K2

Design of bioactive materials for tissue regeneration

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Osteoconductive biomaterials such as calcium phosphate bioceramics have been widely used as scaffolds for bone tissue engineering. However, this kind of materials usually lacks osteoinductivity and is not able to stimulate osteogenic differentiation of stem cells. In addition, angiogenesis plays an important role in tissue regeneration and tissue engineering, and insufficient angiogenesis may result in failure of regeneration of large sized bone defect or reconstruction of bone tissue by tissue engineering approach. Therefore, it is meaningful to develop biomaterials which can enhance both osteogenesis and angiogenesis simultaneously. Previsous studies have shown that the chemical composition and nano-structure are two factors which could affect cell behavior and bone regeneration. In our recent studies, we have designed and fabricated calcium phosphate and silicate based bioactive ceramics and composites, and found that some silicate based bioceramics have the potential to stimulate osteogenesis, and this effect is dependent on the chemical composition of the materials. Furthermore, some of the silicate bioceramics even showed the activity to stimulate angiogenesis in vitro and in vivo. In addition, our studies also showed that the surface nano/micro-structure also affected osteogenesis and angiogenesis. Our results suggest that biomaterials with certain chemical composition and surface structure may be used to design bioactive scaffolds for tissue engineering applications.

V11

Sulfated Hyaluronan Derivatives Impair TGF-β1 Signalling due to Altered Receptor Complex Formation

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Introduction:

Sulfated glycosaminoglycans (GAGs) are multifunctional components of the extracellular matrix influencing cellular processes such as cell migration, proliferation and differentiation. Cell behaviour is either affected by direct interaction with cells or by binding of biological mediators (e.g. growth factors) and modulation of their bioactivity. Chemically sulfated hyaluronan (sHA) derivatives are promising candidates for functional biomaterials since they represent readily available, simplified mimetic structures of naturally sulfated GAGs with a defined sulfation degree and sulfate distribution. Previous studies revealed that differently sHA derivatives interact with TGF-β1 in a sulfation-dependent manner [1] and that the presence of sHA leads to impaired TGF-β1 signalling in *in vitro* cell culture experiments [2]. The purpose of this study was to reveal the influence of sHA on the formation of the TGF-β1:receptor signalling complex.

Materials and Methods:

The influence of sHA on the capability of TGF-β1 to bind to its receptors TGF-β receptor (TGFβR)-I and -II was investigated by surface plasmon resonance (SPR) using a BIACORE^{TM} T100 instrument. Pre-formed complexes of sHA and TGF-β1 were injected over TGFβR-I or TGFβR-II, immobilized on a Sensor Chip C1^{TM} surface. In another experimental setting TGF-β1 and sHA were injected sequentially over the immobilized TGFβR-II, followed by the injection of TGFβR-I as the last component, according to the natural order of binding events. This was supposed to reveal whether the entire TGF-β1:receptor signalling complex is able to form in the presence of sHA.

Results and Discussion / References:

Binding of TGF-β1 to TGFβR-I or TGFβR-II was impaired in a sulfation dependent manner when TGF-β1 was pre-incubated with sHA. However, the recruitment of TGFβR-I to the TGFβR-II/TGF-β1/sHA complex was enhanced compared to TGFβR-II/TGF-β1 when the components were sequentially injected.

In conjunction with *in vitro* cell culture findings [2] our data suggest, that the TGFβR-II/TGF-β1/sHA complex competes with TGFβR-II/TGF-β1 for TGFβR-I. The former probably leads to an inactive signalling complex and thereby impairs TGF-β1 signal transduction. These findings are of particular interest for the selection of sHA derivatives to be included in biomaterial engineering for improved tissue regeneration.

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[2] van der Smissen A *et al.*, Acta Biomater. 9:7775-7786, 2013

Acknowledgement

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V12

Multi-Component Artificial Extracellular Matrices Influence Cells Relevant to Wound Healing

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Introduction:

Collagen and glycosaminoglycans (GAGs) like hyaluronan (HA) are important components of the extracellular matrix (ECM) of skin and bone. The chemical sulfation of HA (sHA) leads to an increased interaction with growth factors relevant to regeneration of these tissues (e.g. BMP-4, TGF-ß1) [1, 2]. Collagen-based artificial ECMs (aECMs) containing high-sulfated HA were shown (i) to enhance the growth of human dermal fibroblasts (dFb) [3], (ii) to support the osteogenic differentiation of human mesenchymal stromal cells [4] and (iii) to inhibit osteoclast differentiation [5]. Combining low- and high-sulfated HA derivatives and collagen type I to engineer multi-component aECMs should lead to a better mimicry of the *in vivo* situation in comparison to aECMs with one GAG derivative. The purpose of this study was to develop these aECMs and to reveal their effects on dFb and osteoclast-like cells.

Materials and Methods:

aECMs were prepared by *in vitro* fibrillogenesis of collagen type I alone or in the presence of fluorescence-labeled low-sulfated (sHA1) and high-sulfated (sHA4) sHA derivatives as well as a combination of both. Fluorescence measurements, dimethylmethylen blue (DMMB), Lowry and ortho-phthaldialdehyde assays were used to characterize the composition and release behavior of the aECMs. The morphology was examined via AFM. The initial adhesion and proliferation of dFb as well as the viability and differentiation of osteoclast precursor-like RAW264.7 cells on aECMs was studied.

Results and Discussion / References:

The aECM composition was analyzed after *in vitro* fibrillogenesis. The highest sHA release occurs during the first hour of aECM incubation in PBS at 37°C, while at later time points the release is only marginal. The results of the fluorescence measurement are in line with these of the DMMB assay. About 90 - 95% of the applied collagen I is integrated into the aECMs and the content remains quite stable over time. The AFM images of the aECMs show a decrease in fibril diameter with increased GAG concentration and sulfation degree as already shown for collagen type II [4].

sHA-containing aECMs lead to an increased adhesion and proliferation of dFb compared to the collagen reference. The proliferation of dFb and the viability of RAW264.7 cells are affected differently by the single- and multi-component-aECMs. These results demonstrate the promising potential of multi-component aECMs to alter the cellular behavior in a more defined manner allowing the precise adjustment of cellular response.

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Acknowledgments

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V13

Nichtklassische Mineralisation von Kollagen

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Introduction:

Zwei wesentliche Funktionen sind dem Knochen inherent: er dient als Calciumspeicher und Lastträger. Die Entwicklung eines artifiziellen degradablen Biomaterials mit diesem Eigenschaftsprofil ist bisher nicht gelungen, weil die Aufbauprinzipien im natürlichen Knochen noch nicht ausreichend verstanden sind und daher durch artifizielles Material nachgeahmt werden können. Bekannt ist jedoch, dass sie der nichtklassischen Kristallisation folgen. In Gegenwart der gelösten strukturdirigierenden Komponenten Kollagen I bzw. Osteocalcin (OC) entstehen in vivo Octacalciumphosphatminerale (OCP), die die Fähigkeit haben, sich durch Wasserabspaltung in Hydroxylapatit (HAP) umzuwandeln und dadurch in der Lage sind, auch hinsichtlich der mechanischen Eigenschaften optimale Verbunde zu bilden. Im Rahmen dieses Beitrages soll gezeigt werden, welchen Einfluss beide organischen Komponenten auf den Kristallisationsprozess von OCP bzw. HAP in Abhängigkeit ihrer Konzentration und des pH-Wertes haben und die Frage beantworten, ob der Bildungsprozess reversibel ist. Die Beantwortung dieser Frage ist wesentlich, um auch den Prozess der Knochenheilung des systemisch erkrankten Knochens fördern zu können. Die nachfolgend beschriebenen Experimente fußen auf der Modellvorstellung des *in vivo* Mineralisationsprozesses *Polymer induced liquid precursor*-Prinzips (PILP). Diese sieht eine zunächst kristallisationshemmende Wirkung von nicht-kollagenen Proteinen wie OC und die anschließende Kristallisation von amorphen Calciumphosphatphasen an der Kollagenfibrille vor [1, 2].

Materials and Methods:

Untersuchungen an Knochenproben des humanen Femurkopfes sowie an *in vitro* hergestellten mineralisierten Fibrillen erfolgten mittels analytischer Transmissionselektronenmikroskopie. Die *in vitro* Mineralisation wurde sowohl an Tropokollagen (GfN) mit anschließender Firbillogenese, als auch an fibrilliertem Kollagen in Form von lyophilisierten Scaffolds vollzogen. Die Inkubation in simulierter Körperflüssigkeit (1fach und 0,1fach) sowie eine Vorinkubatin in 4,5 mM $CaCl_2$-Lösung und anschließende Zugabe von 2,1 mM Na_2HPO_4-Lösung erfolgten bei 37 °C über bis zu 21 Tage. Der Zusatz von OC zur Mineralisierungslösung, als auch die Zugabe zur Calcium-Lösung und anschließende tropfenweise Zugabe der Phosphatlösung zur Initiierung des PILP-Prozesses erfolgte zur anschließenden Inkubation der Kollagenscaffolds. Eine Charakterisierung der Proben wurde mittels Rasterelektronenmikroskop und elektronendispersive Röntgenspektroskopie durchgeführt.

Results and Discussion / References:

Fourier-transformierte HRTEM-Aufnahmen des kortikalen Knochens legen den Schluss nahe, dass auch die Bausteine der anorganischen HAP-Phase eine helikale Struktur aufweisen. Die nanometergroßen HAP-Kristallite sind durch den Prozess der Umwandlung von OCP zu defizitärem (metastabilen) HAP in der Lage, die Kollagenmoleküle in helikaler Form eng zu umschließen. Dies zeigen HRTM-Aufnahmen des gesunden Knochens. Inwieweit dieser Verbund auch bei artifiziellem Material erreicht werden kann, steht derzeit noch offen. Die bisher erzeugten Verbundmaterialien zeigen im Gegensatz zu nach klassischer Kristallisation entstandenen, die Fähigkeit zur Selbstorganisation. Sollte auch die Degradation dieses Materials strukturell und zeitlich umgekehrt proportional zum Aufbauprozess verlaufen, dafür sprechen erste Ergebnisse, dann hat dieses Verbundmaterial das Potenzial für eine neue Generation von Knochenersatzmaterialien.

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V14

Bioverträgliche hermetische Verkapselung für biomedizinische Sensoren

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Introduction:

Die Therapie von Stoffwechselkrankheiten, wie z.B. Diabetes mellitus, benötigt geeignete sensible Messsysteme. Diese sollten die verschiedenen metabolismus-relevanten Parameter (Blutzuckerspiegel, pH-Wert, CO_2-Konzentration) gleichzeitig und kontinuierlich erfassen können. Eine neue Herausforderung dabei ist ein implantierbares drahtloses Sensorarray- Mikrosystem bestehend aus mehreren Hydrogel-basierten piezoresistiven Sensoren, die eine Inline-Überwachung physiologischer Parameter in menschlicher Körperflüssigkeit ermöglichen.

Materials and Methods:

Die besonderen stimuli-responsiven Hydrogele ermöglichen eine Entwicklung von zuverlässigen Biosensoren, die zudem an zahlreiche Analyte angepasst werden können. Hierbei steht als Anwendung vor allem die Inline-Überwachung in der medizinischen Diagnostik im Mittelpunkt. [1, 2] Zusätzlich zu den Untersuchungen und der Modellierung der Quellkinetik sowie der Diffusionsprozesse in Hydrogelen wird die Biokompatibilität des entwickelten Mikrosystems in den Vordergrund gestellt. Die Entwicklung von Sensoren für den medizinischen Bereich erfordert hohe Ansprüche an deren Biokompatibilität. In Anbetracht der Verwendung im biomedizinischen Bereich ist die Medizinprodukte Norm DIN EN ISO 10993 für die Untersuchungen der Schichtkombinationen auf Ihre Bioverträglichkeit heranzuziehen. Hier kommt das Zellmodell für den subkutanen Einsatz mit geeigneten *In vitro* Methoden zum Nachweis der Biokompatibilität zum Einsatz. Für Elektronikanwendungen ist es zudem erforderlich die Beeinträchtigung des Einhausungsmaterials durch zahlreiche Umgebungseinflüsse und Parameter genauer zu untersuchen um einen lang anhaltenden Schutz des Messsystems zu gewährleisten.

Zur flexiblen Verkapselung des verwendeten Silizium-Chips wird Parylene C bei Raumtemperatur als eine dünne Schicht (ca. 5 µm) konform abgeschieden [3]. Die Abscheidung erfolgt im Vakuum aus der Gasphase. Aufgrund dieser gasförmigen Abscheidung erreicht und beschichtet Parylene auch Bereiche und Strukturen, welche mit flüssigkeitsbasierten Verfahren nur kaum erreichbar sind, wie z. B. scharfe Ränder und Spitzen. Das inerte, hydrophobe, optisch transparente, polymere Beschichtungsmaterial wird durch eine zusätzliche physikalische Adhäsion mit amphiphilen Copolymeren funktional ausgestattet.

Results and Discussion / References:

Die Beschichtung des Sensorarray-Messsystems mit Parylene C und unterschiedlichen amphiphilen Copolymeren verändert die Oberflächeneigenschaften. Die topographischen Veränderungen sowie die der Oberflächenspannung wurden ermittelt. Erste Untersuchungen zur Biokompatibilitätsprüfung weisen zunächst keine toxischen Eigenschaften der Copolymere auf. [4] Die Langzeittests der hermetischen Verkapselung des Sensorarray-Messsystems zeigen einen positiven Verlauf und ermöglichen somit eine Empfehlung des verwendeten Messsystems zur medizinisch relevanten Anwendung.

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V15

Ein modulares Perfusionsbioreaktorsystem für das Scale up im Tissue Engineering - Verteilung, Differenzierung und Sauerstoffverbrauch von Mesenchymalen Stroma Zellen in großdimensionierten Konstrukten

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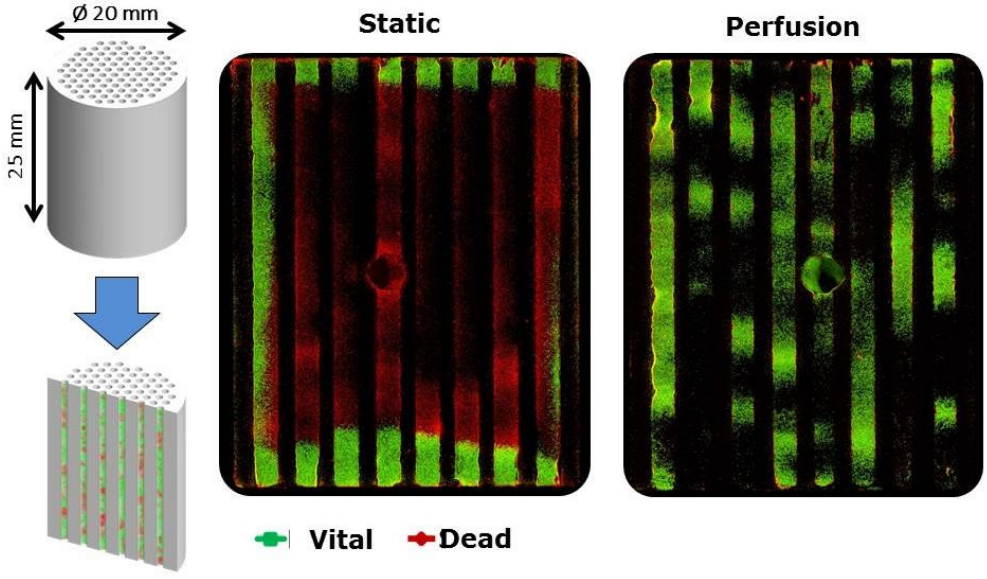
Introduction:

Knochendefekte als Folge von Tumoren, Infektionen oder Lockerung von Implantaten stellen eine große medizinische und sozioökonomische Herausforderung dar. Eine wichtige Voraussetzung ist in diesem Zusammenhang die dynamische Kultivierung von dreidimensionalen porösen zellbeladenen Konstrukten in einer klinisch relevanten Größe. Das Ziel dieser Studie war es, eine Perfusionsbioreaktorsystem für die dynamische Kultivierung von großdimensionierten Zell-Gerüst-Konstrukten zu entwickeln.



Materials and Methods:

Tricalciumphosphat Zylinder (20 mm x 25 mm) wurden mit 6,3 x 106 immortalisierten humanen mesenchymalen Stromazellen (MSCs) beladen und für 14 Tage in einem modular anpassbaren Perfusionsbioreaktorsystem mit integrierter Onlinesauerstoffmessung kultiviert. Unter statischen Bedingungen kultivierte Konstrukte dienten als Kontrolle. Zellverteilung und Vitalität in der zentralen Ebene wurden nach 1, 7 und 14 Tagen mit Hilfe der konfokalen Lasermikroskopie untersucht. Zusätzlich wurde das Zellwachstum anhand der Aktivität der Lactat-Dehydrogenase beurteilt. Die Sauerstoffkonzentration wurde online in der Mitte des Gerüstes und am Ein-und Auslass der Bioreaktorkammer überwacht.

Results and Discussion / References:

Nach 7 Tagen konnten keine signifikanten Unterschiede in Bezug auf die Zellproliferation zwischen statischer Kultivierung und Perfusionskultur beobachtet werden. Nach 14 Tagen zeigten die statisch kultivierten Konstrukte großen nekrotischen Zonen in der Mitte. Gleichzeitig konnte in den statisch kultivierten Proben ein kritischer Abfall der Sauerstoffkonzentration beobachtet werden. Im Gegensatz dazu blieb der Sauerstoffgehalt innerhalb der durchströmten Konstrukte während der gesamten Kultivierungsdauer konstant. Damit blieb die Vitalität der Zellen in der Mitte der dynamisch kultivierten Konstrukte erhalten.

Die Ergebnisse verdeutlichen, wie essentiell die dynamische Kultivierung für die Herstellung von großdimensionierten, mit Zellen beladenen Konstrukten und somit für das Scale up im Bereich des Tissue Engineering ist.

V16

Glycosaminoglycan Sulfation of artificial Extracellular Matrix Coatings enhances Regenerative Potential of Bone Cells

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Introduction:

In light of prolonged life expectancy, the need for biomaterials that govern bone regeneration increases. Improved bone regeneration and osseointegration can be achieved by functionalizing implant materials. The extracellular matrix (ECM) affects differentiation of bone cells and is critical for bone regeneration. Here we assessed the role of the natural occurring bone ECM glycosaminoglycans (GAGs), hyaluronan (HA) and chondroitin sulfate (CS), and their sulfated derivatives. In particular, their effects on differentiation and crosstalk of bone-resorbing osteoclasts, bone-forming osteoblasts and regulatory osteocytes involved in bone remodeling processes were assessed and evaluated for potential implant functionalization.

Materials and Methods:

The impact of native and sulfate-modified GAGs on viability, morphology, differentiation, gene expression and cell signaling was studied using murine bone marrow monocytes, mesenchymal stromal cells and the MLO-Y4/UMR-106 cell lines as a model for osteoclasts, osteoblasts, and osteocytes, respectively. Cells were seeded on culture plates coated with artificial ECMs containing rat collagen type I (Coll)/HA, Coll/CS or Coll/high-sulfated GAGs (sHA3, sCS3). Coatings with Coll alone served as control. Using surface plasmon resonance and a Wnt reporter assay, direct interactions of GAGs with the regulatory proteins in bone, receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and sclerostin (SOST) were evaluated.

Results and Discussion / References:

In response to native and high-sulfated GAGs profound effects on all stages of osteoclast differentiation were observed. GAG sulfate modification increased the viability of osteoclast precursors by 20% ($p<0.05$). However, tartrate resistant acid phosphatase (TRAP)-staining and immunofluorescence of regular sealing zone structures in osteoclasts were profoundly decreased up to 80% ($p<0.05$). This was accompanied by a loss of resorptive activity up to 40% compared to cells exposed to native GAG ($p<0.01$) and decreased mRNA levels of osteoclastic marker genes, such as cathepsin K, osteoclast-associated receptor and TRAP ($p<0.05$). On the other hand, the proliferation and metabolic activity of osteoblasts and osteocyte-like cells treated with GAGs was decreased by up to 40% ($p<0.05$) indicating a shift from the proliferative to matrix formation phase of osteogenic differentiation. Indeed, these cells showed an up to four-fold increased matrix deposition and up to 27-fold increased expression of gene products associated with differentiation, such as the RANKL/OPG ratio, alkaline phosphatase, osteocalcin, Runx2 and decreased SOST expression levels ($p<0.05$). Correspondingly, supernatants collected from these cells profoundly suppressed osteoclastogenesis ($p<0.05$) but did not affect their adhesion and viability. Using surface plasmon resonance, we demonstrated that GAGs can directly bind to OPG, but not RANKL, in a sulfation degree dependent manner resulting in modified OPG bioactivity. Similarly, sclerostin bioactivity was down-regulated when incubated with sulfated GAGs.

Conclusion: In summary, sulfation of GAGs alter the bone cell cross talk by increasing osteogenesis and reducing osteoclastogenesis. Therefore GAG sulfation may represent a useful tool to control osteoclastic activity and prevent bone loss adjacent to implant surfaces.

Acknowledgments:

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V17

Anbindung von Polyglycerol-Schichten auf technischen Oberflächen

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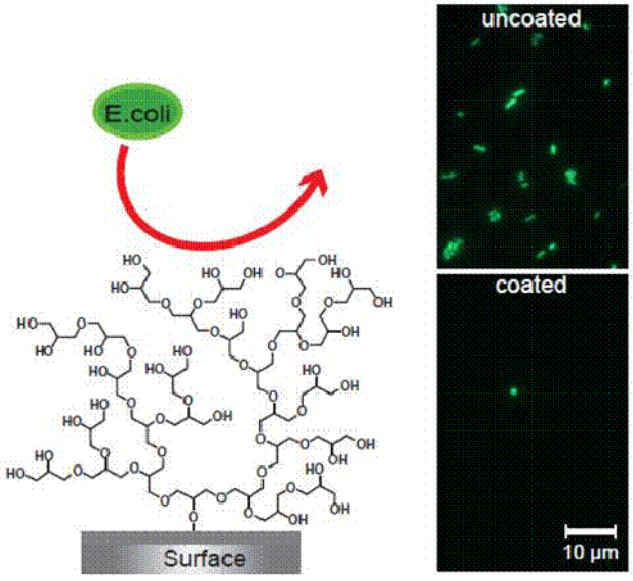
Introduction:
Monoschichten, die Polyglycerol (PG)-Kopfgruppen tragen, unterdrücken die Adsorption von Proteinen und Bakterien ebenso effizient wie die weit verbreiteten Oligoethylenglycol SAMs[1]. Im Gegensatz zu Letzteren sind PG Beschichtungen stabil gegenüber Umwelteinflüssen, Autooxidation und *in vivo* Verdauungsprozessen[2]. Zudem können Oberflächen mit PG-Beschichtungen in Standardprozessen hitzesterilisiert werden, da die PG-Systeme stabil gegenüber thermischen Zersetzungsprozessen sind. Dies und die hervorragende Biokompatibilität machen PG-Beschichtungen attraktiv für vielfältige biomedizinische Anwendungen.
Während in bereits etablierten Standardverfahren hyperverzweigte Polyglycerole über geeignete Ankergruppen auf Oberflächen angebracht werden, zeigen wir ein Verfahren zur direkten Anbindung auf technisch relevanten Oberflächen wie Stahl, Aluminium und Glas. Dafür wird die natürliche Oxidschicht der Materialien als Initiator für die anionische Ringöffnungs-Polymerisation von Glycidol[3] verwendet.



Materials and Methods:
Während in bereits etablierten Standardverfahren hyperverzweigte Polyglycerole über geeignete Ankergruppen auf Oberflächen angebracht werden, zeigen wir ein Verfahren zur direkten Anbindung auf technisch relevanten Oberflächen wie Stahl, Aluminium und Glas. Dafür wird die natürliche Oxidschicht der Materialien als Initiator für die anionische Ringöffnungs-Polymerisation von Glycidol[3] verwendet. Die Oberflächen wurden vor ihrer Verwendung mittels Plasma gereinigt, weitere Vorbereitungsschritte oder Auxiliare sind nicht nötig.
Die Schichten konnten mittels Oberflächen-IR, Ellipsometrie und Kontaktwinkel-Goniometrie charakterisiert werden. Das Verhältnis zwischen der Beschichtungsdicke und der Repulsivität gegenüber verschiedenen Proteinen (Albumine, g-Globulin und Fibrinogen) wurde ermittelt. Substrate, die die Adsorption von Proteinen vollständig unterdrücken, wurden auf ihre Resistenz gegenüber der Adsorption von Bakterien getestet.[4]

Results and Discussion / References:
Auf allen verwendeten Materialien konnten PG Beschichtungen erzeugt werden, die die Adsorption von Proteinen deutlich reduzieren. Zudem zeigten erste Messungen eine nahezu vollständige Unterdrückung der Bakterienadsorption. Dies zeigt, dass die Anwendung von Polyglycerol als inerte Beschichtungen in medizinischen Bereichen möglich ist.

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V18

Tissue engineering of Skeletal muscle on parallel aligned electrospun PCL/collagen fibers *in vitro* and *in vivo*

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Introduction:

Tissue engineering (TE) of skeletal muscle is a promising approach to treat tissue defects resulting from a critical loss of skeletal muscle. The three main issues currently investigated in skeletal muscle TE are: 1. the need for vascularization methods which make the transplantation of the newly engineered tissue feasible. 2. Harvesting enough muscle progenitor cells for the formation of grafts of relevant size. 3. Construction of scaffolds which can be infiltrated by myogenic cells and promote their parallel alignment. To address these issues in our study we co-cultivate and differentiate mesenchymal stem cells (MSCs) with myoblasts to myogenic cells. This is done on scaffolds consisting of parallel aligned PCL/collagen nanofibers interspersed with aligned sacrificial PEO fibers for added porosity. Precultivated scaffolds will later be implanted into an arteriovenous rat loop model for axial vascularization *in vivo*.

Materials and Methods:

PCL/collagen blend-fibers of different mixing ratios were electrospun onto a rotating drum consisting of metal bars, which enforces parallel alignment of the fibers via electrostatic and mechanical forces. Meltspun sacrificial PEO fibers were integrated into the scaffold throughout the production process and leached out afterwards to increase the porosity of the scaffold. MSCs where differentiated into myogenic cells by co-cultivation with myoblasts and addition of an optimized mixture of HGF and IGF-1. Differentiation and proliferation of the cells on the scaffolds as well as infiltration of the cells into the scaffolds were analyzed.

Results and Discussion / References:

A higher ratio of collagen in the blend fibers resulted in better adhesion and proliferation of myogenic cells, while a higher ratio of PCL resulted in better spinnability and structural integrity of the fibers. Fibers were aligned with a median deviation of 5° from absolute parallelism. The alignment of the nanofibers promoted a corresponding alignment and myotube formation of seeded cells. A mixture of 10 ng/ml IGF-1 and 60 ng/ml HGF proved to be the optimal ratio of growth factors for the myogenic differentiation of MSCs. By integrating methods from material science, biology and microsurgery we want to establish a comprehensive process for the *de novo* construction of engineered skeletal muscle tissue.

V19

Osteogenic differentiation of human mesenchymal stem cells on electrospun PCL scaffolds via dynamic compression

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Introduction:

Osteogenic and osteochondral defects such as osteoporosis, osteochondrosis dissecans and bone fractures still remain a challenge for orthopedic surgery because of lack of healthy bone substance. According to the current gold standard missing bone tissue is augmented with autologous cancellous bone grafts from the crista iliaca. This involves a second operation field with associated donor site morbidity. The use of allogeneic spongiosa is less favoured, because unlike autologous cancellous bone, allogenic bone is depleted of live cells due to the risk of graft versus host disease. Here, we developed a human mesenchymal stem cell (MSC)-based bone substitute using electrospun poly--caprolactone (PCL) scaffolds within a mechanoreactor, which induces osteogenic differentiation by dynamic compression without addition of any other inductors.

Materials and Methods:

We used electrospun PCL scaffolds that are biodegradable within 9 month rendering further operations and its risks unnecessary. Moreover, PCL is very robust and can stand the high mechanical forces in the human skeletal system. PCL is already established for clinical use in human medicine.

Human MSC were seeded on PCL scaffolds and on tissue culture polystyrene (TCPS) as a control for 21 days. Scaffolds treated in the bioreactor system undergo dynamic compression with 0.8N (3500 Pa) for four hours a day. They were compared to scaffolds without mechanical stimulation. Furthermore, MSC on scaffolds and on TCPS were cultured either in osteogenic induction medium containing DMEM low glucose, FCS, LGPS, ascorbic acid, dexamethasone and ß-glycerophosphate or in stem cell expansion medium for both, dynamic and static conditions. Cytotoxicity of scaffolds and the bioreactor system for human MSC was analysed by live/dead staining. Osteogenic differentiation was analysed using real time RT-PCR, scanning electron microscopy with EDX analysis, alkaline phosphatase assay, Alizarin red and van Kossa staining.

Results and Discussion / References:

PCL scaffolds were not cytotoxic towards human MSC. Cells underwent osteogenic differentiation during the 21-day-culture period in the mechanoreactor even without osteogenic induction media. Osteogenic differentiation was scored by real time RT-PCR, scanning electron microscopy and EDX analysis as well as alizarin red staining, van Kossa staining and alkaline phosphatase assay.

A bioreactor with mechanostimulation inducing osteogenic differentiation by dynamic compression was developed. Next, we will study the molecular mechanism transducing mechanostimulation to osteogenic differentiation. Therefore, we started a first trial with MEK-inhibitor U0126. To conclude, we established a bioreactor setting to produce allogeneic MSC-based PCL-grafts as bone substitutes, which has no immunogenic potency and can be individually modulated to the defect.

V20

Impact of the chemistry of geometrically designed titanium on cell behavior and alignment

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Introduction:

Interaction of cells with a biomaterial surface is decisive for the acceptance and the success of an implant. The material properties like surface topography and surface chemistry are known to effect cellular processes like adhesion, migration, spreading, proliferation and production of extracellular matrix proteins, i.e. cell behavior and cell fate. The complex interplay of cells on materials is not completely understood despite increasing studies and innovations in the implant technology. Recent studies suggested that an additional plasmachemical modification of a machined titanium surface (Rebl 2012) was dominant over the stochastic topography. But the remaining question was whether chemistry in general is able to disturb the cells alignment due to the machined grooves or whether ligands for adhesion receptors on the surface influence cells in a similar manner.

Materials and Methods:

Therefore, in this study different plasma coating or functionalization processes (-NH_2, -CH_3,-COOH and argon/O_2-plasma) (Finke 2007, Duske 2012) as also immobilized proteins and peptides (RGD, collagen I) (Dubs 2009) on micro-grooved titanium surfaces (ZfM Chemnitz) (Matschegewski 2012) were investigated and compared. We determined early stage cell-interface interactions (adhesion, spreading) as well as medium-long-term studies (actin filament alignment, cell elongation) using confocal laser scanning microscopy and raster electron microcopy.

Results and Discussion / References:

First results showed a significantly different cell behavior of human MG-63 osteoblastic cells, i.e. solely on an NH_2-containing plasma polymer layer cells lost their ability to align along the micro-grooves on the surface. We conclude that a specific chemistry is necessary to overcome the strong influence of the micro-topography. The experiments lead to detailed knowledge of conditions for osteointegration and in consequence to new strategies for implant designs.

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V21

Development of BMP-2/SDF-1a functionalized mineralized collagen matrix scaffolds for bone regeneration applications

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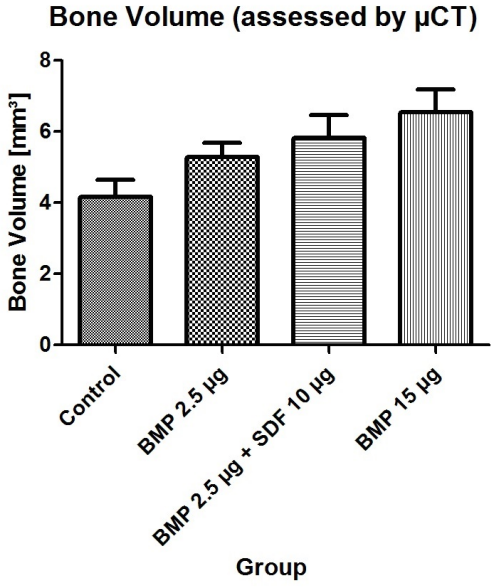
Introduction:

Localized bone loss associated with trauma, tumor, infection, periprosthetic osteolysis, or congenital musculoskeletal disorders represents a major worldwide socioeconomic problem frequently requiring surgical intervention. Previous studies showed that the bone regenerative effect of low doses of Bone-Morphogenetic Protein 2 (BMP-2) can be enhanced by the cytokine Stromal Derived Factor 1 alpha (SDF-1).(1) The aim of this study was to develop a BMP-2/SDF-1 functionalized biomimetic mineralized collagen type I matrix (MCM) scaffold, and to test its bone regenerative potential in a murine segmental critical size defect model.



Materials and Methods:

For the in vivo studies fortyfour 44 12-week-old nu/nu nude mice were randomized to four groups, each containing eleven mice. All experiments were performed in adherence to the National Institutes of Health Guidelines for the Use of Experimental Animals and were approved by the Local Animal Care Committee (protocol no. 24-9168.11-1/2010-29). Critical size bone defects of 3 mm length were created at the right femur of each mouse and stabilized by an external fixator as described before.(2) Control and treatment groups received plain MCM- scaffolds (group 1), and MCM scaffolds loaded with 2.5 µg BMP-2 (group 2), 2.5 µg BMP-2 plus 10 µg SDF-1α (group 3) and 15 µg BMP-2 (group 4). After 6 weeks animals were euthanized and µCT-scans of 20 µm voxel size were done on each femur to analyze regenerated bone volume. Descriptive statistics included means and standard deviations. Unpaired t-tests or Fisher's exact test were used for statistical analysis between the four groups of the in vivo experiment. Differences were considered significant when $p<0.05$.

Results and Discussion / References:

All animals survived the operations, one animal died during observation time due to unkown reason (group 3). Intergroup differences regarding bone volume in and around the defect area were observed between the treatment group 2 (5.3±1.3 mm^3), group 3 (5.8±1.9 mm^3), group 4 (6.5±2.0 mm^3) and the control group 1 (4.2±1.5 mm^3). Statistic significance was shown between group 3 and group 4 compared to the control group (group 3, p=0.0479;group 4, p=0.0080), whereas low-dose BMP treatment group showed no significant result (p=0.0955).

High-dose BMP-2 loaded scaffolds provided the best conditions to form bridging callus. Besides, combination of low-dose BMP-2 and SDF-1α loaded scaffolds showed a better bone regeneration than only low dose BMP-2, whereas the control group resulted more in capping the bony ends.

Combined functionalization of MCM-scaffoldsby BMP-2 and SDF-1 is a promising strategy to enhance bone regeneration.

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V22

Endothelialisierung von PMP-Fasern zur Verbesserung der Antithrombogenität von Oxygenator-Membranen

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Introduction:

Patienten mit schweren Lungenschäden, wie sie beispielsweise bei der chronisch-obstruktiven Lungenerkrankung auftreten, sind auf den Einsatz von Membranoxygenatoren zur Oxygenierung und Kohlenstoffdioxidentfernung angewiesen. Standardmäßig werden hierzu Hohlfasermembranen aus Polymeren, z.B. Polymethylpenten (PMP), eingesetzt, an deren Oberfläche der Gasaustausch stattfindet. Aufgrund der fehlenden antithrombogenen Eigenschaften des Fasermaterials kommt es jedoch zur Thrombenbildung, welche wiederum die Gasaustauschrate und damit die Lebensdauer der Membran verringert. Eine Endothelialisierung der Faseroberfläche soll daher antithrombogene Eigenschaften vermitteln[1].

Materials and Methods:

Aus Hautbiopsaten isolierte humane dermale mikrovaskuläre Endothelzellen (HDMECs) wurden auf der Ober- und der Unterseite einer unmodifizierten PMP-Fasermatte ausgesät. Im Anschluss an die Adhäsion der Zellen, welche unter statischen Zellkulturbedingungen stattfand, wurde die Fasermatte in einen Flussreaktor eingespannt, und eine dynamische Kultivierung der Zellen für zwei bis sieben Tage fand statt. Eine weitere besiedelte Fasermatte wurde parallel als Kontrolle statisch kultiviert. Anschließend wurde die Besiedlung der Fasern, sowie die Viabilität der Zellen, ihre Proliferation, die metabolische Aktivität, die Integrität des Zellmonolayers und die Expression endothelzell-spezifischer Marker analysiert. Hierzu fand eine Lebend-Tot-Färbung mittels Fluoresceindiacetat und Propidiumiodid (FDA/PI), die Färbung des Proliferationsmarkers Ki-67, die Analyse der Metabolisierung des Tetrazoliums MTT, sowie Immunfluoreszenzfärbungen von vaskulärem endothelialem Cadherin (VE-Cadherin) und PECAM-1 statt. Der Anteil der besiedelten Faseroberfläche an der Gesamtoberfläche der Fasermatte wurde mittels einer FDA/PI-Färbung und anschließender Auswertung mit ImageJ bestimmt.

Results and Discussion / References:

Mittels FDA/PI-Färbung und MTT-Assay wurde direkt im Anschluss an die Adhäsionszeit nachgewiesen, dass die Bedeckung großer Teile der Faseroberfläche mit viablen Zellen erreicht werden konnte. Nach der dynamischen Kultivierung war die Zelldichte auf den Fasern reduziert, was auf die mechanische Beanspruchung der Zellen durch den Medienfluss zurückzuführen ist. Der Vergleich beider Seiten der Fasermatte nach FDA/PI-Färbung zeigte jedoch, dass ein Ablösen der Zellen lediglich von der durch Zellkulturmedium direkt angeströmten Seite der Fasern stattfand, während es auf der entgegengesetzten Seite der Fasern über die Kultivierungszeit hinweg zu einer Proliferation der Zellen kam. Sowohl nach statischer, als auch nach dynamischer Kultivierung zeigten die Zellen die Expression der Proteine VE-Cadherin und PECAM-1, welche wichtige Bestandteile der Zell-Zell-Kontakte innerhalb des Endothels darstellen und damit dessen Integrität - die Voraussetzung für die Antithrombogenität - widerspiegeln.

Im Gegensatz zu früheren Studien[2-4] konnte in den vorliegenden Versuchen die erfolgreiche statische Kultivierung primärer humaner Endothelzellen auf PMP-Fasern ohne vorherige Oberflächenmodifikation gezeigt werden. Auch eine dynamische Kultivierung war möglich, es zeigte sich im Vergleich zur statischen Kultur jedoch eine signifikant reduzierte Zellzahl, was auf eine eingeschränkte Zelladhäsion auf dem Fasermaterial schließen lässt. Dieser Umstand macht eine Oberflächenfunktionalisierung der PMP-Fasern notwendig, welche auch unter dynamischen Flussbedingungen die Ausbildung eines antithrombogenen Endothelzell-Monolayers erlaubt.

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V23

Responsive antimicrobial nanosystems targeted for burn wound treatment: compatibility with primary endothelial cells and wound healing processes

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Introduction:

One of the leading challenges in wound management is the prevention of secondary infections. Due to their specific characteristics, thermal injuries are especially vulnerable for infections, often leading to wound healing disorders or even life-threatening conditions such as toxic shock syndrome and sepsis. Here, disinfection steps in parallel with a close monitoring of the wound are currently implemented as preventive measures. However, a continuous exposure to antiseptic drugs and frequent dressing changes may also affect healing processes and cause severe pain and mental trauma in the patient. According to the World Health Organization, major burns occur globally with an incidence of 0.11%, demonstrating the scale of the problem and the urgent need for innovative wound infection prophylaxis systems.

A sophisticated approach to avoid side-effects on the tissue is the incorporation of antimicrobials and colorimetric molecules into biodegradable nanocarriers that release their content only in the presence of pathogens. Following degradation by bacterial enzymes, any infection will thus give both a signal and be effectively treated at its source. In this study we investigated the effect of biodegradable, fluorescent-labeled hyaluronan nanocapsules containing polyhexanide[1] and poly-L-lactic acid nanoparticles loaded with octenidine[2] on primary endothelial cells (HDMEC) as the major cell type making up blood vessels and simultaneously involved in wound healing processes.

Materials and Methods:

To evaluate cytotoxicity the MTS (CellTiter 96 AQ_{ueous} non-radioactive assay) and Crystal Violet Assay were performed on cells that were exposed for 24h and 7d to the nanosamples and comparable concentrations of the corresponding antiseptic agents. Cell-nanocarrier interactions were analyzed by microscopy following immunocytochemistry as well as flow cytometry after exposure for 2, 8, 24 and 72h. To investigate the effects on vascular inflammatory response, the expression of various cell adhesion molecules (CAM) (ICAM-1, VCAM-1, E-selectin) and cytokines (IL-6, IL-8, MCP-1) was determined by enzyme immunoassays (EIA) and enzyme-linked immunosorbent assays (ELISA) after nanosample stimulation. Furthermore, an angiogenesis assay using a co-culture model of HDMEC and dermal fibroblasts (NHDF) was performed to analyze effects of the nanosystems on the potential to form capillary-like structures.

Results and Discussion / References:

Although microscopic and flow cytometric analysis pointed to a time-dependent uptake of both of the nanosystems, CAM-EIAs and cytokine ELISAs after exposure showed no significant increase of inflammatory marker expression, indicating that the samples do not interfere with inflammatory response. In addition, endothelial cell potential to form capillary-like structures in co-culture with dermal fibroblasts was not inhibited. Cytotoxicity experiments after short- (24h) and long-term (7d) exposure to the materials showed that both systems exhibit mild toxic effects on the cells depending on the concentrations applied. However, it was demonstrated that within the range of the effective dose of polyhexanide as well as octenidine against two of the most relevant wound germs *S. aureus* and *P. aeruginosa*[3], both nanocarriers affected the cells much less than solutions of the antiseptic agents alone under comparable concentrations.

These results indicate that responsive antimicrobial nanocomposites may represent an advanced drug delivery system and a promising alternative in burn wound treatment with few side-effects.

This study was supported by the EU-project Bacteriosafe (#245500).

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V24

Dual setting calcium phosphate cement - silica gel system

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Introduction:

Mineral biocements based on calcium phosphate chemistry (CPC) set by a continuous dissolution - precipitation reaction in an aqueous environment. At strong acid conditions ($pH4•2H_2O$)[1] or monetite ($CaHPO_4$)[2] are formed. In contrast to hydroxyapatite, these CPCs are higher soluble under physiological conditions, which result in a rapid resorption *in vivo*, but at the same time, they suffer from their brittle character and low mechanical strength. Here we developed a dual setting system by combining the brushite forming cement paste with an inorganic silica based precursor to enhance the mechanical properties of the composite.

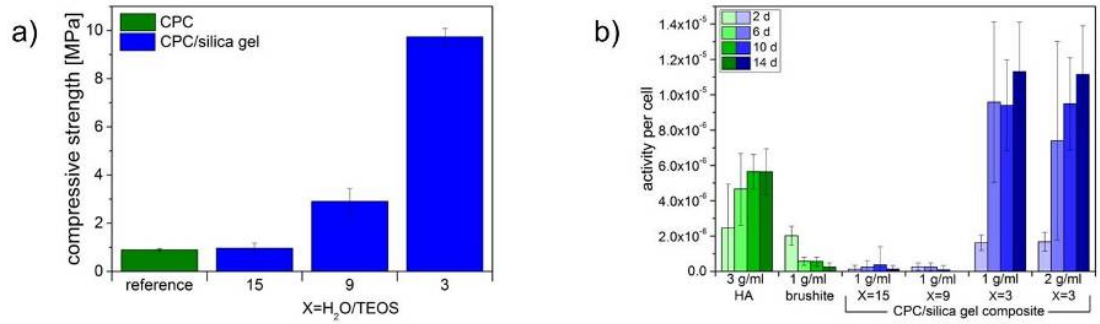

Figure 1: a) compressive strength and b) activity per cell of osteoblast-like MG 63 cells of CPC reference and CPC/silica gel composite

Materials and Methods:

Brushite cements were produced by mixing β-TCP powder in an equimolar ratio with dicalcium phosphate anhydrous. Silica gel was produced by the sol gel process with tetraethyl orthosilicate (TEOS) as precursor under acidic conditions. Here the water:TEOS ratio (X) was set at 3, 9 or 15. Cement pastes were formed by mixing CPC-powder and pre-hydrolysed liquid sol gel at a powder to liquid ratio (PLR) of 1 or 2 g/ml. As reference, the CPC powder was mixed with water or a 0.5 M citric acid solution and treated the same. The setting temperature, pH and time were measured. Cement characterization was done by compressive test, porosimetry (BET and Hg), X-ray diffractometry and scanning electron microscopy. Additionally *in vitro* cytocompatibility testing with osteoblastic cells were performed and the ion release (Ca, P, Si and Mg) during cell culture were analyzed by inductively-coupled-plasma mass-spectrometry.

Results and Discussion / References:

Cement solidification was found to be fast (2-5 min) at higher water:TEOS ratio (X≥9) with a clear increase of the pH during the first hour of setting. In contrast, at the lowest X=3, the setting time of the composites was more than 40 min, which is likely an effect of the slowly increasing pH due to a higher HCl content. The setting temperature decreased with increasing TEOS content. These parameters enabled a control of the cment phase composition and favored the formation of monetite instead of brushite at high water:TEOS ratios.[3] In the set cements the µm-size gaps between the brushite crystals were filled with nanoporous silica gel leading to a bimodal pore size distribution. This compaction results in improved compressive strength 5-10 times higher than the reference (Figure 1a). The composites with the highest silicate content showed the best biological behaviour (Figure 1b), whereas the good cell response did not seem to be attributed to the released silicate ions, but to the release of phosphate and the adsorption of magnesium ions from the cell culture medium.

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V25

Carbon nanotubes used as a drug delivery systems for oncostaics in experimental oncological surgery studies on murine tumor models.

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Introduction:

Background: Nanotechnology gives a new hope and possibility for obtaining the new precisely prepared biomaterials. The innovative drug delivery posibielieties aspects belong nowadays to the one of the most interesting in modern anti-cancer therapeutic strategies development. Particularly important in this development could be the use of carbon nanotubes.

Aim of the work:

The aim of our work was to evaluate the usefulness of carbon nanotubes covered with different types of cytostatics in a two different new experimental concepts. The first one was targeted onto the use of carbon nanotubes as innovative hemostatic dressings for local recurrence prevention in nephron sparing surgery (NSS) on the xenografted murine model, and second targeted onto the use of carbon nanotubes as a novel drug delivery system in experimental hyperthermic intraperitoneal chemotherapy, also onto the tumor induced murine model.

Materials and Methods:

In the first analyzed study the 5 x 10^6 786-o cells were injected under the kidney fibrous capsule, in the region of upper or lower pole of kidney in the BALB/C nude mouse model. When tumors reached the operative size, we have performed the partial nephrectomy NSS - (+/- resection margin) with intraoperative use of carbon nanotubes covered with cisplatin as hemostatic dressings with oncostatic action. During and after operation we analyzed the hemostatic action of used compunds. After the observation time (5^{th} and 6^{th} week) animals were sacrificed and after kidney collection, we performed standard macroscopic and microscopic (HE) analysis for information about the local oncostatic impact of this type of dressing onto potential, local tumor recurence

In the second part of our study we have analyzed the influence and usefulness of different types of materials and substances. We have investigated: 1) cisplatin (standard drug), 2) cis-$[PtCl_2(dbtp)_2]$ 3) $[Pt(C_4H_4O_5)(dbtp)_2]$ 4) modified nanotubes 5) modified nanotubes filled with cisplatin 6) modified nanotubes filled with cis-$[PtCl_2(dbtp)_2]$ 7) modified nanotubes filled with $[Pt(C_4H_4O_5)(dbtp)2]$ 8) nanotubes conjugated with cisplatin 9) nanotubes conjugated with cis-$[PtCl_2(dbtp)_2]$. All of this materials were used in previously induced due the intraperitoneal injection of 1 x 10^6 murine melanoma B16 cells, murine advanced peritoneal cancer model.

Results and Discussion / References:

The search for an ideal carrier matrix for oncostatic drugs is nowadays one of the most intensively considered research problem of current nanomedicine. In different representative research it has been shown that carbon nanotubes are good carriers of very small molecules and could be used as a novel transfer method of selected oncological drugs. In our opinion carbon nanotubes filled with cisplatin could have a positive future significant impact onto surgical oncology development, especially in the field of local tumor recurrence prevention after organ sparing surgery due to cancer or in innovative concepts of hyperthermic intraperitoneal chemotherapy.

Acknowledgments

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