increasing the initial resistance of the structure to compression, whilst the embedded chondrocytes remodel the Type I collagen gel to form a more cartilage like structure. This may offer a potential application of collagen hydrogel-PHA scaffolds for clinical use in support of cartilage regeneration in small and contained osteoarthritic lesions.

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QUANTITATIVE BIOIMAGING OF GLYCOSAMOGLYCANS IN CARTILAGE TISSUE-ENGINEERING USING CONTRAST ENHANCED COMPUTED TOMOGRAPHY

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Purpose: Different strategies can be employed when manufacturing cartilaginous tissue-engineered constructs, e.g. using cells, scaffolds, or stimulatory molecules. Dynamic analysis of the effects of these factors on tissue regeneration is important to understand and finetune tissue growth and development. However, most of the available techniques to assess tissue quality and composition, such as 1,9-Dimethyl-Methylene Blue (DMMB) and Safranin-O histology, are invasive and destructive. Therefore, it is critical to develop techniques that allow quantitative monitoring of the same construct overtime.Contrast enhanced computed tomography (CECT) has been used for imaging of major cartilage constituents, such as the glycosaminoglycan (GAG)-containing proteoglycans. Here, we propose a CECT-based approach using a cationic iodinated contrast agent (CA4+) for non-destructive and real-time quantification and imaging of GAGs in cartilage-engineered constructs.
Methods: Human chondrocytes were harvested from articular cartilage. Cells were pelleted and cultured in the presence or absence of 10 ng/ml TGFb for different periods (7, 14 and 21 days) to generate constructs of varying GAG content. At the endpoint, pellets were incubated for 3 hr in medium containing CA4+ (4 or 8 mgI/ml, q= +4, MW = 1354 g/mol). Subsequently, μCT was performed at voxel size of 20 μm^3 in four different protocols, namely 90 kV tube voltage with i) 3-minute and ii) 26-second scan time, and 70 kV tube voltage with i) 3-minute and ii) 26-second scan time, all under 200 μA tube current. Phantoms (0-40 mgI/ml CA4+) were used to relate μCT grey values to CA4+ concentration. After scanning, DMMB was performed to quantify GAG content. To test the capability of the proposed technique for 3D visualization of GAGs distribution, additional constructs were frozen upon incubation with CA4+ and scanned (voxel size = 10 μm^3) using 90kV tube voltage, 3 minutes scanning time, and 200 μA tube current. Safranin-O was performed on the same pellets, and sections were matched with the μCT images.
Results: High correlation was found between CA4+ concentration and GAG content in the cultured pellets (Figure 1). Furthermore, CA4+ spatial distribution determined by μCT correlated with GAG distribution as shown by Safranin-O staining (Figure 2).
Conclusions: Here we report a method for real-time spatiotemporal monitoring of tissue production in cartilage tissue engineered constructs. CECT using cationic agents proved to be an efficacious technique for quantification and assessment of 3D distribution of GAGs, which can potentially replace currently available methods.

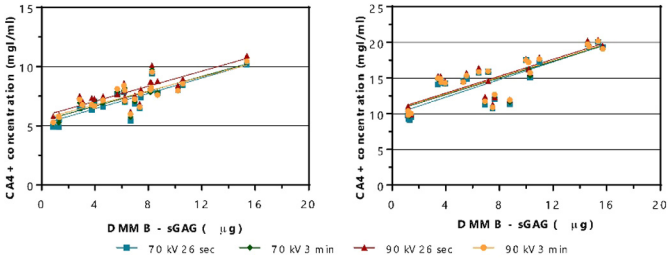

Figure 1. Correlation between CA4+ concentration and total GAG content upon 3 hours incubation with 4 (left) and 8 (right) mgI/mL CA4+

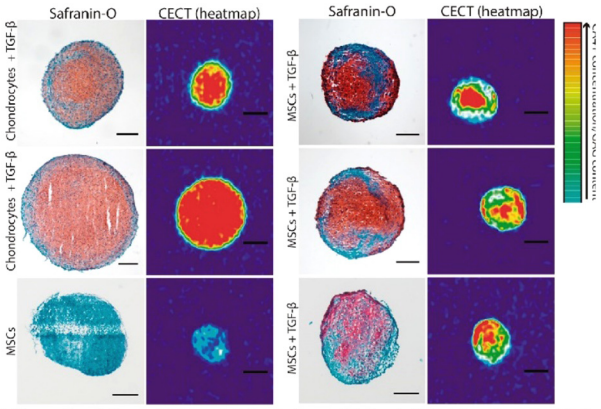

Figure 2. Comparison between CA4+ spatial distribution and Safranin-O histology. Scale bar histology images: 200 μm. Scale bar CECT images: 500 μm.

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CONDROGENESIS OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN AMNIOTIC FLUID IN CHITOSAN-XANTHAN SCAFFOLDS UNDER TGF- BETA 3 STIMULI

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Purpose: There are no effective treatments for pathologies that affect cartilage, such as osteoarthritis. The use of mesenchymal stem cells (MSC) is promising for regenerative medicine and cell therapy for focal lesions. Many sources of MSC have been studied in the last few years. The present study addresses the use of MSC derived from human