Establishment and initial characterization of a simple 3D organotypic wound healing model

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Poor wound healing resulting in chronic wounds affects millions of people worldwide. While the complex biological repair process requiring the regulated interaction of numerous cells in a healing wound is well understood, the number of therapies available to successfully treat chronic wound is still very limited. Development of new therapies is costly and time consuming as it requires tests in a complex tissue context. Thus, defined but simple to use 3D systems reflecting the *in vivo* tissue complexity are urgently needed. A 3D organotypic model (OTC) containing the major cellular component active during wound healing i.e. keratinocytes, fibroblasts and inflammatorycells – specifically macrophages and neutrophils - was established and its use in wound healing studies employing standardized wounding procedures was demonstrated. The model is characterized histologically and by immunofluorescent staining allowing the localization of specific cell types and matrix structures. Soluble mediators of wound healing like MMP-2 and -9 and IL-1β are determined and exhibit an *in vivo* like kinetics of secretion. The system provides a reproducible, simple to use yet sufficiently complex basis that will allow the analysis of molecular effects of therapeutic regimen used in the management of chronic wounds.

Method Comparison of In Vitro Wound Area Measurements

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Wound area is a primary outcome measure in wound healing studies. This method comparison study evaluates differences of wound area measurements of a newly developed image analysis method based on wound edge contour to an existing method based on contrast tolerance. Digital images of 64 wounds were taken immediately after wounding matured in vitro 3D organotypic tissues with a biopsy punch. Wound area measurements were calculated using each image analysis method and then normalized. The method comparison study evaluates the difference of each paired measurements for all 64 wound areas. Measurement differences are demonstrated and evaluated in normalized data boxplots, scatter plots with a line of equality, data histogram and Normal probability plots, and a Bland-Altman plot of paired measure difference against mean. The measured wound areas using the tolerance method have large variability in comparison to the contour method measures. The tolerance method measures often underestimate and overestimate what is assumed to be an approximately repeatable initial wound size. Skewness in comparison plots are due to the 'fat tails' introduced by the variability of measurements of the tolerance method. In contrast, the contour method results in larger wound area measurements on average at a statistically significant level of difference. The relatively less variable range of contour method measurements suggest this method has more potential to agree with the 'true' wound area. Future work to improve the method are proposed for application of image analysis methods to distinguish true wound area and measurement error in time for wound healing treatment-control experiments.

Processing pericardial tissue for cardiovascular surgery
Highlighting the importance of a comprehensive quality management

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Pericardial tissue is widely used as a biomaterial, especially for cardiovascular application. Tissue processing plays a key role in developing future scaffolds derived from biological material, yet standardized protocols are still pending. This study presents a comprehensive in-vitro assessment of different established and advanced treatment protocols of bovine pericardium and compares those findings to commercially available decellularized bovine (CAB) and equine (CAE) pericardial patches.
Native pericardial samples were fixed with glutaraldehyde (GA) or decellularized and subsequently either sterilized (DEC) or treated with GA (DEC-GA). Treatment effects were assessed by histological evaluation of tissue structure and biomechanical properties. Decellularization efficacy and accuracy of the applied sterilization protocol were evaluated. Cell seeding of processed pericardial samples with human endothelial cells constituted as biocompatibility test.
GA-fixed tissue revealed structural deterioration and cytotoxicity. Opposed to popular believe, GA-treatment did not lead to sterility of all samples. DEC samples were successfully sterilized and decellularized, the DNA content was significantly reduced (58.69 ± 4.06 ng/mg, $p < 0.05$) by decellularization compared to native samples (85.19 ± 6.28 ng/mg). Comparative assessment revealed significantly less residual DNA in CAE (38.51 ± 1.82 ng/mg, $p < 0.01$) compared to DEC, while values for CAB (70.60 ± 12.32 ng/mg) showed resemblance to our native samples.
Biocompatibility of DEC, CAB and CAE was confirmed by successful cell seeding. Substantial differences of tissue properties were observed, resulting in considerable varieties within the groups. Decellularized samples displayed a wide range of tissue thickness, e.g. CAB (824.1 ± 159.50 µm) compared to DEC (451.10 ± 64.64 µm).
The results revealed decellularized biomaterials to be more favourable than routinely used GA-fixed tissue due to sterility and biocompatibility reasons. This study also provides a first overview describing consequential variations among biomaterials and illustrates the necessity of multidimensional assessment and tissue quality management for biological scaffold development.

Effects of uniaxial stretching on tenocyte migration behaviour

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Tendon problems represent the most frequent musculoskeletal complaints. As tendon healing is slow and afterwards tendon rarely retains the structural integrity and mechanical strength, treatment of tendon injuries is a significant clinical challenge. Healing of tendon tissue requires tenocyte migration from intact regions of the injured tendon to the repair site, followed by cell proliferation and synthesis of the extracellular matrix [1]. Enhancement of tenocyte migration and proliferation could improve the effectiveness of cell-based therapies in tendon healing. Since tendon tissue is subjected to mechanical loading in its microenviroment, our study aims to investigate the mechanobiological responses of tendon cells using a custom-made tensile bioreactor. Mechanical stretching is an important regulator in functional tendon tissue engineering and it increases the organization and strength of engineered tissues while inducing beneficial cell responses [2]. It has been already observed that mechanical stretching induces the regulation processes of tendon cells such as collagen synthesis and repair activity [3] [4]. Nonetheless, little is known about the effects of mechanical stretching on tenocyte migration behavior. Therefore, our main focus in this study is to examine and enhance the migration behaviour of tenocytes by the appliance of uniaxial mechanical stretch. Uniaxial mechanical stretching will be applied to tenocyte-seeded silicon membranes or alternatively collagen membranes. Understanding the tenocytes cellular mechanisms will improve our knowledge about tendon healing and will provide new approaches for specific treatments.

Reflection on boon and bane of Water Absorbing Components in Active Implants during Package Testing and Operation

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Internal components of active implants with high water absorbencies, like polymer components, are a blessing regarding package lifetime during operation of those implants. They capture intruding water and thus serve as reversible getters and reduce the internal relative humidity. Consequently, the formation of liquid water, leading to corrosion and subsequent failure, is decelerated. During accelerated aging tests of the hermeticity of implant packages, however, water absorbing sensory elements are rather a curse. Their presence reduces the internal humidity and thereby increases the acquired mean time to failure (MTTF). The influence of the water absorbing capacities of these sensory components can be so significant, that the predicted lifetime of the implant package is erroneously exceeded by orders of magnitude in comparison to an empty gas-filled package. And mostly, the sensory elements are not present in the final implant setup due to the limited construction space of active implants.

This paper discusses the phenomena during water intrusion into microsystem packages and the influences of different internal water absorbing elements. For the purpose of illustration, a resistor-capacitor model analogous to electronic components is used. The findings are practically applied to a measurement setup used to investigate packages for an active lens implant, the Artificial Accommodation System. Thereby, the internal water absorption capacities are estimated for both, the measurement setup, including a capacitive humidity sensor and a transducer coil for leakage detection during accelerated aging, and the final implant setup, comprising polymer elements like lens components or casting. As package characterization criterion regarding hermeticity, the permeation resistance is applied, describing the reciprocal of the water permeation rate. Thereby, the evaluation is independent of the measurement setup and of a pre-defined failure criterion. The acquisition of the permeation resistance in combination with the knowledge of the water absorption capacities of the measurement components and of the final implant components allows not only for the correction of the measurement error caused by internal water absorption during the measurement, but also for the lifetime prediction of the final implant package.