TAMPERE UNIVERSITY OF TECHNOLOGY

Texture-property relations of bioamine crosslinked gellan gum hydrogels

Citation
Koivisto, J. T., Koskela, O., Montonen, T., Parraga, J. E., Joki, T., Ylä-Outinen, L., ... Kellomäki, M. (2018). Texture-property relations of bioamine crosslinked gellan gum hydrogels. In *EMBEC and NBC 2017 - Joint Conference of the European Medical and Biological Engineering Conference EMBEC 2017 and the Nordic-Baltic Conference on Biomedical Engineering and Medical Physics, NBC 2017* (pp. 189-192). (IFMBE Proceedings; Vol. 65). Springer Verlag. https://doi.org/10.1007/978-981-10-5122-7_48

Year
2018

Version
Peer reviewed version (post-print)

Link to publication
TUTCRIS Portal (http://www.tut.fi/tutcris)

Published in
EMBEC and NBC 2017 - Joint Conference of the European Medical and Biological Engineering Conference EMBEC 2017 and the Nordic-Baltic Conference on Biomedical Engineering and Medical Physics, NBC 2017

DOI
10.1007/978-981-10-5122-7_48

Copyright
This publication is copyrighted. You may download, display and print it for Your own personal use. Commercial use is prohibited.

Take down policy
If you believe that this document breaches copyright, please contact cris.tau@tuni.fi, and we will remove access to the work immediately and investigate your claim.

Download date:17.04.2021

Texture-property relations of bioamine crosslinked gellan gum hydrogels

J.T. Koivisto[1,2], O. Koskela[1], T. Montonen[1], J.E. Parraga[1], T. Joki[2], L. Ylä-Outinen[2], S. Narkilahti[2], E. Figueiras[1,3], J. Hyttinen[1] and M. Kellomäki[1,2]

[1] BioMediTech Institute and Faculty of Biomedical Sciences and Engineering, Tampere University of Technology, Tampere, Finland
[2] BioMediTech Institute and Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
[3] Ultrafast Bio- and Nano-photonics Group, International Iberian Nanotechnology Laboratory, Braga, Portugal

Abstract— **Gellan gum is a hydrogel with potential for soft tissue engineering but a quick and thorough method is needed for screening of different possible compositions for more extensive studies. Here optical projection tomography in bright field mode was used to image nearly transparent hydrogels to record their optical texture in 3D. The gained Haralick's textural features were then analyzed with multiple discriminant analysis and combined with data from mechanical testing and neuronal cell culturing. We show the usefulness of optical texture analysis in screening of hydrogel compositions when aiming for tissue engineering applications.**

Keywords— **Hydrogel, Gellan gum, Neuron, Optical projection tomography, Haralick's textural features**

I. Introduction

Physically crosslinked hydrogels are an appealing class of biomaterials for tissue engineering applications, since they form a gel in mild conditions, suitable for cell encapsulation and/or minimally invasive therapeutic tissue engineering [1]. One of these promising hydrogels is ionically crosslinked gellan gum (GG) [2]. GG is a bacterial polysaccharide, which is used in pharmaceutical and food applications and has been studied for tissue engineering applications as well [3]. Our recently published results about crosslinking GG with the bioamines spermine (SPM) and spermidine (SPD) show that physical crosslinking with these small molecules is possible and the resulting hydrogels are mechanically biomimicking, cytocompatible and suitable for 3D encapsulation of human neuronal cells [2].

One challenging aspect of hydrogel scaffold development is studying the porosity and microstructure in wet-state. This is a crucial aspect, since cell migration or nutrient diffusion might be hindered through too dense hydrogel network. Electron microscopy or X-ray micro-computed tomography enable studying microstructures, but they require a destructive drying step during sample preparation, which can alter the hydrogel in unknown ways. To circumvent these issues, we developed texture analysis method for wet hydrogels [4] using optical projection tomography (OPT) [5].

OPT is a three-dimensional, nondestructive imaging method for mesoscopic scale transparent samples. In transmission OPT we can see optical texture, which varies between different hydrogel compositions. The information obtained from this texture is not directly the microstructure of the hydrogel but rather density variations in the polymer network, which affect transmission of light enough to be detectable. [4] To quantify these density variations we used Haralick's optical textural features [6]. For analyzing this high dimensional data we used multiple discriminant analysis (MDA) [7], proving that we can optically distinguish different hydrogel formulations of transparent samples indistinguishable with the naked eye. [4]

In this current work, we have a comparison between SPM and SPD crosslinked GG, showing how the two chemically very close bioamine molecules make different hydrogel texture, even if the mechanical properties are the same.

In addition, we compare the textural features in full and in MDA reduced dimensions to find correlations between texture and mechanical properties or grown neurite length, which could be further used for screening of new hydrogel formulations developed in the future

II. Materials & Methods

A. Sample preparation

The reagents GG (GelzanTM, low acyl, Mw 1 kg/mol), SPD (spermidine trihydrochloride), SPM (spermine tetrahydrochloride) and sucrose were all acquired from Sigma Aldrich. Reagents were dissolved in 10 wt-% sucrose deionized water and hydrogels crosslinked at +37°C. All reagents were sterile filtered before use with 0.8/0.2 mm Acrodisc® syringe filter (PALL Corporation, Port Washington, NY, USA). Studied crosslinker concentrations are 1.1 wt-%SPM (GG-SPM-H), 0.6 wt-%SPM (GG-SPM-L), 3.0 wt-%SPD (GG-SPD-H), and 1.5 wt-% SPD (GG-SPD-L).

B. Optical projection tomography bright field imaging

Optical projection tomography (OPT) [5] was done in bright field transmission mode with the previously described

in-house built system [8]. Five parallel hydrogels were prepared into fluorinated ethylene propylene tubes (Adtech Polymer Engineering, U.K.) with parafilm-blocked ends and submerged in water. Projection images using 5x objective were taken around entire 360° rotation at steps of 0.9°, resulting in 400 images [4].

C. Texture analysis, multiple discriminant analysis and linear regression

Image texture analysis was conducted for each projection image using Haralick's textural features [6] as is described in detail in [4] yielding 13-by-8 feature matrix for each projection image. We used here the toolbox originally implemented and used in [9].

Multiple discriminant analysis (MDA) [7] was used to create lower dimensional projection of the Haralick's textural features, such that each dimension is a weighted linear composition of the most different features between different hydrogel compositions.

After analyzing purely the textures, linear regression and artificial neural network regression were applied to find correlation between Haralick's features and compression and cell culture data. Linear model was fitted in the least square sense using all computed Haralick's features. Artificial neural network regression was applied to means of Haralick's features in all 400 projections of each sample. The relation between training, validation and test sets was set to 70%, 10%, and 20% respectively. Both regression methods were computed separately for each compression and cell culture variable.

All computations were performed in MATLAB (MathWorks, Natick, MA, USA).

D. Compression testing

Mechanical testing performed as described in detail in [2], using BOSE Electroforce Biodynamic 5100 machine equipped with a 225 N load sensor and Wintest 4.1 software (Bose Corporation, Eden Prairie, MN, USA). Compression was performed unconfined with a constant 10 mm/min strain rate until 65% strain of original height was reached. Compressive modulus calculated from stress-strain curve slope at perceived linear region according to Hooke's law. Additionally, the fracture strength and fracture strain were recorded as a sudden drop in the stress-strain curve.

E. Cell culture and analysis

BioMediTech has Pirkanmaa Hospital District's ethical approval to derivate, culture and differentiate hESCs (Skottman, R05116) and permission from the National Authority for Medicolegal Affairs (FIMEA 1426/32/300/05) to conduct human stem cell research.

Human pluripotent stem cell derived neuronal cells culture and hydrogel cell culture test were conducted as described previously [2; 10]. Briefly, cells were cultured with hydrogel for two weeks and in the end a LIVE/DEAD® viability assay (Molecular probes, Thermo Fisher Scientific) was used with 1 h incubation in +37°C. Samples were imaged with an Olympus IX51 inverted microscope and an Olympus DP30BW digital camera (Olympus, Finland). The numbers of parallel samples varied between two and four.

Neurite migration was quantified using ImageJ measure tool (Version 1.39, U. S. National Institutes of Health, Bethesda, MD, USA) [11]. Measurement was done with a straight line from the cell surface to the visible end of a neuronal process. Each analyzed cell was measured from longest separately distinguishable neurites. Values of less than 10 µm were considered as representing no migration.

III. Results & Discussion

The ability of OPT to record hydrogels in their wet-state enables studying unaltered hydrogel microstructure and also enables other promising setups, such as live cell imaging inside hydrogels. Here we concentrated on the material characterization and analyzes of the relevance of the hydrogel's optical texture to other properties. Figure 1 shows example projections of all the studied gel formulations.

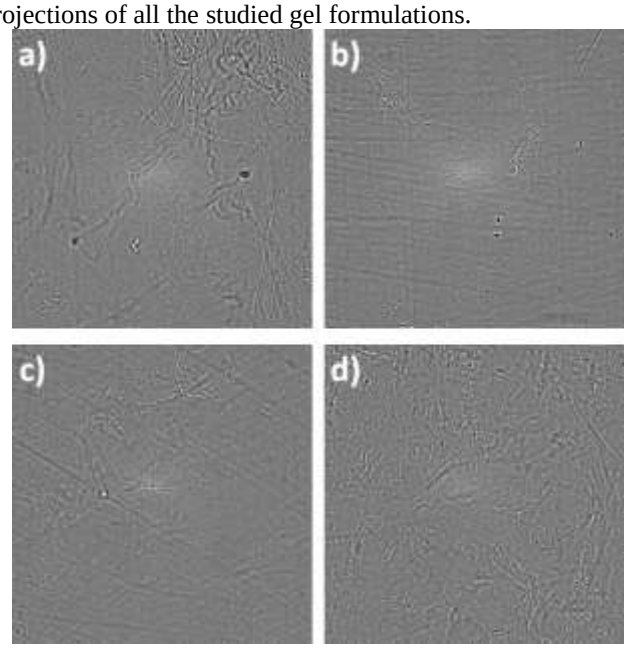

Fig. 1. Representative projections images of the textures of studied hydrogels, a) GG-SPM-H, b) GG-SPM-L, c) GG-SPD-H, d) GG-SPD-L.

Compared to our previous publication [4], we have now applied the Haralick's textural feature for OPT method to yet another gel formulation, the SPD crosslinked GG. In the present case, the MDA requires more features for separation of gels and a minimum three-dimensional space. The highest MDA separation between Haralick's textural feature groups is shown in Figure 2.

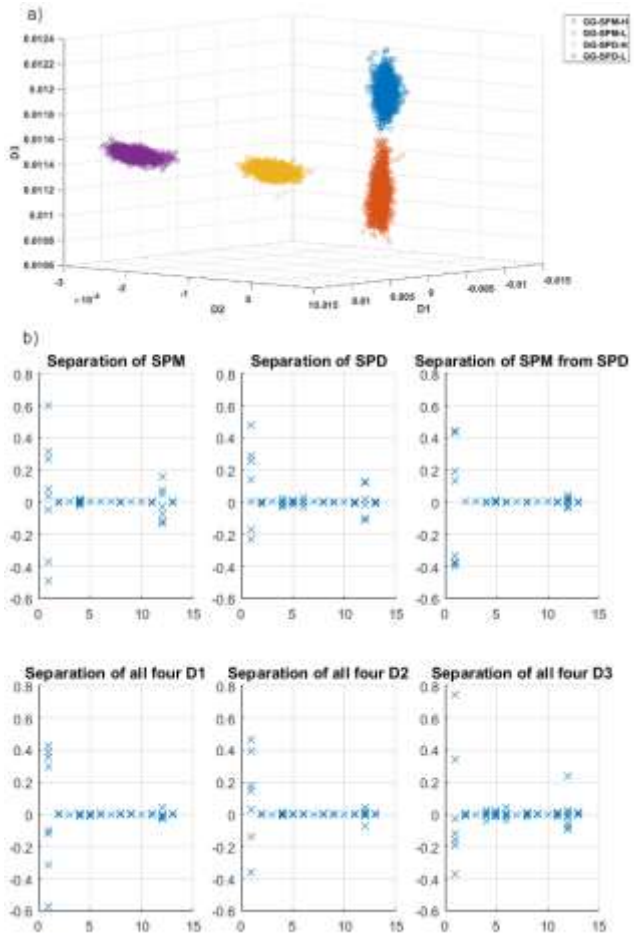

Fig. 2. a) Three-dimensional representation of MDA separation of Haralick's textures in studied hydrogels. b) Weights of Haralick's features on MDA separation.

As can be seen in Figure 2, SPM crosslinked gels differ from each other in dimension D3, which consists mainly of Haralick's features energy, entropy and information correlation 1. However, SPD crosslinked gels introduce additional features: inverse difference moment and sum average, before they can be separated numerically. To separate SPD and SPM crosslinked gels from each other, all these five features are required.

We found that linear regression did not model correlation between textural features and compressive modulus, fracture strength, fracture strain or neurite length. In all of these cases, the behavior was similar to Figure 3a, where linear fit against neurite length is shown. It seems that whereas SPM has an increasing fit, SPD has the opposite, decreasing fit. The same switch in texture profiles can already be noticed in Figure 1 as more visible textural features can be seen in the GG-SPM-H when compared with the GG-SPM-L, and in the GG-SPD-L when compared with the GG-SPD-H.

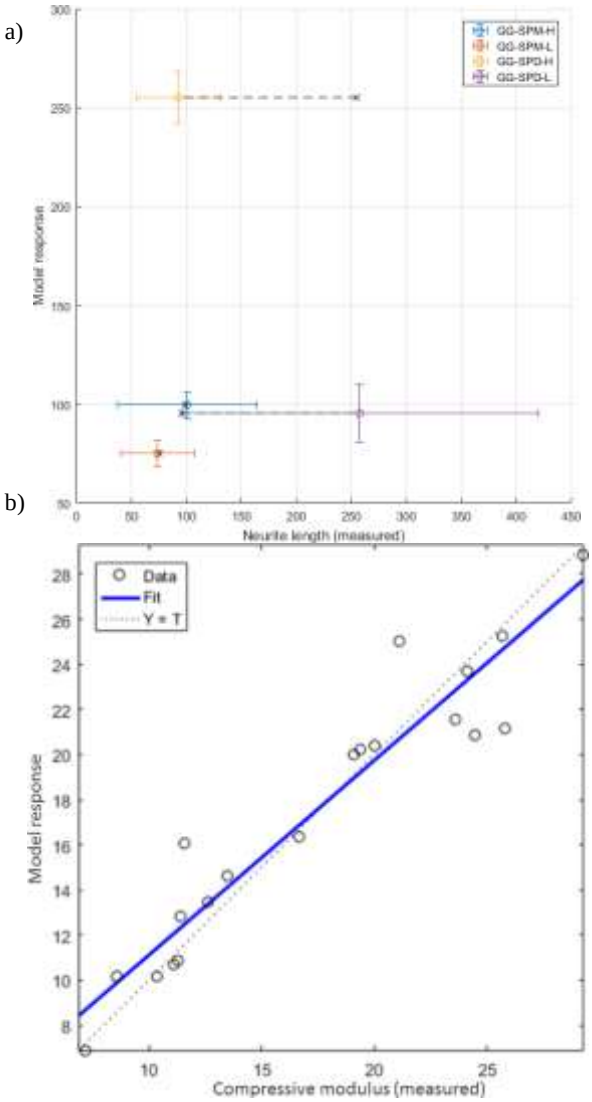

Fig. 3. a) Measured neurite lengths in the x-axis and linear regression fit in the y-axis. Bars show the standard deviations of measures and model response, respectively, while means are in the intersections marked with circles. Black crosses show the difference between measured and fitted means. b) Measured compressive modulus in the x-axis and neural network regression model response in the y-axis.

In neural network regression, correlation factor between output of the network and measured values varied mainly between 0.75 and 0.95. Performance varied depending on the choice of used textural features and random distribution of data to training, validation and test sets. Example neural network regression is shown in Figure 3b.

Linear regression coefficients were highest with the same features as in MDA. In neural network regression, no connection between MDA coefficients and selection of features to use was noticed. For screening purposes, a more detailed study on the predictability of hydrogel properties based on neural network analysis is needed. The future work includes enhancing the predictability by measuring additional hydrogel formulations in multiple concentrations.

IV. Conclusions

We have shown already that 3D textural analysis from projections acquired with OPT can be applied to separate hydrogels from each other. In this study, we made a further step to find correlations between textural features and physical properties. Results suggest that a straightforward linear regression is not sufficient. A classification step and further analysis within separate hydrogel classes are needed. Higher number of samples is required for robust neural network regression.

The effect of the texture or density variations on cell response to the hydrogel will be studied in detail in the future. The heterogeneities of the hydrogel microenvironment likely attract cells, possibly even direct cell growth. This texture analysis method and OPT as a whole are valid methods to study three-dimensional cell growth inside hydrogels.

Acknowledgment

The authors thank Ana M. Soto, M.Sc. (Tech), for the Haralick MATLAB code. The authors wish to thank the Human Spare Parts program of Tekes – Finnish Funding Agency for Innovation and the Jane and Aatos Erkko Foundation for funding of this research.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Annabi N, Tamayol A, Uquillas JA et al. (2014) 25th anniversary article: Rational design and applications of hydrogels in regenerative medicine. Adv Mater 26:85-124 DOI 10.1002/adma.201303233.

2. Koivisto J T, Joki T, Parraga J et al. (2017) Bioamine-crosslinked gellan gum hydrogel for neural tissue engineering. Biomed Mater DOI 10.1088/1748-605X/aa62b0.

3. Bacelar A H, Silva-Correia J, Oliveira JM et al. (2016) Recent progress on gellan gum hydrogels provided by functionalization strategies. J Mater Chem B 4:6164-6174 DOI 10.1039/C6TB01488G.

4. Soto A M, Koivisto JT, Parraga JE et al. (2016) Optical projection tomography technique for image texture and mass transport studies in hydrogels based on gellan gum. Langmuir 32:5173-5182 DOI 10.1021/acs.langmuir.6b00554.

5. Sharpe J, Ahlgren U, Perry P et al. (2002) Optical projection tomography as a tool for 3D microscopy and gene expression studies. Science 296:541-545 DOI 10.1126/science.1068206.

6. Haralick R M, Shanmuga K and Dinstein I (1973) Textural features for image classification. IEEE T Syst Man Cyb SMC3: DOI 10.1109/TSMC.1973.4309314.

7. Duda R O, Hart PE and Stork DG (2001) Pattern Classification. Wiley, New York (NY).

8. Figueiras E, Soto AM, Jesus D et al. (2014) Optical projection tomography as a tool for 3D imaging of hydrogels. Biomedical Optics Express 5:3443-3449 DOI 10.1364/BOE.5.003443.

9. Gupta S and Markey MK (2005) Correspondence in texture features between two mammographic views. Med Phys 32:1598-1606 DOI 10.1118/1.1915013.

10. Lappalainen R S, Salomäki M, Ylä-Outinen L et al. (2010) Similarly derived and cultured hESC lines show variation in their developmental potential towards neuronal cells in long-term culture. Regener Med 5:749-762 DOI 10.2217/rme.10.58.

11. Schneider C A, Rasband WS and Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Meth 9:671-675.

Author: Janne Koivisto
Institute: BioMediTech Institute and Faculty of Biomedical Sciences and Engineering, Tampere University of Technology
Street: Korkeakoulunkatu 3
City: 33720 Tampere
Country: Finland
Email: janne.t.koivisto@tut.fi