Biodegradable poly (lactic acid-co-glycolic acid) scaffolds as carriers for genetically-modified fibroblasts

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Abstract

Recent advances in gene delivery into cells allow improved therapeutic effects in gene therapy trials. To increase the bioavailability of applied cells, it is of great interest that transfected cells remain at the application site and systemic spread is minimized. In this study, we tested clinically used biodegradable poly(lactic acid-co-glycolic acid) (PLGA) scaffolds (Vicryl & Ethisorb) as transient carriers for genetically modified cells. To this aim, we used human fibroblasts and examined attachment and proliferation of untransfected cells on the scaffolds in vitro, as well as the mechanical properties of the scaffolds at four time points (1, 3, 6 and 9 days) of cultivation. Furthermore, the adherence of cells transfected with green fluorescent protein (GFP) and vascular endothelial growth factor (VEGF165) and also VEGF165 protein secretion were investigated. Our results show that human fibroblasts adhere on both types of PLGA scaffolds. However, proliferation and transgene expression capacity were higher on Ethisorb scaffolds most probably due to a different architecture of the scaffold. Additionally, cultivation of the cells on the scaffolds did not alter their biomechanical properties. The results of this investigation could be potentially exploited in therapeutic regiments with areal delivery of transiently transfected cells and may open the way for a variety of applications of cell-based gene therapy, tissue engineering and regenerative medicine.
Introduction

New generation non-viral gene delivery systems, such as nanoparticles, novel cationic lipids and polymers, chemically coupling the nucleic acid to peptides and polymers [1–9] have shown to overcome some of the limitations of viral gene transfer and offer advantages in use of recombinant proteins [10, 11]. On the other side, the localized delivery of genetically modified cells themselves poses another major barrier for efficient and area-specific therapeutic protein expression in gene therapy applications. Challenges regarding the reduction of off-target effects remain to be addressed [12]. Thus, biodegradable synthetic polymer scaffolds may offer a safe and effective option for targeted cell delivery.

Biodegradable scaffolds are routinely used as reinforcement material for various surgical procedures, including hernia repair [13], tendon reconstruction [14], cranio-maxillo-facial surgery or neurosurgery [15–18]. Synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA) and their copolymer poly(lactic acid-co-glycolic acid) (PLGA) are also highly useful for the temporary management of pathologically altered tissue architectures including ligaments, skin, vascular tissues and skeletal muscle [19]. The implanted scaffolds not only provide structural support but also guide new tissue ingrowth and, importantly, are completely absorbed by the body without the need for subsequent surgical removal. As an alternative to fully synthetic polymer-based scaffolds, decellularized biological matrix material of xenogenic or allogenic origin can be used [20]. Biological scaffolds exhibit a tensile strength comparable to that of PLGA-based scaffolds and show superior collagen deposition and organisation [21]. Still, xenografts and allografts have serious constrains due to limited availability, residual α-Gal-mediated immunogenicity [22], as well as the risk of transmission of animal- or human-derived infectious agents [23]. Biodegradability of the matrix material is a crucial issue in the development of tissue engineering scaffold structures. By utilizing easy manufactured off-the-shelf synthetic absorbable polymers, complications associated with antigenicity and disease transmission can be eliminated.

Another important factor in scaffold-aided tissue regeneration is that this process is critically dependant on efficient vascularization [24–28]. Moreover, local delivery of recombinant angiogenic growth factors from scaffold materials presents a promising strategy in promoting formation of a functional blood vessel network within the regenerated tissue [29, 30]. However, the short half-life of the recombinant proteins hinders the unleashing of their full angiogenic potential. Given the essential role in reparation of damaged tissue, fibroblasts are particularly suitable as cellular vehicles for delivery of angiogenic growth factors in tissue engineering [31] as well as for gene therapy applications [32]. Thus, in this study, we applied an expression vector for vascular endothelial growth factor (VEGF165) as a model with which we studied the utility of PLGA-based scaffolds as carriers for genetically modified cells. We used an established transfection procedure on human Hs27 fibroblasts and tested their proliferation and morphology on two PLGA-based scaffolds Vicryl and Ethisorb. The rationale behind using these scaffolds is that they are already established in clinical use and PLGA has been shown to provide excellent conditions for attachment, growth and motility of human skin fibroblasts [33]. Furthermore, we investigated mechanical properties of the scaffolds seeded with cells over the short time frame of 9 days. Finally, Vicryl and Ethisorb were compared in terms of their ability to serve as adhesion stratum for VEGF165-overexpressing Hs27 cells and production of angiogenic VEGF165 protein.

Materials and methods

Cells and Vicryl and Ethisorb scaffolds

Human fibroblast cell line Hs27 (ATCC; CRL-1634) was cultured under standard condition (37°C, 5% CO₂) in Dulbecco’s Modified Eagle Medium (DMEM) containing phenol red, stable
glutamine and 4 g glucose/l (PAA, Coelbe, Germany). The culture medium was supplemented with 10% fetal calf serum (FCS: heat inactivated FCS-Gold, PAA, Coelbe, Germany) and antibiotic/antimycotic solution (AB/AM; PAA, Coelbe, Germany). DMEM containing FCS and AB/AM solution is referred herein as growth medium. For this study, two types of absorbable surgical scaffolds were used: Vicryl (VM 802) and Ethisorb (Patch Typ 6; ZVP 203) scaffold (Ethicon, Johnson & Johnson Medical, GmbH, Norderstedt, Germany). The Vicryl scaffold consists of polyglactin 910, which is a co-polymer of 90% glycolide and 10% L-lactide. Ethisorb is also a polyglactin 910-based implantable scaffold and it consists mainly of polyglactin 910 and a small proportion of poly-P-dioxanon. The resorption time for polyglactin 910 is 45–60 days and for poly-P-dioxanon 90–180 days [34].

Cell growth on Vicryl and Ethisorb scaffolds

For both types of scaffolds, a cell suspension of 5 x 10^5 Hs27 cells in 4 ml growth medium was pipetted in 4-well rectangle plates (Thermo Fisher Scientific, Waltham, MA, USA). Vicryl and Ethisorb patches were immediately placed inside. The scaffolds were incubated overnight in a cell incubator under standard conditions on a horizontal shaker (Duomax 1030, Heidolph, Germany). For controls, the scaffold was put in 4 ml growth medium without Hs27 cells and handled the same way as the scaffold seeded with human fibroblast cells. To remove Hs27 cells which did not attach to the scaffold, 16–24 hours after initial seeding, the scaffold with adherent cells was transferred in new 4-well rectangle plates filled with 4 ml fresh growth medium per well and further incubated under standard conditions. Proliferation rate, morphology, and distribution of attached Hs27 cells, as well as the mechanical properties of the scaffold populated with cells were examined 1, 3, 6 and 9 days after the seeding of cells.

Measurement of Hs27 cell proliferation activity on Vicryl and Ethisorb scaffolds

Cell proliferation activity was measured by ready-to-use colorimetric WST assay (Roche Molecular Biochemicals, Basel, Switzerland). For that, all specimens were transferred in a new rectangle 4-well plate with 3 ml fresh growth medium supplemented with WST solution and incubated for 2 hours at 37˚C. The assay principle is based upon the reduction of the tetrazolium salt WST to formazan by cellular dehydrogenases. The generation of the dark yellow coloured formazan is measured at 450 nm and is directly correlated to cell number. The measurement was performed according to the manufacturer’s instructions by using a microplate reader (Mithras LB 940, Berthold Technologies GmbH, Germany).

Visualisation of morphology of Hs27 cells grown on Vicryl and Ethisorb scaffolds

In order to investigate the cell morphology, scaffolds seeded with Hs27 cells were fixed either in 3% glutaraldehyde or in 3,7% formaldehyde. Two different methods for cell visualisation were applied: Giemsa staining and scanning electron microscopy (SEM). For Giemsa staining, formaldehyde-fixed specimens were stained for 10 min in a 1:20 pre-diluted Giemsa-solution (PAA, Coelbe, Germany) and rinsed 3 times in Ultra pure water. Photos were taken with a CCD-camera under the microscope (Nikon; Eclipse TE2000-S). For SEM, glutaraldehyde-fixed scaffolds were dehydrated with graded ethanol, dried and sputtered with gold (Baltec SCD005; 40 mA; 80 s). Control scaffolds were handled the same way as cell-seeded scaffolds. SEM was carried out using a Jeol-SEM-5400 (Eching) in Hi-Vac mode by applying an acceleration voltage of 5 kV and detecting secondary electrons for imaging.
Measurement of mechanical properties of cell-seeded and control Vicryl and Ethisorb scaffolds

The mechanical properties of Vicryl and Ethisorb scaffolds were tested during tensile testing. A universal test system (zwicki1120, Zwick/Roell, Ulm, Germany) was used, equipped with a 2.5 kN load cell (Type KAF-Z class 0.1, A.S.T., Dresden, Germany). The samples were clamped along their longer side in axial direction. To avoid shear stresses and torsional moments a passive x-y-M-compensation tool was inserted between the upper clamp and the load cell. A free length of $L_0 = 20$ mm was defined as an elongation of 0%. Subsequently, the sample was stretched at a constant speed of 10 mm/min until total failure of the sample. Tensile testing was stopped automatically by the test system after a force drop of 40% occurred, with respect to maximum force. Maximum force was determined from the highest force value of the force-displacement graph. All mechanical results were summarized as mean values (mean) and standard error of the mean (SEM).

Transfection of Hs27 cells with GFP and VEGF165 plasmids and cultivation on Vicryl and Ethisorb scaffolds

For transfection experiments, human fibroblasts were cultivated under standard conditions in growth medium in T175 flasks. Upon reaching confluence, $5 \times 10^6$ cells were transfected with 20 $\mu$g plasmid DNA coding for green fluorescent protein (GFP) provided in the fibroblast transfection kit (Lonza, Cologne, Germany). The transfection was performed by using Nucleofector transfection apparatus (Lonza, Cologne, Germany) and DT130 transfection program. After 16 hours cultivation, the cells were trypsinized, washed and transferred into tubes, which contained pre-cut Vicryl or Ethisorb scaffolds (1.5 cm x 1.5 cm). The tubes were gently rotated in an incubator for additional 16 hours. Subsequently, the scaffolds were taken out and carefully laid in another plate with fresh growth medium and further cultivated for a defined period (1, 3, 6 and 9 days) under standard conditions. By using the same protocol, human fibroblasts were transfected with 20 $\mu$g VEGF165 plasmid DNA [32, 35] and grown on Vicryl or Ethisorb scaffolds. After 1, 3, 6 and 9 days, the supernatant was collected, temporarily stored at -20˚C and used for subsequent measurements of VEGF165 protein concentration.

Detection of intracellular GFP expression and extracellular VEGF165 protein levels

The temporal expression of GFP protein in Hs27 cells was visualised by using the fluorescent microscope (Zeiss Axio Observer.A1, Software AxioVision). The level of VEGF165 protein secreted from Hs27 cells grown on Vicryl and Ethisorb scaffolds was determined by using Human VEGF Quantikine ELISA Kit (R&D Systems) according to the manufacturer’s instructions. The absorbance at 450 nm was measured with a microplate reader (Mithras LB 940, Berthold Technologies GmbH, Germany).

Statistical analysis

The data for the proliferation activity and tensile testing are from single experiments with five technical replicates. The statistical comparison was performed by two-way ANOVA with Tuckey’s post-hoc testing. ELISA experiment was performed three times with three technical replicates. The statistical testing was done by two-way ANOVA followed by Sidak’s multiple comparison tests. For all statistical tests, differences among means were considered significant when the p value was < 0.05.
Results

Proliferation of Hs27 cells on Vicryl and Ethisorb scaffolds

Hs27 cell proliferation could be observed on images of cell-seeded Vicryl and Ethisorb scaffolds captured with the light microscope in the course of 9 days (Fig 1). At day one, the cells were scarcely distributed on both types of scaffolds. After 9 days of cultivation, most of the surface of both scaffolds was covered with Hs27 cells. Furthermore, on the images obtained by light microscopy, it is visible that the textile structure of Vicryl scaffold is woven and contains small pores whereas that of Ethisorb is nonwoven. This implicates the difference in the surface area between Ethisorb and Vicryl scaffolds. In order to quantify the changes in cell number observed by the light microscope, the WST assay was applied. As seen on Fig 2, the increase in cell number is reflected by an increase in dehydrogenase activity over the time course of the experiment. When compared to Vicryl scaffolds, the Hs27 cells on Ethisorb scaffolds showed higher values at all time points, as measured by an increase in optical density of formazan dye at 450 nm, probably caused by the increased surface area available to the cells. The parallel increase of the absorbance in both groups over time reflects a similar rate of proliferation of the attached cells on both scaffolds.

Morphology of Hs27 cells on Vicryl and Ethisorb scaffolds

A suitable way to investigate the topography and morphology of rough surfaces is SEM. In this study, it was used for a close inspection of morphology of adherent cells and the physical state of the scaffold fibers. The SEM did not show any obvious signs of degradation of the polymer fibers of Vicryl and Ethisorb scaffolds (with or without attached Hs27 cells) after 9 days in culture (Fig 3). Furthermore, there was a nearly confluent layer of cells visible on both scaffolds at day 9. The cells grown on scaffolds have maintained their typical polygonal flattened shape. The SEM also demonstrated that the attached cells are predominantly located between the polymer fibers of the scaffolds, bridging the inter-fiber gaps with multiple cell protrusions.

Mechanical properties of Vicryl and Ethisorb scaffolds populated with and without Hs27 cells

Next, the mechanical strength of Vicryl and Ethisorb scaffolds (with and without attached Hs27 cells) was investigated using tensile testing (Fig 4A and 4B). The presence of cells did not affect the mechanical behaviour of the scaffolds for both Vicryl and Ethisorb at any time points investigated (Fig 4C). Furthermore, the mean values of the maximal tensile force for Vicryl scaffolds (with and without cells) only slightly decreased over the course of the 9 days. On the other side, the maximal tensile force of the Ethisorb scaffolds (with and without cells) dropped by almost 50% at day 9. Under the used experimental conditions, Ethisorb scaffolds had significantly higher tensile strength at days 1, 3 and 6 compared to Vicryl.

Transient GFP and VEGF165 expression in Hs27 cells grown on Vicryl and Ethisorb scaffolds

Finally, the difference in the level of GFP expressed in Hs27 cells and the VEGF165 protein secreted from Hs27 cells grown on Vicryl and Ethisorb scaffolds was investigated. By using the GFP expressing plasmid a general transfectability of the Hs27 cells was tested and whether they adhere on the scaffolds upon transfection. Both GFP and VEGF165 transfected cells successfully adhered on the surface of the scaffolds (Fig 5A and 5B). For both Vicryl and Ethisorb, the expression of GFP protein was the highest on day 1 and day 3 and then gradually decreased.
**Fig 1.** Visualisation of Hs27 cell growth on Vicryl and Ethisorb scaffolds. During the co-cultivation period of 9 days, Hs27 fibroblast cells on Vicryl (A) and Ethisorb (B) scaffolds were investigated at four different time points by means of light microscopy with 100-fold magnification. In the upper row of each panel, the scaffolds containing no cells (control) are presented. The fibroblasts were visualized with Giemsa staining and can be seen in the images of the bottom row of each panel as small blue dots.

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on day 6 and day 9 Similarly, the peak of VEGF165 protein expression from cells grown on Vicryl and Ethisorb scaffolds was reached on day 3 with gradual decrease in the course of next 6 days (Fig 6). At all investigated time points, the level of VEGF165 protein secretion from Hs27 cells grown on Ethisorb scaffolds was significantly higher than the level of VEGF165 protein secretion from cells grown on Vicryl scaffolds.

Discussion

Due to significant advances in recombinant DNA technology, cell-based gene therapy is increasingly recognized as an ideal tool to deliver therapeutic proteins to target sites. A typical way of administering genetically modified cells is the direct injection of cells in target areas. However, this mode of administration is accompanied by the spread away from the injection site. The spreading of transfected cells can reduce vector levels at the target site and also lead to low-grade systemic spread in off-target regions [35]. There are different strategies in addressing those concerns. For example, in adoptive T cell therapy, new generation of chimeric
Fig 3. Analysis of scaffold fibers and Hs27 cell morphology by scanning electron microscopy. Hs27 fibroblasts are grown on Vicryl and Ethisorb scaffolds and visualized at day 1, day 3, day 6, and day 9 in culture by means of scanning electron microscopy (Scale bars in the upon two rows in A and B are 100 μm, in the lowest row are 25 μm)

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antigen receptors called TRUCKs (T cells redirected for universal cytokine killing) are used as vehicles to produce and release a transgenic product that accumulates in the targeted tissue [36] and this strategy can potentially yield effective and localized therapeutic effects. Another approach in targeted cancer gene therapy of solid tissues is based on local delivery of NK4.
(hepatocyte growth factor antagonist) secreted from an NK4 gene-transduced oral mucosal epithelial cell [37]. A similar technique by using cellular vehicle for delivery of bone morphogenetic protein-2 has been used to promote bone regeneration [38]. Thus, genetically transfected cells can be used for delivery of therapeutic cells to the intended area in the body.

In general, growing living cells on scaffolds for tissue engineering purposes has been shown to offer significant clinical benefits [39, 40]. The cell-material interaction is very complex and seeded cells respond differently to specific surface chemistry and various architectural parameters [41]. In particular, the choice of scaffold material determines cell adhesion, which has direct effects on cell behaviour including morphology and proliferation. Unchanged cellular phenotype is an important aspect of biocompatibility. The cells show scaffold preferences and, thus, the suitability of the material must be validated beforehand [42, 43]. The results of our study indicate that Hs27 cells grow well on both investigated surgical scaffolds and show apparently unchanged and typical fibroblast morphology. As for the proliferation rate, our results demonstrate that attached Hs27 cells proliferate healthily on both investigated materials. This is indicated by microscopical evaluation as well as by quantification of the metabolic rate of the cells. To measure metabolism, we used a WST-1 test. Similar to the MTT method, the WST-1 system is based on the conversion of tetrazolium to formazan. Another frequently used system to study cell metabolism is based on the resazurin/resorufin conversion (e.g. Alamar blue, CellTiterblue). However, as these systems measure fluorescence instead of absorbance they tend to be influenced by fluorescent parts of the studied system and therefore we preferred WST-1 in this case [44]. In our study, although we started with the same amount of cells on all scaffolds, there were eventually more cells on Ethisorb scaffolds than on Vicryl. In
contrast to Vicryl, which has a woven scaffold-structure with small pores, Ethisorb consists of nonwoven fibres resembling a fleece. This implicates that Ethisorb provides a higher surface area compared to Vicryl allowing a greater number of cells to colonize and expand and, thus, likely explains their greater metabolic activity on Ethisorb scaffolds. Another interesting phenomenon we observed was that cells spread on the entire surface of the scaffolds and at the later time points start to grow between the filaments of the respective materials. Due to the manufacturing process of the materials there are relatively large pores (ca. 300 μm) between the respective cords of the Vicryl mesh, while the surface of Ethisorb mesh is dense. It seems that this leads to a higher area for cell growth on Ethisorb, which may also explain the higher VEGF expression with the Ethisorb mesh. Considering the restricted availability of autologous fibroblasts for clinical use, the pore size of engineered scaffolds could therefore pose one of the limiting factors for gene therapy-based soft tissue reconstructions.

Apart from the influence of the scaffold material on growth, proliferation and differentiation of seeded cells, mechanical and degradation properties of biodegradable scaffolds may be
affected by the cells [16, 45–48]. Surgical scaffold materials and tissue engineering constructs must, first of all, provide sufficient mechanical support at the defect site. Ideally, the mechanical properties of the scaffold material should not be affected by the cells which are, in a pre-implantation phase, grown on their surface. Therefore, in this study, we tested whether Hs27 cells influence the mechanical behaviour of Vicryl and Ethisorb scaffolds in the course of the experiment. Of note, under our experimental conditions, fibroblasts were cultured as a monolayer without adding extracellular matrix components. The results presented here show that attached cells did neither lead to additional deterioration of the used scaffolds after 9 days nor to protection of the fibers from hydrolytic degradation. Interestingly, Ethisorb lost mechanical resistance much faster than Vicryl, although its components (much smaller pore size) should theoretically degrade slower than the components of Vicryl [34].

The ability of cell therapy using genetically modified cells to deliver a bioactive protein at the target site circumvents the limitations associated with conventional protein delivery strategies. In this study, we made use of a well-established transfection method to generate VEGF-F165-overexpressing fibroblast, which colonized efficiently the surface of Vicryl and Ethisorb scaffolds. VEGF is a well-established therapeutic protein for enhancing angiogenesis both in vitro and in vivo. Our and many other groups have previously shown that secreted VEGF can enhance cell proliferation, wound healing and angiogenesis in different pre-clinical models [24, 49, 50]. In present study, our main focus was not on the effect of VEGF on proliferation of cells on scaffolds but rather if scaffolds can be used as carriers for cells which are genetically engineered to secrete VEGF or other therapeutic proteins. The VEGF165 protein expression profiles were similar between the investigated scaffolds showing a gradual decrease in protein levels. The Hs27 cells grown on Ethisorb expressed higher levels of VEGF165 protein, probably due to greater surface-to-volume ratio of Ethisorb scaffolds and higher number of attached cells. In this study, Hs27 were transiently transfected with a VEGF165 expression plasmid. This short-termed expression of VEGF165 protein is thought to stimulate biomaterial vascularisation without negative consequences of prolonged angiogenic stimulation. Our results show that the level of VEGF165 expression of Hs27 cells grown on scaffolds reaches the therapeutic levels as demonstrated in the ischemic hind limb animal model [32]. There are concerns about a possible oncogenic potential of genetically modified cells. These are mainly based on applications with stable viral transfection, because this can induce oncogenic mutations through random integration [51]. To avoid this problem, we have used here a plasmid vector approach, which has an excellent safety profile, because the plasmid is not expected to be integrated into the host genome and therefore the risk is much lower.

Although these in vitro findings present an important step toward construction of bioactive PLGA-based gene delivery cell carriers, further investigations with different therapeutic proteins are needed to determine their clinical utility. Moreover, although it is important to test the in vitro methods in vivo, we think it is not necessary to perform the in vivo study for the current in vitro method. First, several former studies including ours have already proven the use of VEGF as a therapeutic protein for improving angiogenesis and wound healing both in vitro and in vivo [24, 48, 49]. Moreover, we have published a pre-clinical study regarding the genetic-modified fibroblasts expression angiogenic factors including VEGF. In this work we actually applied the same transfection method with fibroblasts and VEGF plasmid as in the current one. We found that genetically modified fibroblasts can enhance angiogenesis and arteriogenesis in a hindlimb ischemia model [32]. This study was a proof-of-concept for our transfection method and in vivo application. Also, both PLGA-based meshes used in our study are clinical level products used for decades. Thus, our current study is mainly focused on the evaluation of a possible carrier material for such applications. We show here that the cells not only are able to survive and proliferate on the scaffolds but more importantly also produce the desired protein.
In summary, in the present study, we show that human fibroblasts seeded on biodegradable Vicryl and Ethisorb scaffolds show excellent biocompatibility. Furthermore, this model system allows successful genetic modification of the cells. The presented methodology could be easily adapted for other proteins and growth factors allowing a broader use of this gene-enhanced engineering technology.

Conclusions
Bioresorbable PLGA scaffolds can be used as vehicle for the delivery of transiently transfected cells and may open the way for a variety of applications of gene therapy, tissue engineering and regenerative medicine. Scaffolds with a condensed structure and smaller pore size might lead to a better cell-scaffold interaction and thus lead to a higher yield of the desired recombinant therapeutic proteins.

Supporting information
S1 Data. Raw data of mechanical property test. (XLSX)
S2 Data. Raw data of cell proliferation test with WST-1. (XLSX)

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References

1. Guo X, Huang L. Recent advances in nonviral vectors for gene delivery. Acc Chem Res. 2012; 45:971–9. https://doi.org/10.1021/ar200151m PMID: 21870813

2. Conde J, Bao C, Tan Y, Cui D, Edelman ER, Azevedo HS, et al. Dual targeted immunotherapy via in vivo delivery of biohybrid RNAi-peptide nanoparticles to tumour-associated macrophages and cancer cells. Advanced functional materials. 2015; 25(27):4183–94. https://doi.org/10.1002/adfm.201501283 PMID: 27340392

3. Conde J, Oliva N, Attilano M, Song HS, Artzi N. Self-assembled RNA-triple-helix hydrogel scaffold for microRNA modulation in the tumour microenvironment. Nature materials. 2016; 15(3):353–63. https://doi.org/10.1038/nmat4497 PMID: 26641016

4. Conde J, Oliva N, Zhang Y, Artzi N. Local triple-combination therapy results in tumour regression and prevents recurrence in a colon cancer model. Nature materials. 2016; 15(10):1128–38. https://doi.org/10.1038/nmat4707 PMID: 27454043

5. Dahlman JE, Barnes C, Khan OF, Thiriot A, Jhunjunwala S, Shaw TE, et al. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. Nature nanotechnology. 2014; 9(8):648–55. https://doi.org/10.1038/nnano.2014.84 PMID: 24813696

6. Dong Y, Dorkin JR, Wang W, Chang PH, Webber MJ, Tang BC, et al. Poly(glycoamidoamine) Brushes Formulated Nanomaterials for Systemic siRNA and mRNA Delivery in Vivo. Nano letters. 2016; 16(2):842–8. https://doi.org/10.1021/acs.nanolett.5b02428 PMID: 26727632

7. Jensen SA, Day ES, Ko CH, Hurley LA, Luciano JP, Kouri FM, et al. Spherical nucleic acid nanoparticle conjugates as an RNAi-based therapy for glioblastoma. Science translational medicine. 2013; 5(209):209ra152. https://doi.org/10.1126/scitranslmed.3006839 PMID: 24174328

8. Lee JB, Hong J, Bonner DK, Poon Z, Hammond PT. Self-assembled RNA interference microsponges for efficient siRNA delivery. Nature materials. 2012; 11(4):316–22. https://doi.org/10.1038/nmat3253 PMID: 22367004

9. Stone GW, Barzee S, Snarsky V, Santucci C, Tran B, Langer R, et al. Nanoparticle-delivered multimeric soluble CD40L DNA combind with Toll-Like Receptor agonists as a treatment for melanoma. PloS one. 2009; 4(10):e7334. https://doi.org/10.1371/journal.pone.0007334 PMID: 19812695

10. Spiro AS, Beil FT, Schinke T, Schilling AF, Eulenburg C, Rueger JM, Amling M. Short-term application of dexamethasone enhances bone morphogenetic protein-7-induced ectopic bone formation in vivo. J Trauma. 2010; 69:1473–80. https://doi.org/10.1097/TA.0b013e3181dc59e4 PMID: 21150526

11. Spiro AS, Beil FT, Baranowsky A, Barvencik F, Schilling AF, Nguyen K, Khamdem S, Seitz S, Rueger JM, Schinke T, Amiling M. BMP-7-induced ectopic bone formation and fracture healing is impaired by systemic NSAID application in C57BL/6-mice. J Orthop Res. 2010; 28:785–91. https://doi.org/10.1002/jor.21044 PMID: 20063306

12. Jang JH, Koerber JT, Gujratty K, Bethi SR, Kane RS, Schaffer DV. Surface immobilization of hexa-histidine-tagged adeno-associated viral vectors for localized gene delivery. Gene Ther. 2010; 17:1384–9. https://doi.org/10.1038/gt.2010.81 PMID: 20508598

13. Bellows CF, Wheatley BM, Moroz K, Rosales SC, Morici LA. The effect of bacterial infection on the biomechanical properties of biological scaffold in a rat model. PloS ONE. 2011; 6:e21228. PMID: 21698179

14. Pendegrass CJ, Oddy MJ, Sundar S, Cannon SR, Godship AE, Blunn GW. The novel use of resorbable Vicryl scaffold for in vivo tendon reconstruction to a metal prostheses. J Bone Joint Surg Br. 2006; 88:1245–51. https://doi.org/10.1302/0301-620X.88B9.17748 PMID: 16943481

15. Buchel P, Rahal A, Seto I, Iizuka T. Reconstruction of orbital floor fracture with polyglactin 910/polydioxanone patch (ethisorb): a retrospective study. J Oral Maxillofac Surg. 2005; 63:646–50. https://doi.org/10.1016/j.joms.2004.11.013 PMID: 15883939

16. Barbolt TA, Odin M, Leger M, Kangas L, Hoiste J, Liu SH. Biocompatibility evaluation of dura mater substitutes in an animal model. Neurol Res. 2001; 23:813–20. https://doi.org/10.1179/0161641011019405 PMID: 11760872

17. Wu G, Liu Y, Iizuka T, Hunziker EB. The effect of a slow mode of BMP-2 delivery on the inflammatory response provoked by bone-defect-filling polymeric scaffolds. Biomaterials. 2010; 31:7485–93. https://doi.org/10.1016/j.biomaterials.2010.06.037 PMID: 20638718

18. Das D, Zhang Z, Winkler T, Mour M, Gunter C, Morlock M, Machens HG, Schilling AF. Bioreosorption and Degradation of Biomaterials. Adv Biochem Eng Biotechnol. 2012; 126:317–33. https://doi.org/10.1007/10_2011_119 PMID: 21975956

19. Dhandayuthapani B, Yoshida Y, Maekawa T, Kumar DS. Polymeric Scaffolds in Tissue Engineering Application: A Review. Int J Polym Sci 2011, Article ID 290602.
Newman MI. Comparison of Surgisis, AlloDerm, and Vicryl Woven Mesh grafts for abdominal wall hernia. Hernia. 2005; 9:252–8. https://doi.org/10.1007/s10029-005-0341-y PMID: 15912258

Wong ML, Griffiths LG. Immunoignecity in xenogeneic scaffold generation: antigen removal vs. decellularization. Acta Biomater. 2014; 10:1806–16. https://doi.org/10.1016/j.actbio.2014.01.026 PMID: 24486910

Zhang Z, Ito WD, Hopfner U, Bo¨hmert B, Kremer M, Reckhenrich AK, Harder Y, Lund N, Kruse C, Machens HG, Ega ¨nja JT. The role of single cell derived vascular resident endothelial progenitor cells in the enhancement of vascularization in scaffold-based skin regeneration. Biomaterials. 2011; 32:4109–17. https://doi.org/10.1016/j.biomaterials.2011.02.036 PMID: 21435711

Hadjipanayi E, Kuhn PH, Moog P, Bauer AT, Kuekrek H, Mirzoyan L, Hummel A, Kirchhoff K, Salgin B, Isenburg S, Dornseifer U, Ninkovic M, Machens HG, Schilling AF. The Fibrin Matrix Regulates Angiogenic Responses within the Hemostatic Microenvironment through Biochemical Control. PLoS ONE. 2015; 10:e0135618. https://doi.org/10.1371/journal.pone.0135618 PMID: 26317771

Hadjipanayi E, Schilling AF. Hypoxia-based strategies for angiogenic induction: the dawn of a new era for ischemia therapy and tissue regeneration. Organogenesis. 2013; 9:261–72. https://doi.org/10.4161/org.25970 PMID: 23974216

Hadjipanayi E, Bauer AT, Moog P, Salgin B, Kuekrek H, Fersch B, Hopfner U, Meissner T, Schlüter A, Ninkovic M, Machens HG, Schilling AF. Cell-free carrier system for localized delivery of peripheral blood cell-derived engineered factor signaling: towards development of a one-step device for autologous angiogenic therapy. J Control Release. 2013; 169:91–102. https://doi.org/10.1016/j.jconrel.2013.04.008 PMID: 23603614

Hadjipanayi E, Cheerna U, Hopfner U, Bauer A, Machens HG, Schilling AF. Injectable system for spatio-temporally controlled delivery of hypoxia-induced angiogenic signalling. J Control Release. 2012; 161:852–60. https://doi.org/10.1016/j.jconrel.2012.04.048 PMID: 22634070

Korenkov M, Yuecel N, Koebke J, Schierholz J, Morsczech Ch, Tasci I, Neugebauer EA, Nagelschmidt M. Local administration of TGF-beta1 to reinforce the anterior abdominal wall in a rat model of incisional hernia. Hernia. 2005; 9:252–6. https://doi.org/10.1007/s10029-005-0341-y PMID: 15912258

Pieper JS, Hafmans T, van Wachem PB, van Luyn MJ, Brouwer LA, Veerkamp JH, van Kuppevelt TH. Loading of collagen-heparan sulfate matrices with bFGF promotes angiogenesis and tissue generation in rats. J Biomed Mater Res. 2002; 62:185–94. https://doi.org/10.1002/jbm.10267 PMID: 12209308

Wong T, McGrath JA, Navsaria H. The role of fibroblasts in tissue engineering and regeneration. Br J Dermatol. 2007; 156:1149–55. https://doi.org/10.1111/j.1365-2133.2007.07914.x PMID: 17535219

Zhang Z, Slobodianski A, Ito WD, Arnold A, Nehlsen J, Weng S, Lund N, Liu J, Ega ¨nja JT, Lohmeyer JA, Müller DF, Machens HG. Enhanced collateral growth by double transplantation of gene-nucleofected fibroblasts in ischemic hindlimb of rats. PloS ONE 2011; 6:e19192. https://doi.org/10.1371/journal.pone.0019192 PMID: 21547081

Yang J, Shi G, Bei J, Wang S, Cao Y, Shang Q, Yang G, Wang W. Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic co-glycolic acid) (70/30) cell scaffolds for human skin fibroblasts cell culture. J Biomed Mater Res. 2002; 62:438–46. https://doi.org/10.1002/jbm.10318 PMID: 12209930

Seidl RO, Todt I, Ernst A. Rekonstruktion von traumatischen Schädeldbasisdefekten mit einem alloplastischen, resorbierbaren Vlies (Ethisorb) HNO. 2000; 48:753–757.

Perisic T, Arnold A, Nehlsen J, Zhang Z, Machens HG, Ito W, Slobodianski A. Biodistribution, pharmacokinetics and safety of plasmids encoding growth factor genes delivered by intramuscular cell transplantation in rats. Gene Ther Mol Biol. 2015; 17:42–61.

Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. Immunol Rev. 2014; 257:83–90. https://doi.org/10.1111/imr.12125 PMID: 24329791

Manabe T, Mizumoto K, Nagai E, Matsumoto K, Nakamura T, Nukiwa T, Tanaka M, Matsuda T. Cell-based protein delivery system for the inhibition of the growth of pancreatic cancer: NK4 gene-transduced oral mucosal epithelial cell sheet. Clin Cancer Res. 2003; 9:3158–66. PMID: 12912968

Laurencin CT, Attawia MA, Lu LQ, Borden MD, Lu HH, Gorum WJ, Lieberman JR. Poly(lactide-co-glycolide)/hydroxyapatite delivery of BMP-2-producing cells: a regional gene therapy approach to bone regeneration. Biomaterials. 2001; 22:1271–7. PMID: 11392999
39. Pape SA, Byrne PO. Safety and efficacy of TransCyte for the treatment of partial-thickness burns. J Burn Care Rehabil. 2000; 21:390. PMID: 10935823

40. Hibino N, McGillicuddy E, Matsumura G, Ichihara Y, Naito Y, Breuer C, Shinoka T. Late-term results of tissue-engineered vascular grafts in humans. J Thorac Cardiovasc Surg. 2010; 139:431–6. https://doi.org/10.1016/j.jtcvs.2009.09.057 PMID: 20106404

41. Chang H-I, Wang Y. Cell Responses to Surface and Architecture of Tissue Engineering Scaffolds, Regenerative Medicine and Tissue Engineering—Cells and Biomaterials, Prof. Daniel Eberli (Ed.), ISBN: 978-953-307-663-8, InTech.

42. Wünsch L, Ehlers EM, Russlies M. Matrix testing for urethral tissue engineering. Eur J Pediatr Surg. 2005; 15:164–9. https://doi.org/10.1055/s-2004-830356 PMID: 15999308

43. Wittenburg G, Flade V, Garbe AI, Lauer G, Labude D. Scaffold preferences of mesenchymal stromal cells and adipose-derived stem cells from green fluorescent protein transgenic mice influence the tissue engineering of bone. Br J Oral Maxillofac Surg. 2014; 52:409–14. https://doi.org/10.1016/j.bjoms.2014.02.023 PMID: 24685477

44. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell Viability Assays. In: Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, et al., editors. Assay Guidance Manual. Bethesda (MD) 2004.

45. Zhang Z, Egana JT, Reckhenrich AK, Schenck TL, Lohmeyer JA, Schantz JT, Machens HG, Schilling AF. Cell-based resorption assays for bone graft substitutes. Acta Biomater. 2012; 8:13–9. https://doi.org/10.1016/j.actbio.2011.09.020 PMID: 21971416

46. Winkler T, Hoenig E, Huber G, Janssen R, Fritsch D, Gildenhaar R, Berger G, Morlock MM, Schilling AF. Osteoclastic bioresorption of biomaterials: two- and three-dimensional imaging and quantification. Int J Artif Organs. 2010; 33:198–203. PMID: 20458689

47. Keller J, Brink S, Busse B, Schilling AF, Schinke T, Amling M, Lange T. Divergent resorbability and effects on osteoclast formation of commonly used bone substitutes in a human in vitro-assay. PLoS ONE 2012; 7:e46757. https://doi.org/10.1371/journal.pone.0046757 PMID: 23071629

48. Winkler T, Hoenig E, Gildenhaar R, Berger G, Fritsch D, Janssen R, Morlock MM, Schilling AF. Volumetric analysis of osteoclastic bioresorption of calcium phosphate ceramics with different solubilities. Acta Biomater. 2010; 6:4127–35. https://doi.org/10.1016/j.actbio.2010.04.015 PMID: 20451677

49. Lohmeyer JA, Liu F, Krüger S, Lindenmaier W, Siemens F, Machens HG. Use of gene-modified keratinocytes and fibroblasts to enhance regeneration in a full skin defect. Langenbecks Arch Surg. 2011 Apr; 396(4):543–50. https://doi.org/10.1007/s00423-011-0761-3 PMID: 21369846

50. Spanholtz TA, Theodorou P, Holzbach T, Wutzler S, Giunta RE, Machens HG. Vascular endothelial growth factor (VEGF165) plus basic fibroblast growth factor (bFGF) producing cells induce a mature and stable vascular network—a future therapy for ischemically challenged tissue. J Surg Res. 2011 Nov; 171(1):329–38 https://doi.org/10.1016/j.jss.2010.03.033 PMID: 20605609

51. Bushman FD. Retroviral integration and human gene therapy. J Clin Invest. 2007; 117:2083–2086. https://doi.org/10.1172/JCI32949 PMID: 17671645