Development of a multi-well-chip for studying 2D and 3D tumor cell migration and spheroid growth in electrical fields

Benjamin K. Naggay, Department of Applied Chemistry at Reutlingen University, Reutlingen, Germany
Benjamin.Naggay@Reutlingen-University.de
Tobias B. Schmidt, Department of Applied Chemistry at Reutlingen University, Reutlingen, Germany
Tobias_Benedikt.Schmidt@Student.Reutlingen-University.DE
Karen Ende, Department of Applied Chemistry at Reutlingen University, Reutlingen, Germany
Karen.Ende@Reutlingen-University.DE
Ralf Kemkemer, Department of Cellular Biophysics at Max Planck Institute for Medical Research, Stuttgart, Germany Department of Applied Chemistry at Reutlingen University, Reutlingen, Germany
Ralf.Kemkemer@mr.mpg.de

Endogenous electrical fields play an important role in various physiological and pathological events. Yet the effects of electrical cues on processes such as wound healing, tumour development or metastasis are still rarely investigated, though it is known that direct current electrical fields can alter cell migration or proliferation *in vitro*. Several 2D experimental models for studying cell responses to direct current electrical fields have been presented and characterized but suitable experimental models for electrotaxis studies in 3D are rare.
Here we report on a novel, easy-to-use, multi-well-based system for studying the response of cells and tumor models (speroids) to electrical fields in 2D and 3D environments. The system allows the application of electrical fields to four experimental chambers laden with cells, either cultured on the bottom of the culture-plate (2D) or embedded in hydrogel filled channels (3D), and simultaneous live-cell-imaging of the samples. Inserts for the multi-well plates are fabricated with polydimethylsiloxane via mold replication of a 3D printed model. For electrical field application, agar-salt-bridges are used to connect electrode reservoirs to the chambers containing the spheroid-laden hydrogel in 3D or cell media for 2D experiments. Multicellular tumours embedded in hydrogels are used as a model for 3D tumour environments and exposed to different direct current electrical fields. Validation tests show stable electrical fields and high cell viabilities inside the channel. Furthermore, the system allows easy handling and imaging, of the tumour spheroids. In addition, results show that tumour spheroids of various diameters can be exposed to direct current electrical fields over several days. Replaceable agar-salt-bridges and electrodes allow direct current electrical field experiments for more than 3 days, not interrupting the live-cell-imaging.

Degradable dual co-electrospun polyester based nonwovens for guided tissue regeneration

Stefan Oschatz, Institute for Biomedical Engineering, Rostock University Medical Center, 18119 Rostock-Warnemünde, Germany, E-Mail: stefan.oschatz@uni-rostock.de
Thomas Reske and Klaus-Peter Schmitz, Institute for Implant Technology and Biomaterials e.V., 18119 Rostock-Warnemünde, Germany
Jana Markhoff, Thomas Eickner, Niels Grabow and Sabine Illner Institute for Biomedical Engineering, Rostock University Medical Center, 18119 Rostock-Warnemünde, Germany

Introduction

Nonwoven materials based on biodegradable polymers have gained growing attention in the field of guided tissue regeneration, e.g. in periodontal and endodontal applications. Those materials initially serve as a barrier for fast-growing cells but may later on provide a necessary supporting structure for cells as well as for apatite formation to regenerate bone defects. The porous nature of nonwovens is in particular beneficial for the formation of cell layers.
In this work, degradable polyester based composite nonwovens were manufactured via dual co-electrospinning. The obtained compounds exhibit separated fibers of each polymer merged into one material. In that way, poly(lactic-co-glycolic acid)/polydioxanone (PLGA/PDO) and polycaprolactone/polydioxanone (PCL/PDO) composite materials were generated.

Methods

The composite nonwovens have been manufactured from commercially available polyesters on a Contipro 4SPIN C4S LAB2. The generated mats have been investigated regarding their suitability for guided tissue regeneration with respect to their inherent degradation behavior. Accelerated degradation in alkaline glycine buffer has been monitored regarding total mass loss, molecular mass loss via GPC measurements as well as macroscopical changes.

Results

The combination of fibers with different degradation properties via electrospinning is shown. The different polymers combined to one composite material exhibit different degradation behaviors under accelerated conditions as it was found in GPC data, whereas on polymer degrades more slowly compared to the other. Moreover, the choice of polymers showed an impact on the degradation behavior of the nonwovens. Although the molecular mass loss is comparable, it was observed that PLGA/PDO showed a distinctly higher degree of fragmentation and total mass loss compared to a PCL/PDO composite.

Conclusion

The described materials offer a potential for tailor-made future applications in guided tissue regeneration. By combining a faster degrading polymer with a more resilient one to a composite, the material may allow for cell infiltration while maintaining its function as a supporting scaffold.

Immobilizing hydrolytic active Papain on biodegradable PLLA for biofilm inhibition in cardiovascular applications

Michael Teske[1], [1]Institute for Biomedical Engineering (University Medical Center Rostock, University of Rostock), Rostock, Germany, Michael.Teske@uni-rostock.
Tina Kießlich[1]/ Julia Fischer[2]/ Hubert Bahl[2]/ Katharina Wulf[1]/ Thomas Eickner[1]/ Niels Grabow[1]/ Sabine Illner[1],
[1]Institute for Biomedical Engineering (University Medical Center Rostock, University of Rostock), Rostock, Germany, [2]Abteilung Mikrobiologie (Institut für Biowissenschaften, University of Rostock) Rostock, Germany, t.kiesslich@hzdr.de, julia.fischer2@uni-rostock.de, hubert.bahl@uni-rostock.de, katharina.wulf@uni-rostock.de, thomas.eickner@uni-rostock.de, niels.grabow@uni-rostock.de, sabine.illner@uni-rostock

Introduction

The use of biomaterials in medicine is becoming increasingly important. One of the main concerns is the foreign body associated infection caused by direct microbial contamination or clinical infections. The bacterial biofilm formation on biomaterials depends on their surface properties. Therefore, several anti-adhesive surface modifications were developed. Nevertheless, the demand for antimicrobial agents that prevent bacterial colonisation is still largely unmet. The immobilization of active antimicrobial agents, such as antibacterial peptides or enzymes, offers a potential approach to achieve long-lasting effectiveness. Therefore, the hydrolytic enzyme papain with its antibacterial activity was covalently immobilized on the biodegradable biomaterial poly-L-lactic acid (PLLA).

Methods

For the enzyme characterization on the PLLA surfaces, the determination of papain amount and activity was determined. A biofilm assay was performed to test the effect of the immobilized papain on the biofilm-forming bacterial strain Clostridioides difficile, one of the most frequently occurring human nosocomial pathogens.

Results

The amount of immobilized papain on PLLA was significantly increased in comparison to the reference (modified PLLA surface with no immobilized papain). The reduction of the biofilm compared to the reference was significant at 24 h and still observable after 72 h.

Conclusion

It was demonstrated that the investigated hydrolytic enzyme papain could be immobilized by coupling via the crosslinker EDC to the PLLA surface and has a significant biofilm reducing effect for C. difficile. Detection was performed by determination of the amount of protein and the reduced biofilm growth.

Initial study on removing cellular residues from hydrostatic high-pressure treated allogeneic tissue using ultrasound

Corresponding author: Christoph Drobek: Universität Rostock, Lehrstuhl für Mikrofluidik, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany, e-mail: christoph.drobek@uni-rostock.de
Janine Waletzko: Universitätsmedizin Rostock, Klinik und Poliklinik für Mund-, Kiefer- und Plastische Gesichtschirurgie, Rostock, Germany
Volker Weißmann: Hochschule Wismar, Kunststofftechnik/Werkstoffe, Wismar, Germany
Stefan Seehafer, Robert Mau: Universität Rostock, Lehrstuhl für Mikrofluidik, Rostock, Germany
Anika Jonitz-Heincke, Rainer Bader: Universitätsmedizin Rostock, Orthopädische Klinik und Poliklinik, Rostock, Germany
Daniela Schwerdt: Hochschule Wismar, Kunststofftechnik/Werkstoffe, Wismar, Germany
Michael Dau, Bernhard Frerich: Universitätsmedizin Rostock, Klinik und Poliklinik für Mund-, Kiefer- und Plastische Gesichtschirurgie, Rostock, Germany
Hermann Seitz: Universität Rostock, Lehrstuhl für Mikrofluidik, Rostock, Germany

Introduction

The regeneration capacity of the human body for large tissue defects is limited. The missing tissue must be replaced with adequate structures, which adopt form and functionality. For this purpose, allografts are becoming increasingly important. However, they can lead to an extensive immune response. The hydrostatic high-pressure treatment (HHD) allows a fast and gentle devitalization of allografts to reduce the immune response after transplantation. After the HHD treatment the devitalized cells have to be removed via an adequate rinsing procedure.

Methods

A ultrasonic rinsing test setup is demonstraded and utilized for bone tissue decellularization after HHD devitalization. The residual DNA content of the bone tissue is determined to evaluate the rinsing quality.

Results

In comparision to untreated control, bone tissue samples treated with the HDD + ultrasound based rinsing showed a significant reduction of DNA content of 21 %. Bone tissue samples treated with ultrasound based rinsing only showed a reduction of DNA concent of about 16% compared to the untreated control.

Conclusion

The Ultrasound rinsing test setup was demonstrated successfully. It enables a significant reduction of the DNA content of the bone tissue for both HHD + ultrasound treated samples.

Protein Adsorption Hysteresis and Transient States of Fibrinogen as Model Mechanisms for Proteome-Binding to Implants.

Herbert P. Jennissen[1], Daniel-Sebastian, Dohle[2], Michael Meißner[1]
[1]Institute of Physiological Chemistry, Biochemical Endocrinology Group,
University of Duisburg-Essen, D-45147 Essen
[2]Klinik und Poliklinik für HTG-Chirurgie, Universität Mainz, D-55131 Mainz

Introduction

The adsorption of proteins on surfaces generally occurs in the form of an adsorption-desorption hysteresis loop [1,2]. The desorption isotherm does not retrace the adsorption isotherm as a result of thermodynamic irreversibility. True Langmuir-type isotherms do not exist in hysteretic systems even if the data can be fitted to a hyperbola. In the two-state domain model of a hysteresis cycle the half-saturation constants (apparent dissociation constants, $K'_{a,0.5}$ and $K'_{d,0.5}$) of the adsorption and desorption branch of a hysteresis loop can be calculated. In a multistate model several transient states can be recorded. The evaluation of hysteretic data is based on the model that that the long-lived metastable states of the local energy minima can be treated thermodynamically as true equilibria (see [1,7]). Under these conditions it is possible to obtain values for the entropy and the molar Gibbs energy ($\Delta_i S$, $\Delta_i G$) of the irreversible process (area of the hysteresis loop) and apparent dissociation constants [1,2].

Methods

Although the proteins phosphorylase [1] and BMP-2 [3]. have been investigated, emphasis is on Fibrinogen which was purified to homogeneity as previously described with >95% clottability [4,5]. Measurements can either be made by evanescent wave technology [5] on quartz glass or radiotracer methods via covalent labelling with ^{3}H or ^{125}I on electropolished titainium. Protein adsorption can be determined in batch procedures [1,6] or flow cells [5]. For curve fitting and statistical analysis the PC program Graphpad Prism Vers. 4. was employed.

Results

It will be shown that the apparent dissociation constants ($K'_{0.5}$) of adsorbed fibrinogen lie in the magnitude of 10^{-7}-10^{-12} M depending on the metastable states 1-3. If one compares the $K'_{0.5}$ of state 1 (encounter complex), state 2 several conformational steps and finally state 3 (apparent final complex) they differ by a factor of 3.1 x 10^{4}, or if the apparent Gibbs free energies are calculated ($\Delta G^{O'} = -RT \ln K$) a difference value of – 25 kJM^{-1} is obtained, which probably corresponds to the Gibbs free energy of the irreversible process ($\Delta_i G$) involving the conformational changes. Differential multiple transient states occur during adsorption. This behavior can be generalized to many proteome proteins.

Conclusion (14 pt bold)

It is concluded that on quartz glass and electropolished titanium fibrinogen adsorption occurs in at least three steps with a dissipation of at least – 25 kJM^{-1} of energy.

[1] Jennissen, H. P. & Botzet, G. (1979) *Int J Biol Macromol*, **1,** 171-179.
[2] Jennissen,H.P. (1985) Protein Adsorption Hysteresis. In *"Surface and Interfacial Aspects of Biomedical Polymers" Vol.2, Protein Adsorption* (Andrade,J.D., ed), pp. 295-320. Plenum Press, New York.
[3] Chatzinikolaidou, M., Lichtinger, T. K., Müller, R. T., & Jennissen, H. P. (2010) *Acta Biomater.*, **6,** 4405-4421.
[4] Jennissen, H. P. & Demiroglou, A. (2006) *J Chromatogr. A*, **1109,** 197-213.
[5] Jennissen, H. P. & Zumbrink, T. (2004) *Biosens. Bioelectron.*, **19,** 987-997.
[6] Dohle, D.-S., Pasa, S. D., & Jennissen, H. P. (2010) *Biomaterialien*, **11 (S1),** 25.
[7] Katchalsky, A. & Spangler, R. (1968) *Quarterly Reviews of Biophysics*, **1,** 127-175

Development of patient-specific drug-releasing round window implants for the treatment of inner ear diseases

F. Matin[1,2], Z. Gao[2,3], F. Repp[4] , S. John[4,5] , T. Lenarz[1,2,3], V. Scheper[1,2,3]

[1] *Department of Otorhinolaryngology, Medical Unisversity Hanover, Hanover, Germany*
[2] *Lower Saxony Centre for Biomedical Engineering, Implant Research and Development, Hanover, Germany*
[3] *Cluster of Excellence"Hearing4all" EXC 1077/1, Oldenburg/Hannover, German Research Foundation*
[4] *OtoJig GmbH, Hanover, Germany*
[5] *Hörsys GmbH, Hanover, Germany*

Matin.Farnaz@mh-hannover.de
Gao.Ziwen@mh-hannover.de
repp.felix@otojig.com
john.samuel@hoersys.de
Lenarz.thomas@mh-hannover.de
Scheper.verena@mh-hannover.de

Introduction

The modern therapy of inner ear disorders is increasingly being determined by local drug delivery. Access to the inner ear is usually found via the round window membrane (RWM). The RWM is located in the bony round window niche (RWN), which allows local deposition of drugs. For safe and controlled drug delivery optimal fitted drug-implants designed for the individual shape of the niche have to be developed. We report about our preparatory work on the patient specific anatomy and model of the RWN.

Methods

Cone beam computed tomography (CBCT) images of 50 patients were analyzed. Based on the reconstructed 3D volumes, the individual structures of the RWN were determined by segmentation using 3D slicer™. A custom build plug-in was used, which allows the determination of the midmodiolar axis by fitting a mean model of the scala tympani and the scala vestibuli in the CBCT-data. This allowed defining a coordinate system relative to the cochlear so that quantities like depth and volume of the RWN, the length of the bony overhang and area of the RWM could be determined in comparable directions.

Results

A large individual anatomical variability of the RWN with a mean volume of 4.54 mm^3 (min 2.28mm^3, max 6.64mm^3) was detected. The area of the RWM ranged from 1.30mm^2 to 4.39mm^2 (mean: 2.93mm^2). The bony overhang had a mean length of 0.56mm (min 0.04mm, max 1.24mm).

Conclusion

Our data prove that there is a need for individually shaped RWN implants due to clinically relevant differences in volume and shape of the niche.

Outlook

Such an individualized, novel implant for minimally-invasive local delivery to the inner ear should be biodegradable and may be produced by 3D printing. Different additive manufacturing processes such as Fused Deposition Modeling, Digital Light Processing, Two-Photon-Polymerization or Micro Injection Molding may address this need.

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