Nederlandse vereniging voor Biomaterialen en Tissue Engineering
Netherlands society for Biomaterials and Tissue Engineering

Program 22nd Annual Meeting, 28 & 29 November 2013

Thursday, 28 November

09.00 – 09.45	***Registration and coffee***
09.45 – 09.50	***Welcome***

09.50 – 11.05	**Oral Presentations** **Chair: Martijn Riool**
01	**Corien Oostendorp,** RUMC-NCMLS-Biochemistry, Nijmegen. *Quantitative Parameters for the Evaluation of Cellular Skin Substitutes*
02	**Wanxun Yang,** RUMC-Biomaterials, Nijmegen. *Modulating Chondrogenic Priming of MSCs to Enhance Endochondral Bone Formation in vivo as a Bone Tissue Engineering Strategy*
03	**Emmanuel Ekwueme**, MIRA-Tissue Regeneration, Enschede. *Tenogenic response of bone marrow stromal cell and tenocyte co-cultures*
04	**Mandy Steinbusch,** MUMC-Lab for Experimental Orthopaedics, Maastricht. *RNase MRP is Involved in Chondrogenic Differentiation*
05	**Paul de Jonge**, RUMC-NCMLS-Urology, Nijmegen. *Tubular Collagen Scaffolds to Regenerate Critical Ureteral Defects in a Porcine Model*

11.05 – 11.30	***Coffee break***

11.30 – 12.45	**Oral Presentations** **Chair: Olga Goor**
06	**Zeinab Tahmasebi,** MIRA-Tissue Regeneration, Enschede. *Effect of Soluble and Incorporated Ions on Proliferation and Differentiation of Human Mesenchymal Stromal Cells*
07	**Kambiz Farbod,** RUMC-Biomaterials, Nijmegen. *Bisphosphonate-functionalized Gelatin Nanoparticles for Bone-seeking Applications*
08	**Payal Balraadjsing**, AMC-Medical Microbiology, Amsterdam. *Dendritic Cell Responses to Biomaterials in Presence of Staphylococcal Infection is nfluenced by the Nature of the Biomaterials*
09	**Jetze Visser**, UMCU-Orthopedics, Utrecht. *Functionalization of Gelatin Methacrylamide with Tissue-derived Matrix for Cartilage Regeneration*
10	**Gouying Si,** MIRA-Controlled Drug Delivery, Enschede. *Near Infrared Light Triggered Gene Delivery Based on Poly(Amido Amine)s-Gold Nanoparticles Hybrid System*

12.45 – 13.45	***Lunch***

13.45 – 15.00	**Oral Presentations** **Chair: Alex Roth**
11	**Michiel Pot,** RUMC-NCMLS-Biochemistry, Nijmegen. *Extending the Surgical Armamentarium for Bladder Reconstructions: a Seamless Spherical Hollow Collagen Bladder*
12	**Sieger Henke,** MIRA-Developmental BioEngineering, Enschede. *Enzymatically crosslinked Dextran-Tyramine micro Hydrogels for improved Beta Cell Function*
13	**Janneke Boere,** UU-Vet Med-Equine Sciences, Utrecht*. Intercellular Communication in the Equine Joint: Lipidome Analysis of Cell-derived Vesicles*
14	**Mellany Ramaekers**, TU/e-Institute complex molecular systems, Eindhoven. *Towards Bioactive Supramolecular Cucurbit[8]uril Hydrogels*
15	**Xiangzhen Yan**, RUMC-Biomaterials, Nijmegen. *Enzymatic control of chitosan gelation for delivery of periodontal ligament cells*

15.00 – 15.25	***Coffee Break***

15.25 – 16.00	**Rapid fire Poster Presentations (1 min sharp, no slides)** **Chair: Sander Leeuwenburgh**
	Jie An, *RUMC-Biomaterials, Nijmegen* **David Barata***, MIRA-Tissue Regeneration, Enschede* **Nick Beijer,** *MIRA-Tissue Regeneration, Enschede* **Bas van Bochove,** *MIRA-Biomaterials, Science & Technology***,** *Enschede* **Marcel Boerman,** *Radboud Univ, Inst. Molec. Mater. (IMM), Nijmegen* **Marjolein Caron,** *MUMC-Orthopaedics, Maastricht* **Maurice van Dalen**, *MESA+ - Nanobiophysics, Enschede* **Andrea Di Luca***, MIRA-Tissue Regeneration, Enschede* **Tony Ekkelenkamp***, MIRA-Controlled Drug Delivery, Enschede* **Olga Goor**, *TU/e-Institute complex molecular systems, Eindhoven* **Milou Groot-Nibbelink***, MIRA-Dev BioEngineering, Enschede* **Dewi Hujaya**, *MIRA-Controlled Drug Delivery, Enschede* **Tom Kamperman,** *MIRA-Dev BioEngineering, Enschede* **Alexey Klymov**, *RUMC-biomaterials, Nijmegen* **Bach Le**, *MIRA-Tissue Regeneration, Enschede* **Loek Loozen***, UMCU-Orthopedics, Utrecht* **Ferry Melchels***, UMCU-Orthopedics, Utrecht* **Ziryan Othman**, *MIRA-Tissue Regeneration, Enschede* **Marloes Peters***, MUMC-Orthopaedics, Maastricht* **Sander van Putten***, UMCG-Pathology and Medical Biology, Groningen* **Alejandra Ruiz Zapata**, *VUMC-Orthopedics, Amsterdam* **Ravi Sinha**, *MIRA-BioMechanical Engineering, Enschede* **Mahshid Vashaghian**, *VUMC-Gynaecology, Amsterdam*

	Rong Wang, *MIRA-Dev BioEngineering, Enschede* **Olaf Wouters***, UMCG-Pathology and Medical Biology, Groningen* **Xiaolin Zhang**, *AMC-Medical Microbiology, Amsterdam*

16.00 – 17.00	***Poster presentations and drinks***

17.00 – 18.00	**Oral Presentations** **Chair: Michelle Poldervaart**
16	**Aliaksei Vasilevich,** MIRA-Tissue Regeneration, Enschede. *Classification of Cell Shapes as Tool to Define Cell Response within a Large Dataset*
17	**Rhandy Eman,** UMCU-Orthopedics, Utrecht. *Establishment of an early Stable Vascular Network Promotes the Induction of Ectopic Bone Formation*
18	**Katrien Brouwer**, RUMC-NCMLS-Biochemistry, Nijmegen. *Regenerative Medicine of the Diaphragm: Development and Preclinical Evaluation of Collagen-based Scaffolds*
19	**Wim Hendrikson,** MIRA-Tissue Regeneration, Enschede. *Biological and Tribological Comparison between PEOT/PBT, PCL and PLDLLA RP plotted Scaffolds*

18.00 – 19.00	***NBTE members Annual Meeting***
19.00 – 20.30	***Dinner***

20.30 – 21.30	**Invited lecture:** *Wearable Smart Systems for Health and Lifestyle Applications* **Sywert Brongersma, Holst Centre/imec The Netherlands, Eindhoven**

21.30 –	***Party time!***

Friday, 29 November

09.00 – 10.15	**Oral Presentations** **Chair: Maarten Leijs**
20	**Corina Ghebes**, MIRA-Tissue Regeneration, Enschede. *Anterior Cruciate Ligament- versus Hamstring-derived Cells*
21	**Michiel Croes,** UMCU-Orthopedics, Utrecht. *Activated T Cells and their Factors Regulate the Osteogenic Differentiation of Human Mesenchymal Stromal Cells in Vitro*
22	**Alex Roth**, MUMC-Orthopedics, Maastricht. *Novel radiopaque UHMWPE sublaminar wires in a growth-guidance system for the treatment of early onset scoliosis: feasibility in a large animal model*
23	**Kristel Boere**, UU-Pharmaceutics, Utrecht. *Native Chemical Ligation as a tool to form Peptide-Functionalized in situ Crosslinkable Thermosensitive Hydrogels*
24	**Lizette Utomo**, Erasmus MC-Otorhinolaryngology, Rotterdam. *Decellularized Ear and Articular Cartilage with Native Architecture for Cartilage Reconstruction Strategies*

10.15 – 10.40	***Coffee Break***

10.40 – 11.55	**Oral Presentations** **Chair: Marije Sloff**
25	**Xingnan Lin**, ACTA-Oral Implantology, Amsterdam. *Ectopic Osteogenesis Induced by Coating-incorporated Depot of BMP-2 in Coral*
26	**Ilaria De Napoli**, MIRA-Biomaterials, Science & Technology, Enschede. *Proof of Concept of Bioactive Double Coating on Polymeric Membranes for Functional Human Proximal Tubule Cell Monolayers Preparation for Bioartificial Kidney Devices*
27	**Willemijn Boot**, UMCU-Orthopedics, Utrecht. *Prophylaxis of Orthopedic Implant-related Infections with Locally Applied Vancomycin using a Hydrogel as Matrix*
28	**Hamdan Alghamdi,** RUMC-Biomaterials, Nijmegen. *Histologic and Genetic Response of Osteoporotic Bone to Implants Coated with Bisphosphonate-Loaded Calcium Phosphate Nanoparticles*
29	**Maarten Janssen,** MUMC-Orthopedics, Maastricht**.** *Human Rib Perichondrial Transplantation: a 20-year Follow-up*

11.55 – 12.20	***Coffee break***

12.20 – 13.35	**Oral Presentations** **Chair: Wim Hendrikson**
30	**Benno Naaijkens,** VUMC-Cardiopathology, Amsterdam. *Development of a New Therapeutic Technique to Direct Adipose Derived Stem Cells to the Infarcted Area using Targeted Microbubbles: StemBells*
31	**Michelle Poldervaart,** UMCU-Orthopedics, Utrecht. *Prolonged Presence of VEGF Promotes Regional Vascularization in 3D Bioprinted Scaffolds with Defined Architecture*

32	**Inge van Loosdregt**, TU/e-Soft Tissue Biomechanics and Engineering, Eindhoven. *Cell-Mediated Retraction Versus Hemodynamic Loading – a Delicate Balance in Tissue-Engineered Heart Valves*
33	**Marije Sloff,** RUMC-NCMLS-Urology, Nijmegen. *A New Tissue-Engineered Urinary Conduit In A Porcine Model*
34	**Agnieszka Bochyńska**, MIRA-Biomaterials, Science & Technology, Enschede. *Biodegradable Hyper-branched isocyanate-terminated Tissue Adhesives for Meniscus Repair*

13.35 – 14.30	***Lunch***

14.30 – 15.45	**Oral Presentations** **Chair: Benno Naaijkens**
35	**Maarten Leijs**, Erasmus MC-Orthopedics, Rotterdam. *Priming human Mesenchymal Stem Cells in Culture to increase Chemotaxis to Osteoarthritic Cartilage and Synovium*
36	**Nathalie Groen,** MIRA-Tissue Regeneration, Enschede. *Exploring Transcriptional Differences Induced by Varying Material Surface Properties in Osteoblasts*
37	**Xin Zhang**, ACTA-Oral Implantology, Amsterdam. *Icariin: is it an efficient Osteoinductive Promoter to Enhance the Effectiveness of BMP-2?*
38	**Anita Krouwels**, UMCU-Orthopedics, Utrecht. *In vitro Tissue Regeneration by Human Regenerated Nucleus Pulposus Cells in Hyperosmotic Culture Medium*
39	**Martijn Riool**, AMC-Medical Microbiology, Amsterdam. *Towards Understanding the Molecular Basis of the Foreign Body Response and Biomaterial-Associated Infections*

	Presentation of the poster, presentation, and NBTE best thesis awards
	Closure of the meeting

Keynote Lecture

Wearable Smart Systems for Health and Lifestyle Applications

Sywert Brongersma

Holst Centre / imec the Netherlands – High Tech Campus 31 – 5656 AE Eindhoven – The Netherlands, **www.imec-nl.nl**, www.holstcentre.com

Western societies all see their healthcare costs outpace GDP growth. To reverse this trend, several public and private initiatives were taken in the last years to make healthcare more cost efficient. It is anticipated that micro and nano-system technology will help enable an increase in the functionality of lifestyle and healthcare devices to gradually reduce cost. This builds on the scaling of microelectronics that additionally provides opportunities for reducing both form factor and power requirements. Recent history has seen the emergence of wearable sensor systems that monitor our physiology and activity. Many players are introducing cardiac, brain, sleep, or energy expenditure monitoring systems while recent results show that a combination of such systems can already give an indication of our stress level and even emotional state. Although this area will clearly lead to a significant increase of sensing technologies in applications such as healthcare and gaming, they are still largely based on simple electrical measurements and physical sensors such as accelerometers.

The next generation of wearable, distributed, and interconnected sensor systems will add additional functionality such as the ability to smell. Miniaturized low power chemical sensors are starting to emerge and will revolutionize our access to information on a very personal level. The composition of breath is indicative of a wide range of conditions ranging from halitosis (bad breath) to early stages of cancer, COPD, and many others. The impact of air quality on our wellbeing is of great concern to all and causes symptoms such as respiratory problems, fatigue, and eye irritation. Sharing such information in the cloud will lead to detailed mapping of air quality in your vicinity based on a high sensor density and helps the asthmatic and allergic population. The ability to assess the quality of our food can not only prevent consumption of bad meat or fish, but can also reduce the vast amount of food that is wasted on a daily basis.

Imagine that you have this capability built into your smartphone or wristwatch. These are the technologies that are being developed today and will revolutionize the way we perceive our surroundings and ability to stay healthy.

Quantitative Parameters for the Evaluation of Cellular Skin Substitutes

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Dept. of Biochemistry, NCMLS, Radboud University Medical Centre, Nijmegen, The Netherlands

Introduction

The skin is the largest organ of the human body and forms a physical and biochemical barrier between the body and the environment [1]. When the skin is extensively damaged and needs to be reconstructed, the golden standard is transplantation of split-thickness skin, composed of the epidermis with a part of the dermis. However, the lack of full dermal tissue results in scarring and wound contraction. In addition, the problem of donor site shortage has to be dealt with when the wound exceeds 50% of the total body surface area [2]. This emphasizes the need for engineered skin substitutes. Currently, these skin substitutes vary greatly in composition and structure. In general, quality control for cellular skin substitutes is not well developed. Next to qualitative parameters e.g. histology, quantitative parameters are needed for unbiased interpretation of the quality of the skin substitutes. Therefore the commercialization of cultured skin substitutes asks for new quantitative standards. In this study, we probed a number of different quantitative parameters to evaluate cellular skin substitutes.

Methods

The skin substitutes were made from compressed bovine type I collagen hydrogels, loaded with either autologous fibroblasts or a combination of autologous fibroblasts and keratinocytes. The skin substitutes were evaluated at DNA, RNA and protein level. Cellular proliferation was assessed by DNA measurements. Transcriptional activity of the cells was investigated with qPCR. Dermal and epidermal gene expression profiles were analyzed. The following genes were chosen for the dermis: alpha smooth muscle actin (ASMA), type III collagen (COLIII), elastin (ELN), fibrillin (FBN), lysyl oxidase (LOX) and vimentin (VIM), and for the epidermis caspase 14 (CASP14), β defensin 2 (DEFB4), fillagrin (FLG), involucrin (IVL), Ki67, keratins 1, 2, 5, 10, 14, 15 (KRT1, KRT2, KRT5, KRT10, KRT14, KRT15), laminin β 3 (LAMB3), loricrin (LOR), proliferating cell nuclear antigen (PCNA), transglutaminase 1 (TGM1) and tyrosine related protein 1 (TYRP1) were chosen. As control genes RPL13, RPLP0 and GAPDH were used.
For quantitative analysis at the protein level, extracellular matrix molecules type III collagen and laminin 5 were evaluated using ELISA assays.
As a functional readout parameter, activity of lysyl oxidase, an enzyme responsible for the crosslinking of collagens and elastin in the extracellular matrix, was investigated.

Results and discussion

Cell counting via a quantitative DNA measurement provided a reliable measurement to assess the proliferative activity of keratinocytes and fibroblasts.
In the dermal construct, elastin mRNA expression was most prominent compared to abdominal skin, indicating the active regeneration of the ECM by the fibroblasts. Epidermal gene expression profiles gave information on the differentiation state of the dermo-epidermal skin substitute.
Using ELISA, laminin 5 and collagen III were evaluated. However, poor extraction of these extracellular matrix proteins probably compromised accurate measurements. The lysyl oxidase assay was found to be unreliable.

Conclusion

DNA and RNA based assays provide quantitative data on the cellular skin substitutes. Future experiments will assess the clinical relevance of these parameters retrospectively after implantation.

Acknowledgements

This work was financially supported by EU-FP7 project EuroSkinGraft (Grant agreement no:279024)

1. Proksch, E., J.M. Brandner, and J.-M. Jensen, *The skin: an indispensable barrier*. Experimental Dermatology, 2008. **17**(12): p. 1063-1072.
2. Böttcher-Haberzeth S., B.T., Reichmann E., *Tissue engineering of skin*. Burns, 2010. **36**: p. 450-460.

Modulating Chondrogenic Priming of MSCs to Enhance Endochondral Bone Formation *in vivo*

as a Bone Tissue Engineering Strategy

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Introduction: The use of engineered bone constructs by combining mesenchymal stem cells (MSCs) and biocompatible scaffolds for the treatment of bone defects has received much attention. However, lack of sufficient vascular supply, resulting in immediate cell death after implantation, is generally thought to be the cause of failure in patients. A new concept of generating bone tissue via the endochondral route, comprising a cartilage intermediate as an osteoinductive template *in vivo*, might have superiority over the standard intramembranous ossification approach. Implantation during the metabolically less-demanding chondrocyte phase may tolerate low oxygen/nutrition condition, thereby allowing for a longer temporal vascularization window. Subsequent hypertrophic stage of the chondrocyte will induce the secretion of angiogenic factors, which promotes vascular invasion to the implant. However, the increased metabolic demand of this stage may again shorten the survival period of the implants. Till now no consensus has been reached on the implantation time of the cartilage templates. The aim of this study was to investigate the effect of chondrogenic priming on the quality and quantity of the endochondral bone formation *in vivo*, thereby selecting an optimal implantation time.

Materials and Methods: Two types of scaffolds were used in this study as cell carrier, i.e. a porous hydroxyapatie/tricalcium phosphate composite (HA/TCP) and a fibrous poly(lactic-co-glycolic acid) combined with poly(ε-caprolactone) electrospun scaffold (PLGA/PCL). Rat MSCs were seeded on the scaffolds and chondrogenically primed *in vitro* for 2, 3 or 4 weeks. The formation of cartilage templates was characterized by matrix quantification and gene analysis. Simultaneously, these cell/scaffold constructs were ectopically implanted in rats for 4, 6 and 8 weeks to assess the spatial pattern of bone formation and cartilage remains via the immunohistological stainings and histomorphometrical analysis.

Results: On both PLGA/PCL and HA/TCP scaffolds, the cartilage matrix deposition increased with the *in vitro* chondrogenic culture time and comparable amounts of DNA was detected. *In vivo* results revealed that, on PLGA/PCL scaffolds, the new bone formation was mixed with cartilage remnants and resembled a woven bone structure at all *in vivo* time points. In contrast, 4 weeks of implantation was sufficient to ossify nearly the complete constructs of HA/TCP. The bone tissue was observed to mature over time *in vivo* and displayed a lamellar bone like structure with obvious marrow development after 8 weeks (*Fig.1*). Histomorphometrical analysis (*Fig.2*) demonstrated that, the length of *in vitro* priming time did not show significant effects on the amount of *in vivo* bone formation on both scaffolds. However, longer *in vitro* priming time resulted in higher amount of cartilage remaining and more homogeneous distribution of the bone/cartilage complex in PLGA/PCL scaffolds.

Conclusions: Chondrogenic priming of rat MSCs for different time periods lead to bone formation *in vivo* through cartilage template remodelling on PLGA/PCL and HA/TCP scaffolds. A longer chondrogenic priming time resulted in better distribution of bone formation *in vivo* while the quantity and quality of endochondral bone tissue was scaffold-dependent. Fibrous PLGA/PCL appeared to maintain cartilage phenotype. Porous HA/TCP supported progressive bone formation with bone marrow development.

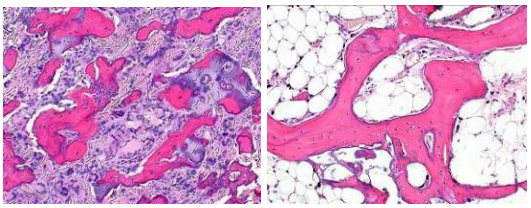

Fig.1 *In vivo* bone formation pattern on PLGA/PCL (left) and HA/TCP scaffolds (right) after 8 weeks of implantation (HE staining).

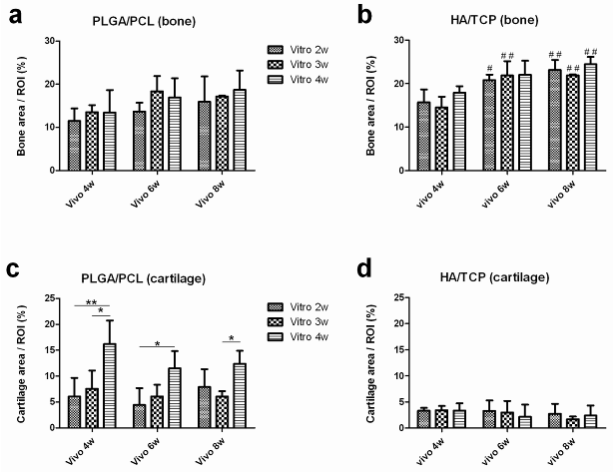

Fig.2 Histomorphometrical analysis of *in vivo* bone formation and cartilage remains.

Tenogenic response of bone marrow stromal cell and tenocyte co-cultures

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[2]Department of Tissue Regeneration, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

Introduction: Mesenchymal stromal cells (MSC) have emerged as potential tools for tissue repair due to their self-renewal and multipotency. Recent work has shown that localized MSC delivery may be beneficial for ligament and tendon repair by increasing cell number, enhancing collagen deposition and maturation, and increasing tissue biomechanical properties after injury [1-3]. However, few studies have attempted to determine the optimal mode of MSC delivery required for enhanced ligament and tendon regeneration. The goal of this work is to characterize the cell response of MSC and tenocytes under direct and indirect co-culture. Utilizing an *in* vitro rat model, we assess the effect of co-culture conditions on the cell metabolic activity, collagen deposition, and gene expression of tenogenic markers in both cell types.

Materials and Methods: Primary tenocytes (PT) and rMSC were harvested and expanded from the bone marrow and patellar tendons of Sprague-Dawley rats.
Indirect co-culture system: After culturing PT for 48 hours in basal medium (BM) supplemented with 1% pen-strep and 2 mM L-glutamine, $5x10^3$ cells/cm^2 rMSC were cultured with the tenocyte-conditioned medium (PT-CM) for up to 7 days. Simultaneous indirect co-culture of the two cell types was achieved using permeable well plate inserts. rMSC were cultured at $5x10^3$ cells/cm^2 on well-plates while PT were seeded on the inserts at a 1:1 ratio. This configuration was reversed to study the effect of co-culture on PT.
Direct co-culture system: To examine the effect of direct contact between the two cell types, populations of PT and rMSC were cultured together at $5x10^3$ cells/cm^2 at a 1:1 ratio. Control groups for both co-culture systems consisted of both cell types cultured individually.
Cell metabolic activity: At 3 and 7 days, the metabolic activity of the cells was assessed using the MTS assay. Cell activity was measured via spectrophotometry.
Collagen quantification: After 7 days, collagenous and non-collagenous protein deposition was assessed using Sirius Red/Fast Green staining. Protein quantification was achieved by measuring absorbance via spectrophotometry.

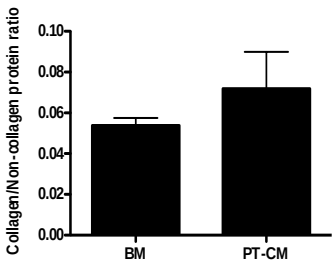

Figure 1. Ratio of deposited collagen to non-collagen protein by rMSC in basal medium (BM) and tenocyte-conditioned medium (PT-CM) for 7 days.

Real-time qPCR: For rat conditioned medium studies, samples were subjected to qPCR using TaqMan Gene Expression Master Mix and Assays for tenogenic markers including collagen types I and III. Relative gene expression was determined using the $\Delta\Delta C_T$ method, normalized to GAPDH.

Results and Discussion: After culturing in tenocyte-conditioned medium, rMSC experienced enhanced collagen deposition (Fig. 1) and higher collagen I/III gene expression (Fig. 2).

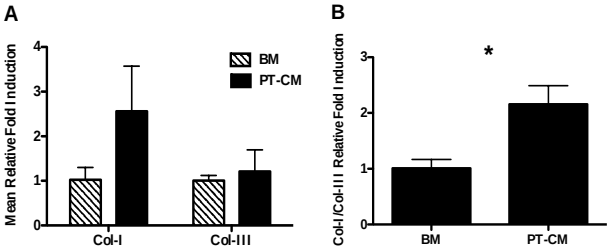

Figure 2. (A) Mean relative gene expression of collagen types I and III and (B) mean ratio of the genes in rMSC cultured in basal and tenocyte-conditioned medium for 5 days.

Preliminary results from the cell metabolic activity data suggest that PT have higher metabolic activity than rMSC. Additionally, the indirect co-culture of rMSC and PT did not lead to a significant increase in cell activity in either cell type. The direct co-culture of the two cell types exhibited a trend towards higher cell activity in comparison to control populations (Fig. 3).

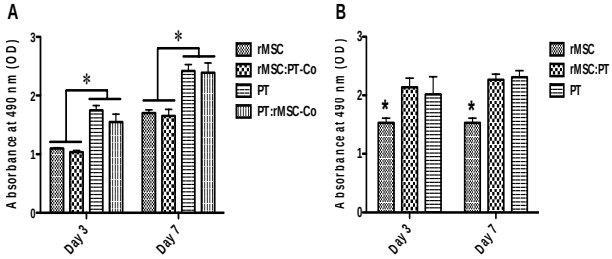

Figure 3. MTS cell metabolic activity of rMSC and PT co-cultured indirectly (A) and directly (B) at a 1:1.

Conclusions: These initial results support reports of the effect of resident cell secretome on MSC function and vice versa. On-going *in vitro* work further investigating this response in a human model has yielded similar results. Future studies will explore inter-donor variability in the gene expression of several tenogenic markers and TGF-β signalling during co-culture and the effect of MSC delivery method on tenogenic response in an *in vivo* Sprague-Dawley Achilles tendon stretch injury model.

References:
[1] Awad et al. Tissue Eng. 1999, 5(3), 267-277.
[2] Watanabe et al. Microsc. Res. Tech. 2002, 58, 39-44.
[3] Lee et al. PLoS One. 2011, 6(3), e17531.
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RNase MRP is Involved in Chondrogenic Differentiation

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Introduction: The ribonuclease mitochondrial RNA processing (RMRP) gene encodes the RNA component of a multi-protein-RNA complex called RNase MRP. This small nucleolar ribonucleoprotein particle is implicated in various cellular processes, including ribosomal biogenesis, mitochondrial RNA cleavage, cell cycle regulation and has been linked to telomerase. Mutations in the RMRP gene cause McKusick-type metaphyseal chondrodysplasia, also known as Cartilage Hair Hypoplasia (CHH). The phenotypic hallmark of CHH is dwarfism. We therefore hypothesized that RNase MRP is involved in chondrogenic differentiation of the growth plate during skeletal development.

Methods: To investigate the potential role of RNase MRP during chondrogenic differentiation *in vivo*, RMRP protein subunits were detected in growth plates of 6 week old mice by immunohistochemistry. To verify these results, expression of RNase MRP subunits and important chondrogenic genes (e.g. Sox9, Col2a1, Runx2, Col10a1) was determined by RT-qPCR in chondrogenically differentiating ATDC5 cells. Moreover, the functional role of RMRP during chondrogenic differentiation was investigated by genetically targeting RMRP by RNAi and analysing the outcome on important chondrogenic markers. As primary chondrocytes or bone marrow stem cells from CHH patients are rarely available, we established a differentiation protocol where human dermal fibroblasts acquire a chondrocyte-like phenotype. This allows us to investigate the chondrogenic capacity of CHH patient-derived primary cells.

Results: To investigate the expression of RNase MRP during growth plate development, RNase MRP protein subunits Rpp25, Rpp38 and Rpp40 were immunohistochemically detected in growth plates of 6 week old mice. Identical distribution patterns were observed: resting zone chondrocytes expressed RNase MRP proteins. Weak expression levels were observed in proliferative chondrocytes. Expression of RNase MRP proteins was clearly detectable in hypertrophic chondrocytes. This temporospatial expression was confirmed using *in vitro* culture models for chondrogenic differentiation. In differentiating ATDC5 cells RMRP and RNase MRP subunits Rpp25 and Rpp40 expression was upregulated from day 14 in differentiation onward, simultaneously with markers for chondrocyte hypertrophy. Chondrocyte proliferation and differentiation is tightly regulated by parathyroid hormone-related peptide (PTHrP), by delaying end-stage differentiation of chondrocytes. We therefore investigated the effect of PTHrP on RMRP expression during chondrocyte differentiation in ATDC5 cells. PTHrP-mediated inhibition of chondrogenic differentiation displayed a decrease in RMRP levels, indicating a relation between PTHrP, chondrogenic differentiation and RMRP expression. Interfering with RMRP expression using RMRP-specific RNAi resulted in impaired chondrogenic marker expression. In addition, RMRP knockdown resulted in increased expression of early chondrogenic transcriptional regulator Bapx1/Nkx3.2, suggesting a regulatory role for RMRP in determining chondrocyte differentiation. Moreover, bone morphogenic protein-2 (BMP-2), a known stimulator of hypertrophy, increases RMRP expression during chondrogenic differentiation of ATDC5 cells. Notably, RNase P expression was not affected by BMP-2. Finally, transdifferentiation of CHH patient-derived dermal fibroblasts into chondrocyte-like cells indicated reduced expression of Col2a1 and Col10a1 in patient-derived fibroblasts as compared to control cultures, as well as deregulated expression of PTHrP.

Discussion and Conclusion: In conclusion, our data indicate that RNase MRP is involved in chondrogenic development of the growth plate and appears to be predominantly associated with terminal hypertrophic differentiation. In future experiments we aim to elucidate which function of RNase MRP is involved in chondrogenic differentiation. These data provide novel insight into the underlying molecular mechanisms causing the CHH-associated skeletal phenotype and suggest RNase MRP as a novel regulator of chondrogenic differentiation in skeletal development.

Tubular Collagen Scaffolds to Regenerate Critical Ureteral Defects in a Porcine Model

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Introduction: Iatrogenic damage is the main cause of ureteral injury due to ligation, cauterization, transection, avulsion, devascularization or radiation therapy. When end to end anastomosis is not feasible, the repair of long ureteral segments can be a challenge for the urologist. Major reconstructive surgeries are often required, each with their associated risks for complications. Biomaterials have the potential to provide an alternative to these major reconstructive surgeries, benefiting both patient and surgeon, by providing an off the shelf alternative. In this study we examined the applicability and compared the effectiveness of cell seeded and non cell seeded tubular collagen scaffolds in a preclinical porcine model.

Methods: Tubular collagen scaffolds (6cm long, Ø=6 mm) were prepared from highly purified bovine type-I collagen, by subsequent freezing, freeze-drying and crosslinking. Scaffolds were sterilized by gamma irradiation (25 kGy). Eleven pigs were used in this study. Bladder biopsies were taken from all animals for urothelial and smooth muscle cell isolation and culture. Characterized scaffolds were seeded with primary bladder smooth muscle cells on the inner and outer surface and urothelial cells in the lumen. A 6 cm long ureteral segment was excised, to create a clinically significant defect, and replaced by a tubular collagen scaffold, with and without cells, over a ureteral 6Fr double-j splint. The pigs were sacrificed one month after implantation for macroscopic and microscopic analysis.

Results: Highly porous tubular collagen constructs were successfully implanted. Implantation of the scaffolds was challenging due to weak mechanical properties. Macroscopic analysis showed regenerated defects in 4 pigs. Six pigs showed a partial or full dissection of the ureter. Microscopic analysis showed urothelial lining of the neo-ureter, with neo-vascularization throughout the tissue and smooth muscle cell ingrowth. No differences were found between cell seeded and non cell seeded constructs.

Conclusion: Implantation of tubular collagen scaffolds in a large animal model, while being a demanding procedure, is feasible. Tubular collagen scaffolds have the capacity to induce urothelial lining, neo-vasculature, and muscle ingrowth in the neo-ureter. However, insufficient mechanical strength was demonstrated both intraoperatively and during regeneration. To facilitate the implantation procedure and to be able to handle intracorporeal dynamics during ureteral regeneration, improvements to the mechanical properties are imperative.

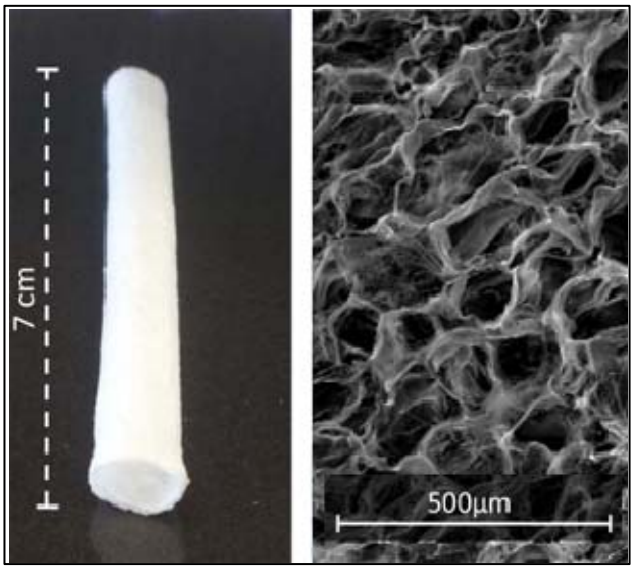

Figure 1. *Left:* Macroscopic view of a dry tubular collagen scaffold. *Right:* Scanning electron microscopy image of the tubular scaffold. A highly porous and homogenous structure can be observed.

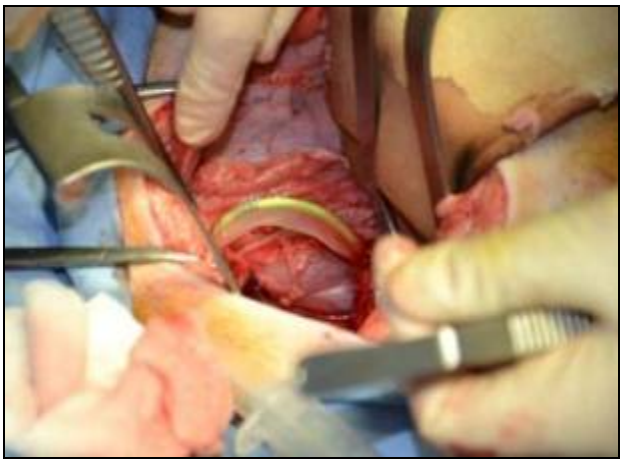

Figure 2. A 6 cm segment of the ureter was resected to create a clinically significant ureteral defect in the pigs. The tubular collagen scaffold was implanted by end to end anastomosis.

Effect of Soluble and Incorporated Ions on Proliferation and Differentiation of Human Mesenchymal Stromal Cells

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Introduction

Incorporating bioinorganics, which are either present in bone or known to affect bone formation and remodeling mechanisms, into bone graft substitutes is an interesting approach to improve their biological performance while retaining their synthetic character [1]. Cobalt ions (Co^{2+}), previously shown to be hypoxia-mimicking factors, can affect proliferation and function of osteoclasts and osteoblasts, as well as the process of vascularization [2-4]. Fluoride ions (F^-) are well-known for their anti-cariogenic properties [1]. A dose-dependent effect of F^- on the behavior of a variety of cell types has previously been demonstrated *in vitro* [4]. In the present study, the effects of Co^{2+} and F^-, in solution, or incorporated into calcium phosphate (CaP) coatings, on proliferation and differentiation of human mesenchymal stromal cells (hMSCs) were investigated.

Materials and Methods

Wells of treated tissue culture well plates were coated with CaP, without or with Co^{2+}, F^- or both, , using a two-step biomimetic method as described elsewhere [5]. hMSCs were seeded at a density of 10000 cells/cm^2 into uncoated and coated well plates and cultured in in either basic or osteogenic medium. Appropriate volumes of a Tris buffer solution containing $CoCl_2$ and NaF were added to the cell medium in the wells without CaP coating to reach the desired concentrations of Co^{2+} and F^- ions in the wells. Proliferation and osteogenic differentiation of cells were studied at different time points by quantifying DNA and ALP activity, respectively.

Results and Discussion

Co^{2+} ions did not have a significant effect on proliferation of hMSCs. Similarly, no effect of F^- ions on proliferation of cells was observed after 7 days of culture. However, after 14 days of culture in osteogenic medium, cells showed a significantly higher DNA content when treated with 10 µM of F^- compared to other conditions ($p<0.05$) (Figure 1). The DNA content of the cells cultured on CaP coatings was not affected by the presence of either ion, independent on the type of medium used (data not shown here).

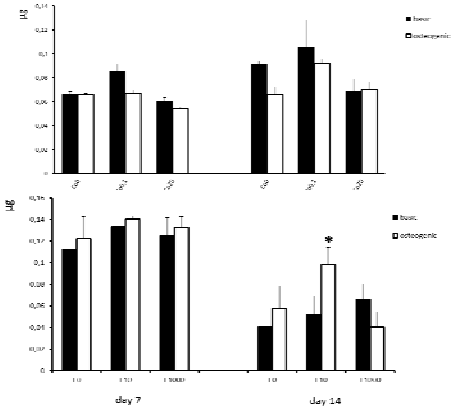

Figure 1. DNA content of hMSCs cultured with cobalt and fluoride ions in basic and osteogenic medium after 7 and 14 days.

Under osteogenic conditions, normalized ALP activity results (Figure 2) showed a decreasing trend in ALP activity of hMSCs by addition of F^- to the cell medium after 14 days of culture, with significant difference between the control and the high concentrations. F^- ($p<0.05$). Similar results were obtained on CaP coatings into which F^- was incorporated (data not shown here).

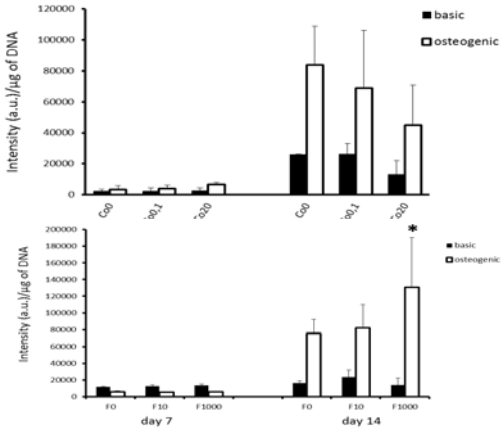

Figure 2. ALP activity of hMSCs cultured with cobalt and floride ions in basic and osteogenic medium after 7 and 14 days.

The dose-dependent data obtained so far in this study suggest that cobalt ions ions do not have any major effect on viability, proliferation and ALP expression of hMSCs in the examined range of concentrations. These findings are consistent with the data presented by Andrews et al. [3]. They observed that the presence of Co^{2+} ions at a higher concentration (100 µM) significantly decreased the proliferation, ALP activity and mineralization of human osteoblastic cells

F^- showed a dose-dependent effect on both proliferation and osteogenic differentiation of hMSCs, an effect that was more pronounced at later time points. F^- ions released from CaP coatings did not show a significant effect on proliferation of hMSCs. It was also observed that 1000 µM of F^- increased the normalized ALP activity of the cells after 14 days (data not shown here). Yang et al. showed a higher proliferation of cells on CaP coatings with higher F^- content.. However, this study was performed using murine osteoblasts unlike our study where hMSCs were used.

Conclusion

The results presented here showed a minor dose-dependent effect of Co^{2+} on proliferation and osteogenic differentiation of hMSCs. However, F^- was shown to positively affect proliferation, but to decrease differentiation of hMSCs in a dose dependent manner.

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Acknowledgement

This research is part of BioMedical Materials Inistitute Reserach Program (BMM, Bone-IP project), co-funded by Dutch ministry of economic affairs, agriculture and innovation.

References

1- L. Yang, B. Harink, P. Habibovic, Comprehensive Biomaterials V. 1, Elsevier, 299-312, 2011.
2- S. Patntirapong, P. Habibovic, P.V. Hauschka, Biomaterials 30, 548–555, 2009.
3- R.E. Andrews, K.M. Shah, J.M. Wilkinson, A. Gartland, Bone 49, 717–723, 2011.
4- W. Fan, R. Crawford, Y. Xiao, Biomaterials 31, 3580–3589, 2010.
5- L. Yang, S. Perez-Amodio, F.Y.F. Barrere-de Groot, V. Everts, C.A. van Blitterswijk, P. Habibovic, Biomaterials 31, 2976–2989, 2010.

Bisphosphonate-functionalized Gelatin Nanoparticles for Bone-seeking Applications

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Introduction: An emerging concept in biomaterials research involves the development of colloidal hydrogels, which are materials made solely of cohesive nanoparticles as building blocks for macroscopic scaffolds.[1] At the Department of Biomaterials, Radboud University Nijmegen Medical Center, a novel colloidal gel has been developed for bone-substituting purposes, which is made of both gelatin and calcium phosphate nanoparticles to mimic the chemical composition of bone tissue. Due to its favorable clinical handling, ease of functionalization, and cost-effectiveness, this nanoparticles-based gel has great potential for application as injectable formulation for tissue regeneration and/or programmed drug release of multiple biomolecules at predetermined release rates. Still, the mechanical integrity of these hydrogels in physiological fluids is largely unknown, although preliminary cell culture and degradation studies have shown that these colloidal gels appear to have a remarkable cohesive capacity. In order to increase the interaction between gelatin and calcium phosphate nanoparticles – and thus the cohesion of these composite gels – this study focuses on developing gels consisting of bisphosphonate-functionalized gelatin nanoparticles and calcium phosphate nanoparticles, since bisphosphonate groups bind strongly[2] to calcium phosphate. Furthermore, linking bisphosphonate molecules onto the surface of gelatin nanoparticles renders them bone seeking, as a potential drug carrier for cancer treatment.

Materials and Methods: Using a two-step desolvation method, gelatin nanoparticles were prepared as described previously,[1, 3] which were subsequently functionalized with aminobisphosphonate alendronate after glutaraldehyde crosslinking. After this crosslinking step, residual aldehyde groups were hypothesized to react with the amine groups of alendronate.

Results and Discussion: Fourier Transform Infrared Spectroscopy (FTIR) qualitatively indicated the presence of phosphorous atoms and phosphonate groups on the gelatin nanoparticles surface, and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) further confirmed that aminobisphosphonate alendronate molecules comprise at least ~ 4% of the total mass of functionalized gelatin nanoparticles (Fig. 1a shows the concentration of phosphorus for samples of 10 mg digested gelatin nanoparticles different functionalities, 4 mg dissolved aminobisphosphonate alendronate as reference, and 10 ml of HNO3 1% as reference used as medium for all the samples). Swelling ratio of the nanoparticles increased from ~ 9 up to ~ 30 after the bisphosphonate functionalization, which is equivalent to a 5% increase in their water content from ~ 90% up to ~ 95% (Fig. 1b). Scanning electron microscopy (SEM) also showed that the morphology and size of the lyophilized nanoparticles did not undergo a significant change and remained intact in comparison to the gelatin nanoparticles without phosphonate groups (Fig. 1c).

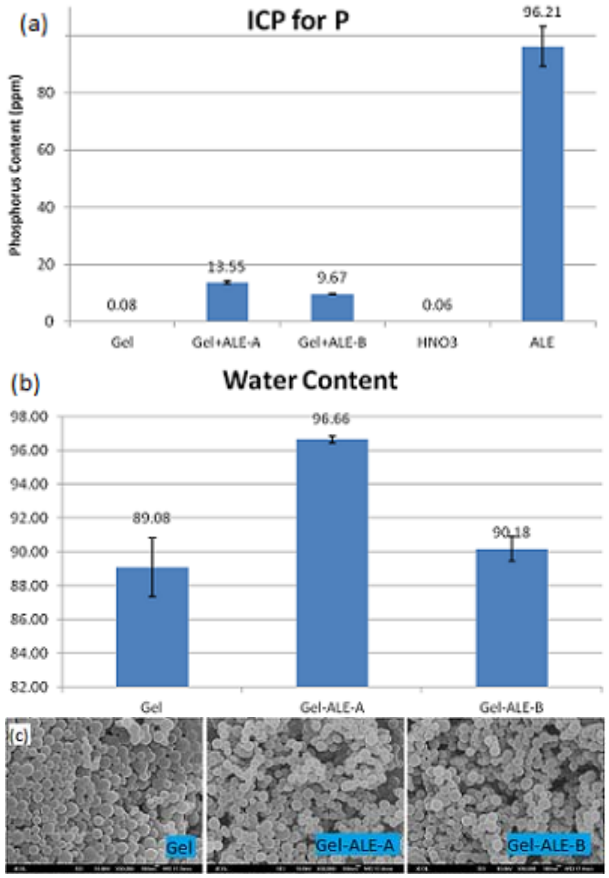

Fig. 1: (a) ICP-OES, (b) water content, and (c) SEM micrograph of bisphosphonate-functionalized gelatin type B nanoparticles (Gel-ALE-A and Gel-ALE-B refer to early and late-stage addition of alendronate, respectively) compared to non-functionalized gelatin nanoparticles (Gel).

Conclusions: Although the presence of phosphorous atoms on the gelatin nanoparticles was approved, additional investigations need to be performed to further recognize and evaluate the optimal condition and the extent in which alendronate can be linked to the gelatin nanoparticles.

References:

[1] H. Wang, M. B. Hansen, D. W. Lowik, J. C. van Hest, Y. Li, J. A. Jansen, S. C. Leeuwenburgh, Advanced materials 2011, 23, H119.

[2] S. Zhang, G. Gangal, H. Uludag, Chemical Society reviews 2007, 36, 507.

[3] a) S. Balthasar, K. Michaelis, N. Dinauer, H. von Briesen, J. Kreuter, K. Langer, Biomaterials 2005, 26, 2723; b) C. J. Coester, K. Langer, H. van Briesen, J. Kreuter, Journal of microencapsulation 2000, 17, 187; c) T. G. Shutava, S. S. Balkundi, P. Vangala, J. J. Steffan, R. L. Bigelow, J. A. Cardelli, D. P. O'Neal, Y. M. Lvov, ACS nano 2009, 3, 1877.

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Dendritic cell responses to biomaterials in presence of staphylococcal infection is influenced by the nature of the biomaterials

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Introduction: *Staphylococcus aureus* and *Staphylococcus epidermidis* are the major pathogens in medical device (biomaterial)-associated infection. The combination of a biomaterial and bacteria can provoke inflammatory reactions of non-expected nature. A major cell type orchestrating these immune responses are dendritic cells (DCs). To fully understand the immune responses in biomaterial-associated infection, insight needs to be obtained into maturation of DCs induced by staphylococci, by biomaterials and by the combination. The aim of this study is to assess how *S. epidermidis* and *S. aureus* influence DC cytokine secretion induced by three different materials; poly(L-lactic acid) (PLLA), poly (DL-lactic acid) (PDLLA) and poly(trimethylene carbonate) (PTMC).

Methods: Monocyte-derived DCs were cultured on PLLA, PDLLA or PTMC disks in the presence or absence of *S. epidermidis* or *S. aureus*. The level of cytokines secreted by DCs was assessed. The experiments were performed with cells of different donors.

Results: DCs cultured on PLLA, PDLLA and PTMC did not induce cytokine secretion. DCs cultured with *S. epidermidis* or *S. aureus* in absence of the biomaterials or on PLLA and PDLLA, secreted high levels of IL-10, IL-6 and IL-23. Significantly lower cytokine levels were measured when DCs were cultured on PTMC in presence of *S. aureus* and *S. epidermidis*.

Conclusion: In presence of *S. aureus* or *S. epidermidis* biomaterials differ in their capacity to induce DC cytokine secretion. The effect of the combined presence of biomaterials and staphylococci on DC maturation marker expression is in progress.

Functionalization of gelatin methacrylamide with tissue-derived matrix for cartilage regeneration

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Introduction
Decellularized tissue has been widely used as a promoter for cellular differentiation in regenerative medicine. In this project, we created a crosslinkable hydrogel derived from cartilage, meniscus and tendon tissue. The chondrogenic potential of chondrocytes and Mesenchymal Stromal Cells (MSCs) embedded in these tissue-derived matrix (TDM) hydrogels was evaluated.

Methods
Equine knee joints (n=8) were dissected and the cartilage, both menisci and the patellar tendons were harvested. All donors were pooled and the three types of tissue were separately milled, decellularized and digested with pepsin. When clear suspensions were obtained, methacrylic anhydride was added followed by dialysis. The success of methacrylation prodecure was evaluated with nuclear magnetic resonance. The TDM digests were freeze-dried and resolubilized in phosphate-buffered saline (PBS) at 10% (w/v) containing photoinitiator. Next, they were UV-crosslinked and the compressive modulus was measured. For cellular differentiation experiments the TDM digests were separately blended with gelatin methacrylamide (gelMA) (20:80%) and equine chondrocytes and MSCs were embedded (both 1 donor, 20 million cells/ml). These gels were cultured *in vitro* for 6 weeks in chondrogenic medium after which mechanical properties were determined. GelMA was used as a control group. Gene expression and cartilage matrix formation was analyzed by histology, biochemistry and immunohistochemistry.

Results
Cartilage, meniscus and tendon tissues were successfully decellularized and digested. Methacrylation of these digests resulted in crosslinked hydrogels with a stiffness ranging between 20-100 kPa. When the digests were blended with gelMA and cells were embedded, the stiffness ranged between 12-24 kPa. After six weeks of culture with chondrocytes, the stiffness of the gelMA only group remained unaltered, whereas the cartilage and meniscus group were significantly lower; the tendon group disintegrated during culture. In addition, the production of glycosaminoglycans (GAGs) and collagen type II was low in the cartilage and meniscus group, in contrast to matrix production in the gelMA control group. On the other hand, the stiffness of all TDM groups, as well as the control group cultured with MSCs, increased significantly. This was in line with abundant GAG and collagen type II formation in all MSC groups. Gene expression profiles of both cell types are currently being analyzed.

Discussion
Novel, crosslinkable hydrogels were created from matrix derived from cartilage, meniscus and tendon tissues. The *in vitro* chondrogenic differentiation of MSCs was unaltered by the addition of TDM, however chondrocytes were negatively affected. This confirms findings in earlier studies that show a poor performance of chondrocytes cultured on collagen type II matrices. The mechanisms behind the cell-matrix interactions are currently being studied.

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Near Infrared Light Triggered Gene Delivery Based on Poly(Amido Amine)s-Gold Nanoparticles Hybrid System

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Aim. A novel near infrared (NIR) light responsive gene delivery vehicle through incorporation of gold nanoparticles into the poly(amido amine)s (PAAs) and plasmid DNA complexes to achieve multifunctional theranostics (Fig. 1). The aim of this research is to evaluate *in vitro* and *in vivo* colloidal stability of the nanoparticles, NIR responsiveness on gene delivery, cytotoxicity, and transfection efficiency.

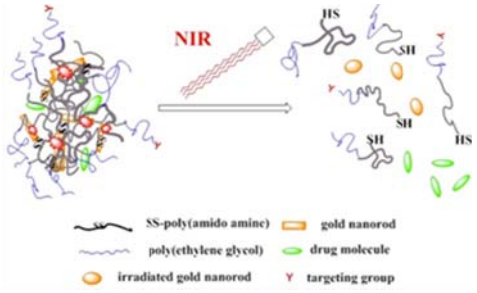

Fig. 1 Schematic illustration of near-infrared light triggered release system

Methods. Gold nanorods (GNRs), were successfully synthesized via the seed mediated growth method [1]. Poly(amido amine)s were obtained through Michael-type pol-addition of the primary amine monomer 1-amino-4-butanol (ABOL) to *N, N'*-cystamine-bisacrylamide (CBA) in the presence of $CaCl_2$ as a catalysis in a mixture of H_2O and CH_3OH (Fig.2 a). Polymer was obtained via ultrafiltration and lyophilization [2]. Different weight ratios of PAAs and DNA, with different amounts of GNRs were evaluated to find the optimal composition of the GNR polyplexes. The hybrid polyplexes were characterized with dynamic light scattering (DLS), zeta-potential, UV-Vis spectrometry and transmission electron microscopy (TEM) (Fig. 2 b).

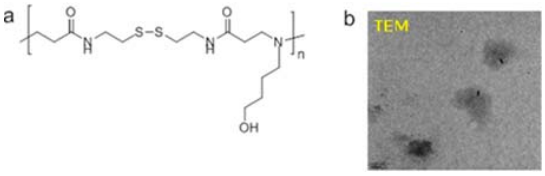

Fig. 2 a: Structure of PAAs; b: TEM of hybrid polyplex.

Results. Well-defined GNRs have been prepared with good uniformity (~15 nm in width, ~50 nm in length). The longitudinal surface plasmon resonance (LSPR) in the NIR range is at approximately 800 nm. DLS results showed that upon addition of poly(amido amine)s hybrid polyplexes were successfully prepared with nanosized dimensions ranging from 80 nm to 200 nm and positive charged. The presence of GNRs stabilized the polymer/DNA polyplexes. UV-Vis spectrometry showed a typical absorbance peak in the NIR window (around 800 nm), suggesting that the GNRs were present in the hybrid polyplexes without compromising their colloidal stability. TEM results confirmed that the size of hybrid polyplexes ranges from 100 nm-200 nm and that GNRs were indeed incorporated into the polyplexes (Fig. 2 b).

Conclusions. The results show that stable hybrid polyplexes consisting of PAAs, GNRs, and DNA were successfully prepared without compromising the surface plasmon resonance properties of GNRs. We are currently further characterizing the resultant hybrid polyplexes; cell transfection and toxicity studies will be carried out, and the response of hybrid polyplexes to NIR laser irradiation will be explored.

References.

[1] Nikoobakht, B. and El-Sayed, A.M., Chem Mater, 2003, 15, 1957-1962
[2] Zintchenko, A., van der Aa, L. J. and Engbersen, J. F. J., Macromol. Rapid Commun., 2011, 32, 321-325

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Extending the surgical armamentarium for bladder reconstructions: a seamless spherical hollow collagen bladder

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Introduction

One treatment option of bladder-related diseases, e.g. in case of cancer, infections and traumatic injuries, is radical cystectomy in combination with urinary diversion techniques and construction of an autologous new 'bladder'. Currently, surgical interventions rely on the use of autologous tissues harvested from the gastrointestinal tract, but this procedure is associated with complications[1]. The field of tissue engineering and regenerative medicine may offer alternatives by constructing biological substitutes[2]. In this study we present an adjustable one-step procedure to construct standardized resorbable seamless hollow scaffolds, including seamless anatomical branching, for reconstruction of bladder tissue.

Materials and Methods

Insoluble type I collagen suspensions (0.7% (w/v) collagen in 0.25 M acetic acid) were poured into a custom aluminum mold with three tubular appendices (Fig. 1), designed to mimic the bladder of an adolescent human. Three appendices were included to provide anastomosis sites for the urethra and ureters. To construct scaffolds with a hollow lumen and a wall thickness of 10 mm, the mold containing the collagen suspension was frozen in a computer-controlled freezing bath for 3 min. at -80 °C or 12 min. at -20 °C. Non-frozen collagen was removed from the mold, the frozen construct was lyophilized, and subsequently γ-irradiated (25 kGy).

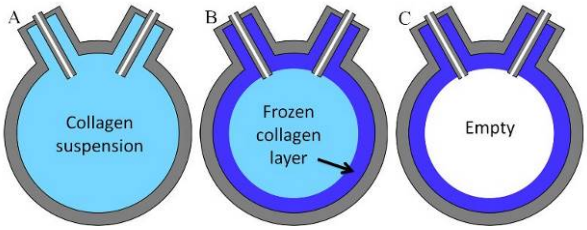

Fig 1. Schematic overview of scaffold production. A) A mold (grey) mimicking the shape of a bladder, including appendices. The mold was filled with collagen and frozen (B) in a freezing bath. After removing non-frozen collagen (C), the frozen construct was removed from the mold. This yielding a hollow frozen construct. A scaffold was constructed by lyophilization.

Magnetic resonance imaging (MRI) was used to visualize the scaffold structure and subsequently generate a 3D model of the scaffold. Scanning electron microscopy was used to characterize scaffold morphology. Temperature measurements during the freezing process were performed using thermocouples. Biocompatibility was evaluated by culturing primary porcine urothelial cells on the lumen for 7 days.

Results

Hollow spheres were successfully constructed using the technique described. This was confirmed by MRI; the volume of the hollow sphere was ± 380 cm^3. Collagen suspensions were frozen with freezing rates of 3.8 ± 0.4 and 0.5 ± 0.0 °C/min, which resulted in pore sizes of 38 ± 2 µm and 70 ± 17 µm, for freezing at -80 °C and -20 °C, respectively. SEM analysis reveal a unidirectional pore structure, indicating the inward growth of ice crystals. Perpendicular cross-sections showed that pores had a hexagonal morphology. The inside of the scaffold was mostly closed and had only few pores. The outside had open pores. The morphology and pore sizes in appendices were comparable.

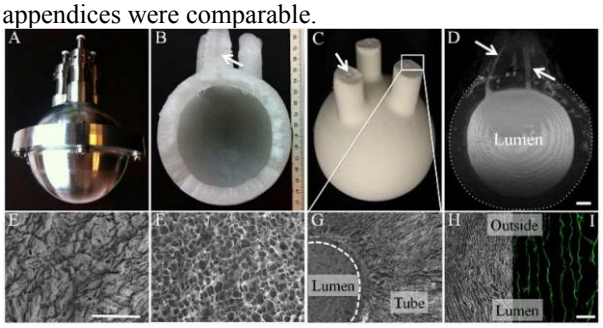

Fig 2. Hollow sphere overview. A) mold with 3 appendices; B) cross-section of frozen construct; C) freeze-dried scaffold; D) 3D model of MRI images (white arrows indicate lumen of the appendices, white dotted line indicates outside of the scaffold, grey color indicates lumen); E-H: SEM of the scaffold after crosslinking; E) inside; F) Outside; G) an appendix; H) cross-section; I) type I collagen staining of scaffold cross-section 50x, bar = 200 µm.

Cells seeded onto the scaffolds were able to attach, proliferate, and differentiate, as visualized by confluent layers of urothelial cells expressing cytokeratin 18.

Discussion

The methodology to construct hollow collagen spheres is reproducible and aids adjustments in e.g. size, volume, wall thickness and pore size. The unidirectional structure and open outside may favor *in vivo* influx of cells and nutrients. Omentum wrapping of the construct may result in good vascularization and improved treatment options for cystectomy patients.

Conclusion

The technique to construct hollow collagen spheres is an easy, versatile and standardized methodology to develop scaffolds for bladder regeneration.

References

1. Atala A et al. J. Urology,1993
2. Langer R et al. Science, 1993

Enzymatically crosslinked Dextran-Tyramine micro hydrogels for improved beta cell function

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Introduction

Tissue engineering aims at re-creating functional implants to replace or improve biological tissues, such as insulin responding implants to treat diabetes type I patients. However, creating the required complexity to enable a target tissue's function *ex vivo* has remained a grand challenge. Generally, cells, biomaterials and growth factors are combined into a single homogeneous construct. This construct is expected to be optimal for both encapsulated cells e.g. beta-cell function and host-implant interactions e.g. vascularisation. Unfortunately, most encapsulated cell types require substantially different conditions than those desired for host-implant interactions. We aim to resolve this lack of biological complexity by first encapsulating cells in cell-instructive micro-hydrogels (from now called microgels), which can be incorporated in a bulk material that is optimal for host-implant interactions. In this study, we first investigated the suitability of enzymatically crosslinked Dextran-tyramine (Dex-TA) microgels to ionically crosslinked alginate and photocrosslinked Poly(ethylene glycol)-diacrylate (PEGDA) as biomaterial for the microgels. Subsequently, we encapsulated beta cell lines in Dex-TA microgels in order to create islet-like building blocks.

Materials and methods

We developed a droplet generating microfluidic chip that allows enzymatic, ionic and photo-initiated crosslinking. In the chip, droplets of cell laden hydrogel precursor were formed using emulsification, and crosslinked in a delay channel, which together results in the production of cell seeded microgels. Human mesenchymal stromal cells (hMSCs) and two beta-cell lines, MIN6 and INS1E, were investigated on cell survival and metabolic activity in PEGDA, alginate and Dex-TA microgels.

Results and Discussion

The cell survival of encapsulated hMSCs in both alginate and PEGDA microgels was approximately 80%. In contrast, hMSCs survival in Dextran-tyramine microgels was over 95%. Furthermore, hMSCs remained metabolically active in Dextran-tyramine and alginate microgels, while the metabolism of hMSCs in PEGDA strongly decreased. Finally, where the alginate microgels are mechanically weak and easily disassemble under mild manual manipulation, the Dextran-Tyramine and PEGDA microgels possess sufficient mechanical strength for downstream handling. The initial survival in Dex-TA of both beta-cell lines was between 80 and 90 percent. However, encapsulated INS1E cells became metabolically inactive after 7 days, and the metabolic activity of MIN6 cells decreased to 35% after 11 days. This suggests that the biomaterials lack relevant biological stimuli that are required by the cells for their continued functioning. Based on the cell survival, metabolic activity, and need for inclusion of biological stimuli, Dex-TA was found the most suitable biomaterial to create the islet-like microgels.

Conclusions/summary

Our work shows that microfluidic encapsulation of cells in Dex-TA based microgels does not adversely affect hMSC viability and metabolic activity, compared to PEGDA and Alginate. Furthermore, to ensure continued functioning of the beta-cells, modifications of the Dex-TA is needed.

Intercellular communication in the equine joint: lipidome analysis of cell-derived vesicles

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Introduction

Cell-derived vesicles (nanometer-sized carriers released by most cell types) are increasingly recognized as major vehicles for intercellular communication. This is probably also true in the joint, where such vesicles in the synovial fluid can supposedly play important roles in the communication between chondrocytes and other cells in the surrounding articular tissues during health and disease.

The aim of this study is to verify the presence of cell-derived vesicles in native equine synovial fluid and analyze the membrane lipid composition thereof. We hypothesize that the lipidome could be specific for vesicle subsets and can reveal markers by which different vesicle types can be discriminated. These particular markers are not yet available in the vesicle research field.

Similar analyses will be planned for cell-derived vesicles produced by chondrocytes and synoviocytes during *in vitro* cell culture. In this way we hope to gain more insight into the different vesicle types present in the joint and their cellular origin.

Materials and Methods

Equine synovial fluid samples of healthy horses and culture media from chondrocyte and synoviocyte cell culture underwent a multistep centrifugation protocol in order to pellet cell-derived vesicles according to size. Subsequently, vesicles were floated into a linear sucrose gradient for separation according to buoyant density. Total protein samples of vesicles were analyzed for general vesicle markers by use of SDS-PAGE and Western Blotting. For lipidome analysis of these vesicles, lipids were extracted (Bligh & Dyer method) and run over an HPLC column to separate lipid classes, followed by mass spectrometry (LCMS) to detect specific lipids.

Additionally, vesicles were fluorescently labeled with PKH67 and co-cultured with either chondrocytes or synoviocytes. Binding and/or uptake of vesicles by cells was subsequently measured with FACS.

Results

Different subsets of cell-derived vesicles are present in equine synovial fluid, defined by differences in size and buoyant density. Analysis of vesicle membrane lipids showed distinct lipid profiles for so-called ‘exosomes’ (typical size range: 40-100 nm) versus ‘microvesicles’ (typical size range: 100-1000 nm) (Figure 1). Analysis of cell-derived vesicles from cell culture media is still ongoing, as well as the vesicle-cell co-culture experiments.

Conclusion

Up to now, no discriminating markers for specific vesicle subsets are available. According to the preliminary results of this study, lipidome profiling may provide such markers. The results of the *in vitro* cell culture experiments might provide new insights into the (sub)cellular origin of vesicles in the joint and might lead to the identification of discriminatory markers for different vesicle subsets. Ultimately, studying the communication between cells in the joint may be of great benefit for the progress of cartilage regenerative medicine.

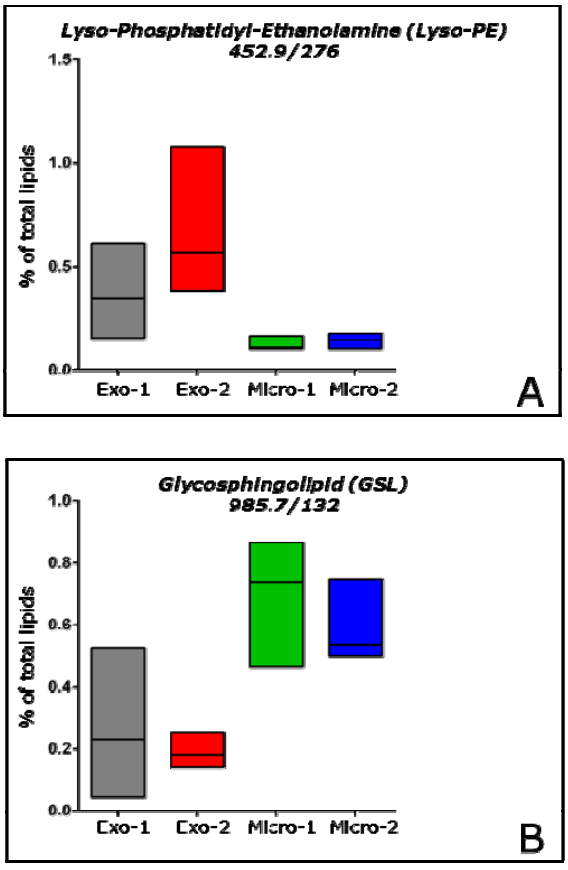

Figure 1. *Pilot study for lipidome analysis (LCMS) of synovial fluid derived vesicles from two horses. Graphs are examples of lipids present in different concentrations between ‘exosomes’ (Exo-1; Exo-2) and ‘microvesicles’ (Micro-1; Micro-2). Each data group represents single measurements of 3 vesicle containing sucrose fractions. Data are normalized for total lipids in each sample.* ***A)*** *Lyso-PE (452.9/276) is found at higher concentrations in exosomes compared to microvesicles.* ***B)*** *GSL (985.7/132) is more pronounced in microvesicles compared to exosomes.*

Towards Bioactive Supramolecular Cucurbit[8]uril Hydrogels

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Introduction

Within the field of tissue engineering hydrogels are investigated in order to obtain an ideal cell culture environment. This is because the extracellular matrix (ECM) also has gel-like properties. Supramolecular hydrogels are a good choice as ECM mimics, because they are formed via well-defined, directed, supramolecular interactions. This makes these hydrogels unique in their structural control. Furthermore, bioactivity can be introduced via directed, supramolecular interactions.

The aim of this research is to develop a supramolecular hydrogelator network based on the host-guest interactions of cucurbit[8]uril (Q8) and the phenylalanine – glycine – glycine (FGG) peptide motif[1]. It is proposed that by functionalization of poly(ethylene glycol) (PEG) with the FGG motif, a cross-linked network is formed due to the host-guest interactions of two peptides with Q8 (Figure 1). Finally we aim at the introduction of bioactivity by addition of FGG-tagged proteins or peptides to this host-guest system.

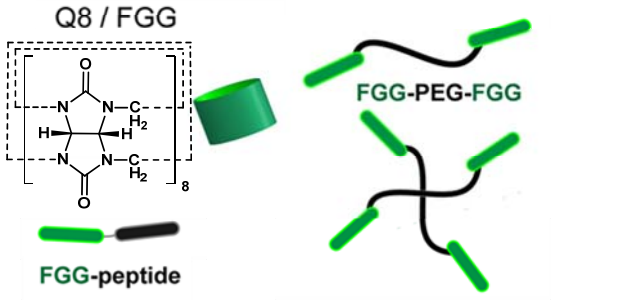

Figure 1. General approach to the Q8-FGG hydrogel system. Q8 is proposed to function as a supramolecular cross-linker between PEG-FGG polymers to form a 3D network.

Library design

A library of FGG-PEG compounds was designed containing both bifunctional and 4-armed PEGs with molecular weights of 2k and 10k. For a detailed study of the complexation with Q8, a bifunctional monodisperse compound with 5 ethylene glycol monomers was synthesized (Figure 2).

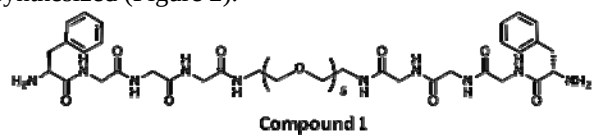

Figure 2. Monodisperse oligo(ethylene glycol) end-functionalized with FGG.

Model compound complexation

Various techniques, such as ITC, NMR, QTOF and GPC, were used to determine the thermodynamic parameters of the complexation, and the size of the complexes over a concentration range of 0.05 to 1 mM. A 1:1 complex of model compound **1** to Q8 is formed which consists of ring structures. Based on the binding affinity determined by ITC, the effective concentration of the bivalent compound was calculated to be in the range of 750 µM.

Introduction of bioactivity

For the supramolecular introduction of bioactivity, FGG-tagged proteins were used. It is necessary that these proteins can compete with the FGG-PEG guests for complexation with Q8. To obtain insight in the dynamics of this competition, the exchange of FGG-tagged monomeric yellow fluorescent protein (FGG-mYFP) with the complexes of Q8 and compound **1** was studied using fluorescence anisotropy measurements. It was shown that formation of ring structures of Q8 and **1** is more favorable than the dimerization of FGG-mYFP by Q8.

Towards a hydrogel

For the formation of a hydrogel, a 3D network of FGG-PEG constructs and Q8 has to be formed. This can be achieved by using solely a 4-armed PEG construct, or by combining both the bifunctional and 4-armed PEG constructs. Gelation experiments were performed with the 10k polydisperse 4-armed FGG-PEG. A 10 wt% gel was formed and confirmed by vial inversion test.

Conclusions and outlook

In the concentration regime measured, the complexes of model compound **1** and Q8 form rings with a high K_{ass}. Furthermore it was shown that compound **1** is a good protein dimerization inhibitor.

The 4-armed FGG-PEG constructs form hydrogels. In a next step bioactivity will be introduced in the host-guest hydrogels, and the dynamics of protein incorporation will be studied. This opens the door to specific protein functionalization of these hydrogels.

References

1. H.D. Nguyen, D.T. Dang, J.L.J. van Dongen, L. Brunsveld, *Angew. Chem.* **2010**, *122*, 907-910

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Enzymatic control of chitosan gelation for delivery of periodontal ligament cells

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Introduction

Periodontitis, an inflammatory disease characterized by the loss of periodontal tissues, is the main cause of tooth loss in adults. Periodontal ligament cell (PDLC) based strategies hold the promise for periodontal tissue regeneration. To deliver PDLCs in the periodontal defects, injectable in situ forming chitosan hydrogel is a potential candidate due to its antimicrobial activity, anti-inflammatory effects, easy handling, and good conformation to irregular defects. The aim was to optimize enzymatic control over gelation of chitosan-based hydrogels for the delivery of periodontal ligament cells (PDLCs). Chitosan gels can be formed by increasing the pH of acidic chitosan solutions by means of hydrolysis of urea using the enzyme urease, but the kinetics of this process have not been investigated in detail before.

Materials and methods

The effect of urea and urease concentrations on the pH and osmolarity of chitosan-based hydrogels as well as their gelation time, rheological properties, morphology, water content, swelling and degradation rate. Further, the suitability of the optimized hydrogel for delivery of PDLCs was evaluated by assessing the viability of encapsulated PDLCs as well as the proliferation and differentiation of cells collected upon hydrogel degradation.

Results

The gelation time, strength and degradation rate of the chitosan hydrogels could be controlled precisely by variation of the urea and urease concentrations. Hydrogels formed at low urease concentrations revealed higher storage moduli (Fig. 1).

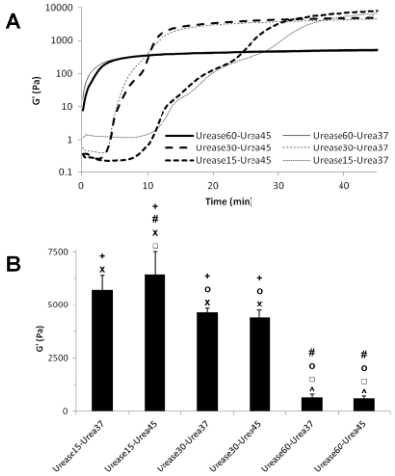

Fig. 1 Rheological characterization of chitosan hydrogels

The higher strength of hydrogels formed at lower urease concentration also reduced their degradation rate, which resulted in a remaining weight of up to 60% after soaking in lysosyme-containing PBS for 30 days (Fig. 2).

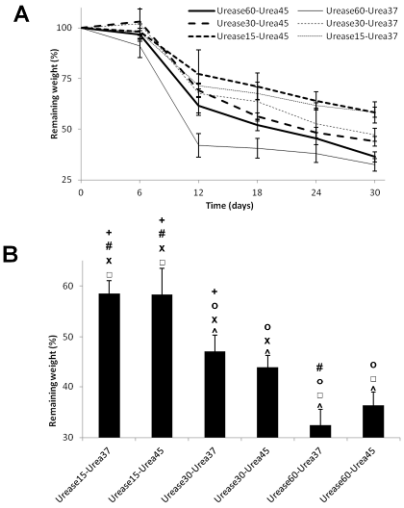

Fig. 2 Degradation curves for the various hydrogels after soaking for 6-30 days in PBS containing 1.5 µg/ml lysozyme.

PDLCs remained viable inside these hydrogels for up to 30 days. Cells released from the hydrogel upon degradation and collected after 3, 15 and 30 days were able to proliferate and osteogenically differentiate.

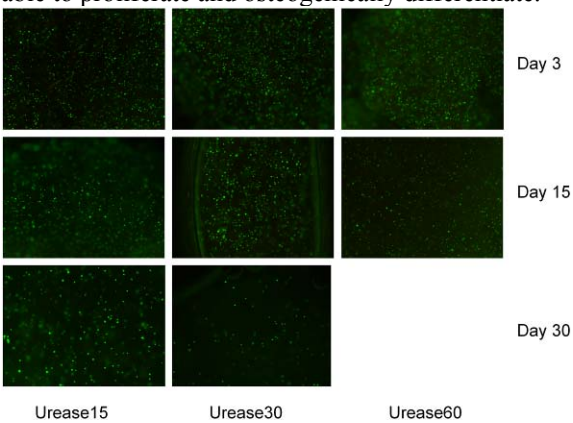

Fig. 2 Viability of PDLCs incorporated into chitosan hydrogels containing 15, 30 or 60 U/ml urease after 3, 15 and 30 days, visualized by LIVE/DEAD staining.

Conclusion

The current study demonstrated that gelation time, strength and degradation rate of chitosan hydrogels can be fine-tuned by controlling the pH upon gelation through variation of the concentrations of urea and urease. PDLCs remained viable upon encapsulation within these hydrogels for up to 30 days. Cells that were collected from the hydrogels after 3, 15 and 30 days were able to proliferate and osteogenically differentiate. In summary, the enzymatic control over the gelation of chitosan hydrogels offers options for the delivery of periodontal ligament cells via an injectable cytotherapeutic system.

Classification of cell shapes as tool to define cell response within a large dataset

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Introduction

It is well known that cell shape has an effect on cell function, and that by manipulating cell shape, we can direct cell fate. This phenomenon opens new opportunities for the development of biomedical materials. Growing cells on different topographies induces a great variety of different cell shapes, and this can thereafter modulate cellular function. To obtain a variety of cell shapes, we applied a high-throughput screening approach to determine cell response to more than 2000 randomly generated topographies. Cell morphology was captured by high content imaging and analysis of images generated a large dataset with different descriptors of cell shape. The aim of this research was to classify cells with different shapes based on combinations of these descriptors.

Method

We used a software package (CellProfiler Analyst) that provides us the possibility to randomly select an image from the whole dataset, no matter its size, and to put that image in a specific window that corresponds to a certain cell class. While the researcher visually classifies the images, the algorithm defines the corresponding descriptors, which are set of measured parameters linked to the images. As soon as enough data are provided, an algorithm can select a set of descriptors that correspond to cell class. The advantage of this approach is that a human controls the analysis and the results can be always verified by eye and corrected if needed.

Results

The dataset from the screen contained images of human mesenchymal stromal cells in 16 replicates on more than 2000 different topographies. Classes were determined based on cell shape, nuclear shape, and actin structure. We found a large variation in topography-induced cell shape, but it was possible to distinguish common features.

In total, we defined 10 different classes. Below, we provide information about two of them, based on cell shape that we find within our screen data. The first class is referred to as “sticks” (Fig 1A), which are elongated cells that do not create any branches. In this class, the cytoskeleton follows the outlines of the features and adopts its shape to the space between the primitives.

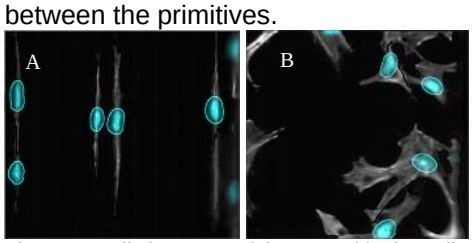

Figure 1. Cell shape: A: sticks; B: multipolar cells.

A second class that we defined are "multipolar" cells (Fig 2B), in which the cytoskeleton is not restricted by topographical features and protrusions are observed in various directions.

Discussion

CellProfiler Analyst was able to find “sticks” with an accuracy of 86% for true positive and 97% for true negative cells, while the accuracy for multipolar cell type was 58% for true positive and 78% for true negative. The difference in the accuracy can be explained by two factors. Firstly, the complexity of the cell phenotype affects classification, where sticks are much less complex and are not so variable in their shape as branched cells. Secondly, the subjectivity of researcher also can lead to a lower accuracy for complex phenotypes. In this case, the advantage of a human assessing the data by eye becomes a disadvantage. However, these two classes are mutually exclusive, such that once highly accurate classes are identified, classification of very complex classes can be done on the rest of data.

Conclusion

This study shows that using a machine-learning approach, it is possible to classify very complex data, in which cell morphology is affected by topographical features. Our ability to use this classification is very important as it enables a systematic study of big data, and distinguishes diverse cell response for further analysis.

In the next phase of this project, we use this classification to select topographies that tend to maintain a certain cell phenotype, and test the universality of the instructions from topography. Furthermore, the selected topography will be used for microarray experiments. Results of this study will lead to new advances in our understanding of how surface cues can influence cell behavior, enabling the improved design of materials for biomedical applications.

Acknowledgement

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Establishment of an early stable vascular network promotes the induction of ectopic bone formation

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Introduction:

Adequate vascularisation is crucial for the induction of bone formation and has been one of the main research focuses the past years. It has been shown that endothelial progenitor cells (EPCs) can contribute to the induction of vascularisation. Notably, two types of EPCs can be distinguished, early EPCs (E-EPCs) and late EPCs (L-EPCs), each with their specific characteristics. Whereas L-EPCs have been used extensively in combination with multipotent stromal cells (MSCs) to induce vascularisation in bone constructs, there is a lack of knowledge about the use of E-EPCs in vascularized bone tissue engineering. Therefore, we investigated the use of both EPC subtypes in MSC-based constructs to induce stable vessel formation *in vitro* and *in vivo* and subsequent bone formation in subcutaneous pockets.

Methods:

Early- and late goat EPCs were assessed for their tubular network forming capacity by 3D angiogenesis assays.[1] In addition, the effect of combining both subtypes with or without MSCs was evaluated. To evaluate vessel formation and bone formation *in vivo*, cells were embedded in 200µl Matrigel (BD Biosciences) plugs and implanted subcutaneously in nude mice (n=23). Five groups were formed: 1) MSC1, 2) MSC2, 3) E-EPC/MSC1, 4) L-EPC/MSC1 and 5) E-EPC/L-EPC/MSC1. Cell loading was kept equal in all groups (except group 1, which received half of the dosage). Each mouse received all constructs. All constructs contained 20% (w/v) of biphasic calcium phosphate (BCP) particles of 100-200 µm diameter. Implants were retrieved after 2 weeks and 6 weeks and evaluated for vessel- and bone formation, respectively. Implants were processed for paraffin embedding and stained with Goldner's trichrome to evaluate vessel formation. Basic fuchsin/methylene blue stained MMA sections revealed bone formation.

Results:

When early goat EPCs (E-EPCs) and late goat EPCs (L-EPCs) were combined in Matrigel plugs *in vitro*, no network formation was observed. However, combining both cell types with goat MSCs resulted in partially connected networks. *In vivo* studies revealed that 50% of implanted E-EPC/MSC constructs showed the formation of perfused vessels after 2 weeks whereas percentages in constructs including only MSCs were slightly lower. Moreover, replacement or partial replacement of E-EPCs by L-EPCs inhibited the formation of perfused vessels after 2 weeks. After 6 weeks, the numbers of formed vessels was similar in all EPC-based constructs. Bone was formed in all constructs; a higher incidence was found in MSC2 constructs (100%) and E-EPC/MSC1 constructs (80%) compared to MSC1 constructs (40%), L-EPC/MSC1 (40%) and E-EPC/L-EPC/MSC1 constructs (40%). Altogether, our findings indicate establishment of vascular networks by E-EPCs and L-EPCs at different time points and the possible importance of early vasculogenesis to induce ectopic bone formation.

Discussion:

We demonstrate that early- and late goat EPCs are able to form vascular networks in the presence of goat MSCs *in vitro* and *in vivo*. Upon implantation, early EPCs outperform late EPCs in terms of formation of fully erythrocyte-perfused vessels at an early time point (2 weeks). Vasculogenesis induced by both subtypes at a later time point (6 weeks) is similar. However, a higher incidence of bone formation is found when constructs showed a high density of perfused vessels after 2 weeks. In conclusion, our findings show the application of two subtypes of EPCs that induce a differential timing of -vascularisation in ectopic bone constructs.

References:

[1] Fedorovich et al. Tissue Engineering Part A. (2010) 16(7): 2355-2367

Acknowledgements:

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Regenerative Medicine of the Diaphragm: Development and Preclinical Evaluation of Collagen-based Scaffolds

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Introduction

Large congenital diaphragmatic defects are repaired using patches of most often polytetrafluoroethylene or decellularised porcine small intestinal submucosa, but both materials give rise to a high percentage of reherniations. As porous type I collagen scaffolds have previously been shown to be successful in the (preclinical) treatment of several other congenital birth defects, we hypothesised that they may be useful in the treatment of diaphragmatic defects, as these scaffolds may be replaced by the patient's own tissue and thus grow with the child, We therefore investigated the use of collagen-based scaffolds in animal models for diaphragmatic hernia.

Materials and Methods

Random round-pored porous type I collagen scaffolds were prepared using freezing at -20°C and lyophilisation, and strengthened by crosslinking. Scaffolds with radially oriented pores, to mimic the original diaphragm muscle fibre orientation, were created by using an inward-out freezing technique. To improve muscle cell and blood vessel ingrowth the glycosaminoglycan heparin and the growth factors vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were attached to the round-pored scaffolds. Scaffolds were implanted in a surgically created diaphragmatic defect in rats and analysed with histology up until a maximum of 24 weeks. Finally, collagen scaffolds were strengthened with Vicryl and analysed in a surgical lamb model for diaphragmatic hernia.

Results and Discussion

Random-round pored crosslinked type I collagen-based scaffolds proved feasible for use in closure of diaphragmatic defects in rat, as no reherniations were observed. Moreover, cellular infiltration and replacement of the scaffolds with collagen was observed, as was occasional muscle cell ingrowth.

To further improve cellular infiltration into the scaffold, scaffolds with a pore orientation mimicking the diaphragmatic fibre architecture were developed. By creating scaffolds with a radial pore orientation, cellular infiltration into the scaffold was increased in the rat model for diaphragmatic hernia. Cells were found to align to the pores. However, as muscle and blood vessel ingrowth into the scaffolds remained rather limited, we aimed to increase this by the addition of the bioactive molecules heparin, VEGF and HGF. Indeed, heparin induced blood vessel ingrowth into the round-pored scaffold at two weeks after implantation. Growth factor addition led to more growth of blood vessels over the scaffold macroscopically, but this could not be confirmed using immunohistochemistry. Muscle ingrowth remained limited. The use of growth factors should be further investigated in terms of optimal dose and release pattern.

As analysis of materials in a large animal model is needed for better translation to the human situation, collagen scaffolds strengthened with a Vycril polymer were implanted in the muscular part of the diaphragm of 2-3 weeks old lambs. Although scaffold attachment to the surrounding tissue was good, scaffolds proved too weak as bulging of the abdominal content into the thoracic cavity, covered by a thin layer of tissue (eventration) was found in all animals after 6 months of implantation. Stronger scaffolds, for instance using a slower-degrading polymer, should thus be used in further studies.

Conclusion

In this project we aimed to develop collagen-based scaffolds for treatment of diaphragmatic defects. Although shown feasible in a rat model, especially after adjustment of the pore orientation and the addition of heparin, we should now focus on a slower-degrading scaffold before use in children may be considered.

Acknowledgements

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Biological and tribological comparison between PEOT/PBT, PCL and PLDLLA RP plotted scaffolds

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Introduction. In tissue engineering, the goal is to regenerate and/or restore malfunctioning tissue. In this aspect, a scaffold provides a temporary structure for cells to attach to and form tissue. Common biomaterials used for scaffolds are ceramics and polymers. While ceramics are known to have excellent osteo-inductive and osteo-conductive properties, polymers do not – or only marginally – possess these properties. However, polymers can be processed with various techniques, therefore allowing for any overall scaffold shape. One such technique is Rapid Prototyping (RP) with which highly controllable and reproducible scaffolds can be made. For load bearing applications, the scaffold needs to be stiff enough and ideally have a low friction coefficient, thus enabling smooth articulation. The biomaterial itself can elicit a specific biological response in-vitro and/or in-vivo when implanted and has a specific intrinsic friction coefficients. RP scaffolds were fabricated from PCL, PLA and PEOT/PBT, which are well described (1-3). They were biologically compared by seeding hMSCs in the scaffolds and exposing them to basic, osteogenic or chondrogenic medium for 28 days. Cell free scaffolds were also tested for their tribological performance. The best performing biomaterial from both analyses was chosen to determine the tribological performance with cell cultured scaffolds.

Materials and Methods. Scaffolds of 8 mm were obtained through 3D fiber deposition. 300 PEOT 55 PBT 45 (PolyVation), PCL (Mw 65.000, Sigma Aldrich) and Poly L-DL-Lactic Acid 80/20 (Purac) were used as biomaterials. Collagen type I coated scaffolds were seeded with hMSCs (P3, 500.000/scaffold) and kept in culture for 7 days in proliferative medium followed by 28 days in either basic, osteogenic or chondrogenic medium. For each timepoint (7, 14 and 28 days), three scaffolds were harvested for DNA, GAG and ALP quantification and one for cell localization and ALP staining. A Nanotribometer CSEM was used to obtain the friction coefficients with a load of 1N at 2 mm/s in PBS. Empty scaffolds were used to determine the biomaterial's friction coefficient. For the final tribology study, scaffolds with hMSCs (same donor, P3) were cultured in basic or chondrogenic medium.

Results and Discussion. Irrespective of the polymer, the amount of ECM formed was highest when exposed to osteogenic medium, while chondrogenic medium had the least ECM. Although differences are small, SEM images showed slightly more ECM in the 300/55/45 scaffolds compared to PCL and PLDLLA. The amount of GAG/DNA was highest in 300/55/45 after 28 days (17.6 ug GAG/ug DNA) while for PCL and PLDLLA it was 7.7 and 6.1 ug GAG/ug DNA respectively (figure 1a). ALP/DNA showed again highest values for 300/55/45 compared to PCL and PLDLLA (figure 1b). Interestingly, the highest values were not seen in the osteogenic medium cultured, but in the chondrogenic medium cultures (~ 3.5 fold). ALP staining confirmed this finding for PCL but not for 300/55/45. The tribological performance of the scaffolds showed again 300/55/45 as the best performing material, with a friction coefficient of 0.5 vs 0.9 and 1.7 for PCL and PLDLLA respectively, table 1. Scaffolds from 300/55/45 cultured with cells were chosen to do additional friction studies. When cultured in basic medium, the friction was 0.08, while in chondrogenic medium it was 0.19.

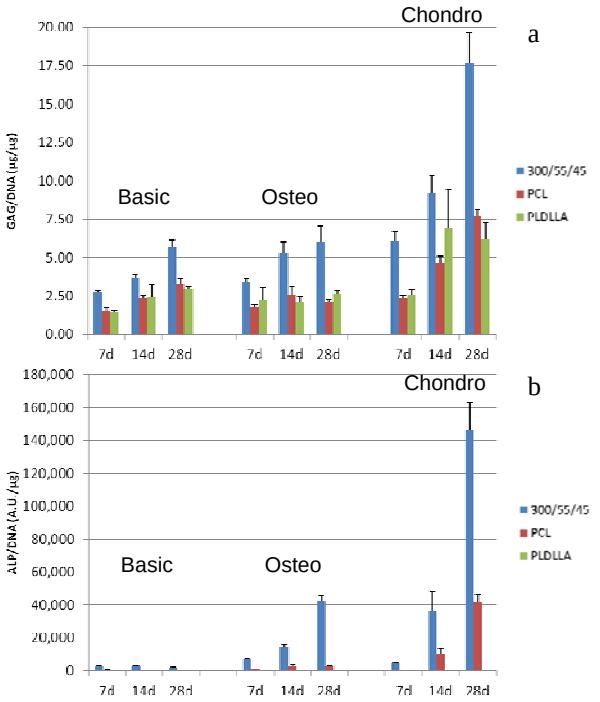

Figure 1 GAG/DNA (a) and ALP/DNA (b) for 300/55/45 (blue), PCL (red) and PLDLLA (green)

Table 1 Friction coefficient

	PBS	Basic medium	Chondrogenic medium
300/55/45	0.5	0.08	0.19
PCL	0.9	X	X
PLDLLA	1.7	X	X

Conclusions/Summary. These results show that the biological response of hMSCs seeded scaffolds, varies with the polymer used. Overall, 300/55/45 showed to have more ECM and higher GAG and ALP values than PCL and PLDLLA. The tribological performance of 300/55/45 was also better than other polymers and improved when hMSCs were cultured in the scaffold.

Acknowledgements. We would like to acknowledge funding from the Netherlands Institute of Regenerative Medicine, contract grant number: FES0908.

References.

1. Shor, L., et al., Biofabrication, 2009
2. Duan, B., et al., Acta Biomaterialia, 2010
3. Woodfield, T.B.F., et al., Biomaterials, 2004

Anterior Cruciate Ligament- versus Hamstring-derived cells

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Background: Anterior Cruciate Ligament (ACL) injuries are common clinical problems that impair the functionality of the knee joint resulting in improper gait. Due to the poor healing potential of the ACL, ACL reconstruction is the treatment of choice. The torn ACL is replaced by a new graft, often a Hamstring (HT) and the functionality of the knee joint is regained. Clinical outcome and functional follow-up assessment after the surgical treatment proof that the HT graft is suitable for replacing the torn ACL. Even so, although it is described that both ACL and HT are similar in structure, biology and mechanical properties [1], cells derived from these two tissues might be intrinsically different as result of variation in anatomical location and function. Both ACL and HT are described by closely packed collagen fibers and low cellular content, with cells aligned with the fibers. On the opposite, the ACL is located between the femur and the tibia, transmitting force from one bone to the other, while the HT is located on the back of the knee joint attaching the hamstring muscle group to the tibia and thereby transmitting force from the muscle to the bone. Understanding the differences and similarities between the two cell types could have a great impact on refining ACL reconstruction surgeries, such as accelerating the process of healing.

Aim: The aim of this study was to analyze the phenotypic differences between ACL- and HT-derived cells.

Methods: ACL- and HT-derived cells were isolated from tissue harvest from patients undergoing total knee arthroplasty or ACL reconstruction upon informed consent. The self-renewal and multilineage potential, as well as the expression of surface markers and gene expression profile of both cell types were analyzed.

Results: Both ACL- and HT-derived cells showed limited self-renewal potential and had a surface marker profile distinct from mesenchymal stromal cells (MSCs). The colony forming units (CFUs) from ACL- and HT-derived cells did not show the circular shape that is characteristic of self-renewing colonies obtained from MSCs. Surface marker expression showed high expression of CD90 but limited expression of other established MSCs markers, such as CD73, CD105 and CD146.
Differences in ACL- and HT-derived cells were observed on multilineage potential. While ACL-derived cells showed high potential to differentiate into chondrocytes and adipocytes but not osteoblast, HT-derived cells showed limited multilineage potential. The expression of tendon/ligament related genes (COL1A1, COL3A1 and TNC) was similar on both cell types.

Conclusion: Based on our findings, we support the use of HT graft as a replacement for a torn ACL, as the intrinsic properties of the ACL-derived cells can be to a certain extent replaced by HT-derived cells. Nevertheless, our findings also indicate that HT-derived cells would need some modulating factors to reduce the differences between the two cell types and consequently improve the clinical outcome.

1.Zhu, J., et al., *Ultrastructural and Morphological Characteristics of Human Anterior Cruciate Ligament and Hamstring Tendons.* The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 2012. **295**(9): p. 1430-1436.

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Activated T cells and their factors regulate the osteogenic differentiation of human mesenchymal stromal cells in vitro

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Introduction

There is general agreement that uncontrolled chronic inflammatory processes have a catabolic effect on bone. Several observations from clinical practice however provide clues that acute inflammation may trigger new bone formation. The role of the adaptive immune system in this process is still poorly understood. It is known that T cells are recruited in human fractures during the early phase of repair [1] and although fracture healing occurs adequately in mice lacking lymphocytes, it is delayed compared to wild type mice [1, 2]. It is thought that T lymphocyte (subsets) and their secreted cytokines are involved in bone regeneration through either direct effects on the osteogenecity of bone precursor cells, or by regulating the polarization of macrophages from the early pro-inflammatory to the healing/angiogenesis-promoting phenotype [3]. In this study, we aim to elucidate the effect of T lymphocytes on human MSC osteogenecity in vitro. This knowledge may be used to optimize the local inflammatory response towards regeneration in bone replacement strategies.

Materials & Methods

Bone marrow-derived human MSCs (p3-p5) were used for experiments. Pan T cells were isolated from peripheral blood of healthy donors by positive selection with CD3 MicroBeads (Miltenyi) and subsequently stimulated using CD3 and CD28 mAbs (Sanquin Reagents). MSCs were osteogenically differentiated in the presence of activated T cells (T_{ac}), either in a direct or indirect co-culture, or in activated T cell conditioned medium (ATCM). Resting T cells were used as a control. After 10 days, alkaline phosphatase (ALP) activity was measured using a biochemical colorimetric assay with p-nitrophenyl phosphate. The Quant-iT PicoGreen assay (Invitrogen) was used to measure DNA content. Alternatively, CountBright beads (Invitrogen) were used to count MSCs in the direct MSC-T cell co-culture system by flow cytometry. For statistical analysis of ALP expression, p-values for triplicate measurements were calculated by repeated measured ANOVA. A p-value of <0.05 was considered significant.

Results

In the direct co-culture, the presence of activated T cells resulted in significant, 2- to 3- fold, increases in MSC ALP activity, with increasing effects for larger T/MSC cell ratios. These effects were not observed for resting T cells. Although BMP-2 induced ALP activity was enhanced by T lymphocytes in a direct culture, this was not observed for culture in ATCM or for indirect culture in a Transwell system, suggesting the presence of cell-cell contact dependent mechanisms. Enhanced MSC ALP activity was accompanied by T_{ac} cell-induced inhibition of MSC proliferation.

Discussion & Conclusion

This preliminary data demonstrate a role of inflammatory T-cell (factors) in inducing the osteoblast phenotype. Although the action of several cytokines released by activated T-cells is likely the most important mediator of MSC osteogenecity, we show that cell-cell contact dependent effects may also exist. As BMP-2 induced differentiation is only enhanced in the case of direct cell contact, alternative pathways may be involved here.

Next, to gain more understanding in the interplay between T cells and MSC osteogenic differentiation, a wider range of osteogenic markers will be quantified. Furthermore, future experiments will determine which pathways are involved in T_{ac} cell-mediated MSC osteogenic differentiation and also establish possible differential roles of pro- and anti-inflammatory T cell subsets. This will elucidate the key factors involved.

To conclude, although pro-inflammatory T cells can induce bone loss by stimulation of osteoclastogenesis, T cell cytokines also regulate osteoblast differentiation in MSCs. Modulating the adaptive immune response may be a way to enhance bone replacement strategies.

References

[1] Nam et al. T-lymphocytes enable osteoblast maturation via IL-17F during the early phase of fracture repair. PLoS One 2012.
[2] Toben et al. Fracture healing is accelerated in the absence of the adaptive immune system. J Bone Miner Res 2011.
[3] Mills et al. M1 and M2 Macrophages: Oracles of Health and Disease. Crit Rev Immunol. 2012.

Novel radiopaque UHMWPE sublaminar wires in a growth-guidance system for the treatment of early onset scoliosis: feasibility in a large animal model

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Introduction

Growth-guidance or self-lengthening rod systems are an alternative to subcutaneous growing rods and the vertical expandable prosthetic titanium rib (VEPTR) for the surgical treatment of early onset scoliosis (EOS). The main perceived advantage in comparison to growing rods is the marked decrease in subsequent operative procedures. Therefore growth-guidance systems are especially suitable for neuromuscular EOS patients, who often suffer from significant comorbidities. The Shilla growth-guidance system and a modern Luque trolley are examples of such systems; both depend on gliding pedicle screws and/or sliding titanium sublaminar wires. However, the unknown consequences of metal-on-metal wear debris are reason for concern in young patients. Ultra high molecular weight polyethylene (UHMWPE) or other polymeric sublaminar cables have already been introduced for spinal deformity surgery; the soft and flexible structure of woven UHWMPE wires decreases the risk of neurological injury. Until now, radiolucency of UHWMPE wires has limited the possibility for postoperative radiological assessment and subsequent clinical use. The development of novel radiopaque UHMWPE wires, with bismuth-oxide particles blended into each fiber, allows for clinical application. The goal of this study consists of two parts: (1) to test the stability and biocompatibility of novel radiopaque UHMWPE wires as sublaminar wire, and (2) to assess the potential of using UHMWPE sublaminar wires in a growth guidance system for EOS.

Materials and Methods

An intervention group of twelve immature sheep (18 weeks old) received posterior segmental spinal instrumentation; pedicle screws were inserted at L5 and UHWMPE wires were passed sublaminarly at each level between L3 and T11. Dual cobalt-chromiun rods were placed along the spine between levels L5 and T11 and fixed by set-screws at the pedicle screw attachment sites. The UHMWPE sublaminar wires were secured using a double-loop sliding knot, tightened to 100N, and secured with multiple granny knots. The control group consisted of four age-matched, unoperated animals in order to determine ‘normal’ spinal growth. Lateral radiographs were taken at 4-week intervals to evaluate growth of the instrumented segment. After 24 weeks, the animals were sacrificed and the spines were harvested for histological evaluation and high-resolution peripheral quantitative computed tomography (HR-pQCT) analysis. The occurrence of spontaneous spinal fusion was assessed by manual palpation.

Results

No neurological deficits occurred during the postoperative period. One animal died during follow-up (7 weeks postoperatively) due to unknown cause. At sacrifice, none of the cables had loosened and all instrumentation remained stable. Substantial growth occurred in the instrumented segments (L5-T11) in the intervention group (2.67± 0.16 cm). Spinal growth was slightly higher in the control group, (2.96 ± 0.35cm), but no statistical comparison could be made due to lack of power. Manual palpation revealed a solid, spontaneous fusion across all instrumented levels. HR-pQCT analysis showed interlaminar ossification, possibly a result of periosteal stripping. Typical bone formation encircling the posterior rods coupled with degenerative facet joint changes were also seen. Histological analysis revealed fibrous encapsulation of the novel radiopaque UHMWPE sublaminar wires in the epidural space (typical physiological response to foreign materials), with no evidence of chronic inflammation or wear debris.

Discussion

Despite the occurrence of spontaneous fusion in all cases, UHMWPE sublaminar cables allowed for almost normal continued growth of the instrumented spinal segments during follow-up. Extrapolation of these results to the pediatric patient population is difficult due to a much higher growth velocity in animals, but also due to the exaggerated bone formation response typical for quadrupedal animal models in which higher spinal loads lead to stronger mechanical stimuli to form new bone (Smit2002). Fibrous encapsulation of the cable and preservation of instrumentation stability during the course of this study show that the application of these novel radiopaque UHMWPE sublaminar cables in spinal deformity correction surgery is safe. Further research into the fate of bismuth-oxide particles however is ongoing.

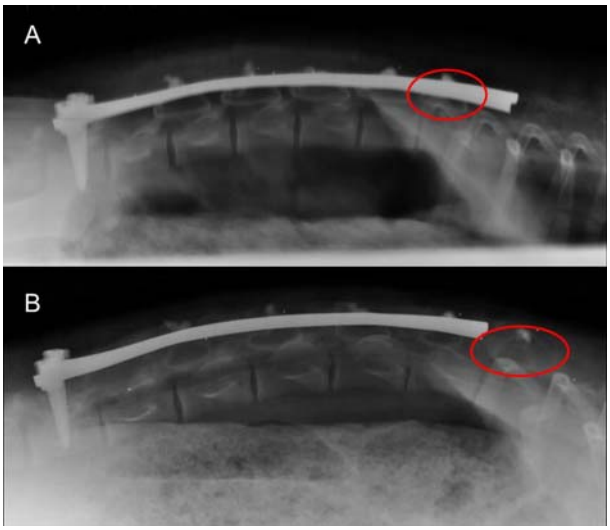

Figure 1: (A) Direct postoperative and (B) 24-weeks postoperative lateral X-ray of the instrumented sheep spine. Marked growth of the instrumented segment has occurred as illustrated by sliding of the most cranial UHMWPE sublaminar wire (circled).

Native Chemical Ligation as a tool to form Peptide-Functionalized in situ Crosslinkable Thermosensitive Hydrogels

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Introduction

Injectable hydrogels are able to fill irregularly shaped tissue defects using minimal invasive treatment. Thermoresponsive polymers are attractive as injectable systems since they can form hydrogels upon temperature changes. However, networks built from thermosensitive physical interactions alone form mechanically weak scaffolds resulting in fast erosion or degradation before new tissue is formed. To enhance the mechanical properties an additional chemical cross-linking mechanism can be introduced in the thermogelling polymers. Here, we aim to crosslink thermosensitive hydrogels in situ using Native Chemical Ligation (NCL). In NCL, a thioester and a N-terminal cysteine undergo a reversible transthioesterification, whereafter an N-to-S rearrangement occurs, resulting in the irreversible formation of a native peptide bond (Figure 1). NCL was chosen because this ligation method has many advantages over currently applied crosslinking methods for biomedical applications: 1) NCL is a chemoselective crosslinking method, eliminating the risk of unwanted side reactions with functional amino acids[1]; 2) NCL can take place in physiological buffers without the use of ultraviolet (UV) light and/or toxic radicals or catalysts; 3) NCL allows for easy functionalization of hydrogels with bioactive peptides.

Materials & Methods

A novel monomer HPMAcys (Figure 2) was synthesized that can copolymerize with other (meth)acrylamides to yield (meth)acrylamide backbone with cysteine functionalities. Thermoresponsive ABA-triblock copolymers consisting of a poly(ethylene glycol) (PEG) middle block, flanked by random blocks of *N*-isopropylacrylamide (pNIPAm) and HPMAcys were synthesized. This polymer was deprotected to obtain free amine and thiol groups and characterized by NMR and GPC. Two thioester crosslinkers were synthesized from poly(ethylene glycol) (PEG) and hyaluronic acid (HA), respectively. Gelation kinetics in PBS were studied by rheology. Furthermore, a functional peptide was covalently attached to a PEG thioester crosslinker by NCL reaction.

Results

A thermoresponsive polymer with cysteine functionalities and two thioester crosslinkers were successfully synthesized. These two components were combined to form hydrogels. The polymers were shown to be soluble at 25°C and immediately form a network when heated to 37°C. This network was strengthened in time by covalent crosslinking due to the NCL reaction, resulting in hydrogels with up to ten times higher storage modulus than hydrogels formed from only the thermosensitive component. Furthermore, a bioactive collagen mimicking peptide was successfully ligated to the thioester crosslinker. Confirmation of successful ligation was obtained by ^{1}H NMR and GPC.

Conclusion

A novel injectable two-component hydrogel was developed that selectively forms hydrogels in situ by a dual gelation mechanism without using additives. Network formation proceeded through an initial thermal gelation due to the thermosensitive pNIPAAm chains and further gelation by chemoselective native chemical ligation between the cysteine moieties and the thioester-functionalized crosslinkers. Additionally, we showed the ability to ligate bioactive peptides to this hydrogel, making this system attractive for tissue engineering purposes.

References

[1] Hu, B.-H.; Su, J.; Messersmith, P. *Biomacromolecules* 2009, 10, (8), 2194-2200.

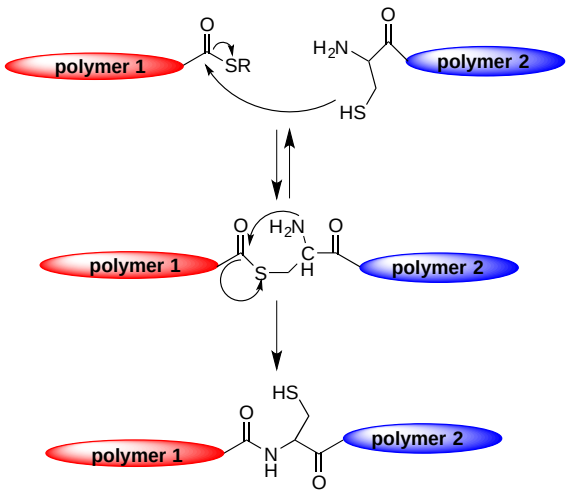

Figure 1: Native Chemical Ligation reaction scheme

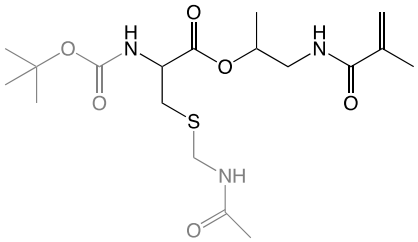

Figure 2: Chemical structure of monomer HPMA-cys. Protective groups are displayed in grey.

Decellularized ear and articular cartilage with native architecture for cartilage reconstruction strategies

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INTRODUCTION Scaffolds are widely used in cartilage engineering, yet the fabrication of a scaffold with a highly organized architecture similar to that of native cartilage remains a challenge. Scaffolds derived from acellular extracellular matrix from native cartilage tissue, however, are able to provide such a microenvironment. Till now, no method to specifically decellularize full thickness cartilage has been described in literature. Therefore, the goal of this study was to decellularize full thickness cartilage of specific cartilage subtypes and characterize the obtained scaffolds for their use in cartilage engineering.

METHOD Full thickness cartilage samples (Ø 8 mm) were obtained from the ears and metacarpophalangeal joints of three calves less than 8 months old. A decellularization method based on literature [1] was optimized to obtain decellularized ear (EC) and articular (AC) cartilage scaffolds. This SDS-based decellularization method was modified by including an extra enzymatic treatment to remove cell remnants from the matrices. Removal of nuclear material was assessed by DNA quantification and histology. Matrix integrity was assessed by glycosaminoglycan (GAG), collagen and elastin biochemical assays as well as histology. Viscoelastic properties were determined by stress-relaxation-indentation [2] and scaffold toxicity was evaluated with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Decellularized bovine scaffolds were then seeded with $2 \cdot 10^6$ human bone-marrow days in chondrogenic medium.

RESULTS No DNA was detected after decellularization in both EC and AC compared to untreated cartilage. Furthermore, histological analysis showed the removal of cell remnants (Fig. 1). The total collagen and elastin contents were retained after decellularization, while GAG content and viscoelasticity reduced to <20% in both EC and AC. The MTT assay showed that the scaffolds were not toxic for BMSCs after decellularization. BMSCs were seeded on the scaffold but migration was limited. After 21 days of culture, expression of the chondrogenic markers *SOX9*, *ACAN* and *COL2A1*, showed the capacity of the scaffolds to support chondrogenesis *in vitro*.

DISCUSSION AND CONCLUSION We obtained scaffolds by decellularizing EC and AC in which the collagen and elastin contents were retained as well as the native collagen structure and architecture. Migration of BMSCs throughout the matrices needs to be further optimized, which should in turn lead to synthesis of GAGs and restored biomechanical properties. The fabrication and characterization of decellularized ear and articular cartilage scaffolds is an important step towards an improved cartilage reconstruction strategy.

REFERENCES

1. Kheir E, Stapleton T, Shaw D, Jin Z, Fisher J, Ingham E. Development and characterization of an acellular porcine cartilage bone matrix for use in tissue engineering. J Biomed Mater Res A. [Evaluation Studies Research Support, Non-U.S. Gov't]. 2011 Nov;99(2):283-94.
2. Stok KS, Lisignoli G, Cristino S, Facchini A, Muller R. Mechano-functional assessment of human mesenchymal stem cells grown in three-dimensional hyaluronan-based scaffolds for cartilage tissue engineering. J Biomed Mater Res A. 2010 Apr;93(1):37-45.

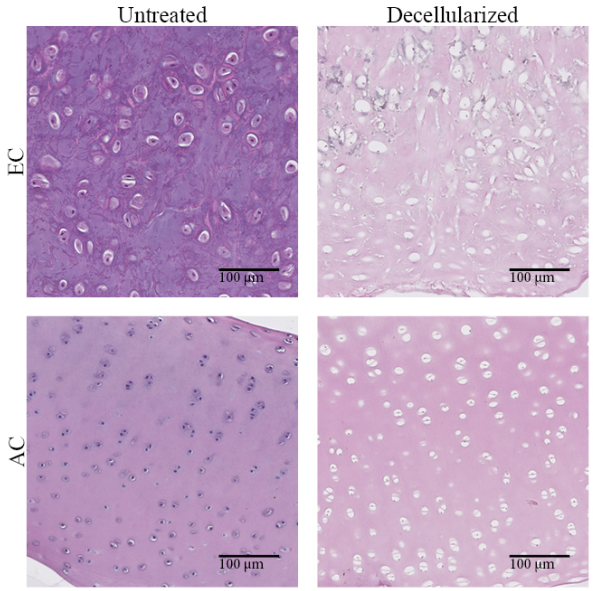

Fig. 1: Decellularization of bovine ear and articular cartilage. Histological H&E stain after decellularization compared to untreated cartilage. EC: ear cartilage; AC: articular cartilage.

Ectopic Osteogenesis Induced by Coating-incorporated Depot of BMP-2 in Coral

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Introduction: Porous coral hydroxyapatites (CHA) granule is a good alternative for the treatment of large bone defects. However, it lacks intrinsic osteoinductivity. In the previous studies, osteoinductivity could be conferred by coating bone substitutes with a layer of calcium phosphate into which bone morphogenetic protein 2(BMP-2) was incorporated.

Purpose: To ascertain whether CHA granules bearing a calcium phosphate (CaP) coating into which BMP-2 had been incorporated could be rendered efficient osteoinductivity.

Materials and Methods: CHA granules (0.25g per sample) bearing a CaP coating into which BMP-2 (20μg, 40μg or 60μg per sample) was incorporated or bearing no BMP-2 were implanted subcutaneously in rats (n=6 per group). Five weeks later, the implants were retrieved for a micro-CT evaluation and histomorphometric analysis. The volumes of ectopic bone and the degree of CHA degradation were estimated by volume density of bone and volume density of CHA, respectively. All data were analyzed with a one-way analysis of variance (ANOVA) and the level of significance was set at $P<0.05$.

Results: Newly formed bone was observed along the surface of CHA granules that bore a CaP coating-incorporated depot of BMP-2, as well as bone marrow. There was a tendency for a higher osteoinductive efficacy of BMP-2 when its amounts increased. However, no significant differences were found in the volume of bone among the three dosages of BMP-2. Although the CHA revealed certain degree of degradation in all the groups, no statistically significant differences were found.

Conclusions: These findings indicate that the CaP coating-incorporated BMP-2 confer porous CHA granules with the property of osteoinductivity and thus dually enhancing its clinical potential in the repair of bony defects.

Proof of concept of bioactive double coating on polymeric membranes for functional human proximal tubule cell monolayers preparation for bioartificial kidney devices.

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Introduction:

Chronic kidney disease patients suffer from diverse clinical complications mainly due to the accumulation of protein-bound uremic toxins. The Bio-artificial Kidney (BioKid) aims at improving toxins removal by coupling renal epithelial cells to dialysis membranes. Biokid development is limited by poor renal cell functionality in vitro, that is primarily guaranteed by monolayer integrity. In this study, human conditionally immortalized proximal tubule epithelial cells (ciPTEC) where grown on polyethersulfone (PES) based membranes coated with L-DOPA and collagen IV and monolayer integrity was evaluated.

Material and Methods:

Polyetheresulphone (PES) 2D membrane flat sheet and hollow fiber (HF) membranes were coated with L-DOPA and collagen IV at different concentrations. Optimal Dopa and Coll IV concentration and time exposure to both the chemicals was Expression of tight junction protein zonula occludens-1 (ZO-1) was detected using laser scanning confocal microscopy after 7 days culture on membranes. Cell function was studied in real-time using ciPTEC cultured on HF membranes by uptake of ASP+, a known substrate of Organic Cation Transporters (OCTs), under flow conditions.

Results and Discussion:

Optimized PES membranes coating with L-DOPA and collagen IV resulted in high BSA and IgG rejection together with good hydraulic permeability. CiPTEC seeded on coated membranes showed a clear ‘cobble stone’ and tight epithelial morphology on both flat and HF membranes, and ZO-1 was abundantly expressed along cell tight junctions. CiPTEC exposed to ASP+ demonstrated a clear fluorescent intracellular signal, indicating active ASP+ uptake. In the presence of metformin, an OCT inhibitor, cells reduced ASP+ uptake of 57 % indicating specific.

Conclusions:

Optimized L-DOPA and collagen IV coating favours the formation of functional ciPTEC monolayer on PES membranes, making it a promising coating for Biokid membranes for the excretion of uremic toxins by ciPTEC.

References

1. Wilmer M.J. et al, Cell and Tissue Research 339, 2010, p.449 – 457
2. Ni M. et al, Biomaterials 32 (6), 2011,p.1465–1476

Prophylaxis of Orthopedic Implant-related Infections with Locally Applied Vancomycin using a Hydrogel as Matrix

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Currently, no options are available for local delivery of antibiotics to uncemented orthopedic implants for prophylaxis of infection. The present study investigates the efficacy of a vancomycin-loaded hydrogel (Novagenit – Italy) as coating for prevention of early postoperative infections.

For this purpose, 18 New Zealand White rabbits received a titanium rod in the proximal tibia after local contamination with *S. aureus*. In the experimental group, a vancomycin-loaded hydrogel was coated on the rods (N=6). Two control groups were used: no-hydrogel (N=6) and hydrogel-only (N=6). Blood parameters (erythrocyte sedimentation rates (ESR) and neutrophil counts) and weights were measured during the experimental period of 28 days. Following explantation, the anterior tibia was processed for detection of viable bacteria. The posterior part containing the rod was used for histological purposes: bone-implant contact, grading severity of infection, and fluorochrome deposition to follow bone formation.

The blood values and weights remained stable over time in the 'vancomycin-hydrogel' group, suggesting the absence of infection in the animals. In contrast, the hydrogel-only group showed decreased weights during the study and increased ESR on day 7. Furthermore, both the hydrogel-only and no-hydrogel groups showed elevated neutrophil counts after implantation. No viable bacteria were found in the tibiae in the vancomycin-hydrogel group, indicating absence of local infection, whereas in the 'hydrogel-only' group 5/5 (one rabbit died, cause unknown), and in the 'no-hydrogel' group 5/6 tibiae contained bacteria. Currently, samples are processed to obtain histological results.

Concluding, these preliminary results showed that local application of vancomycin-loaded hydrogel could successfully prevent implant-related infection.

The study was performed under the multicenter Collaborative Project "*I.D.A.C.*", funded by the European Commission, within the 7th Framework Programme on Research Technological Development and Demonstration, grant no. 277988.

Histologic and Genetic Response of Osteoporotic Bone to Implants Coated with Bisphosphonate-Loaded Calcium Phosphate Nanoparticles

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Introduction

The prevalence of osteoporosis will increase within the next decades due to the aging world population, which can affect the bone healing response to dental and orthopedic implants. Consequently, local drug targeting of peri-implant bone has been proposed as a strategy for the enhancement of bone-implant integration in osteoporotic conditions.

This study aimed to evaluate the efficacy of an electrostatic spray deposition (ESD)-derived nCaP/BP coating on peri-implant bone response in osteoporotic as well as healthy conditions using an established rat femoral condyle implantation model.

Materials and Methods

For animal experiments, male Wistar rats (12-weeks old, weight ~350 g) underwent orchidectomy (ORX) surgery to induce osteoporosis through a loss of gonadal function (hypo-gonadism). After confirmation of the osteoporotic condition, titanium implants coated with nCaP, bisphosphonate, or bisphosphonate-loaded nCaP were installed bilaterally in the femoral condyles of hypo-gonadism (osteoporotic) and sham-operated (healthy) rats. Non-coated implants served as control.

Results

After 4 weeks of implantation, peri-implant bone volume (%BV; by micro-CT) and bone area (%BA; by histomorphometry) were significantly increased within a distance of 500 µm from implant surfaces functionalized with BP compared to control implants in osteoporotic and healthy conditions.

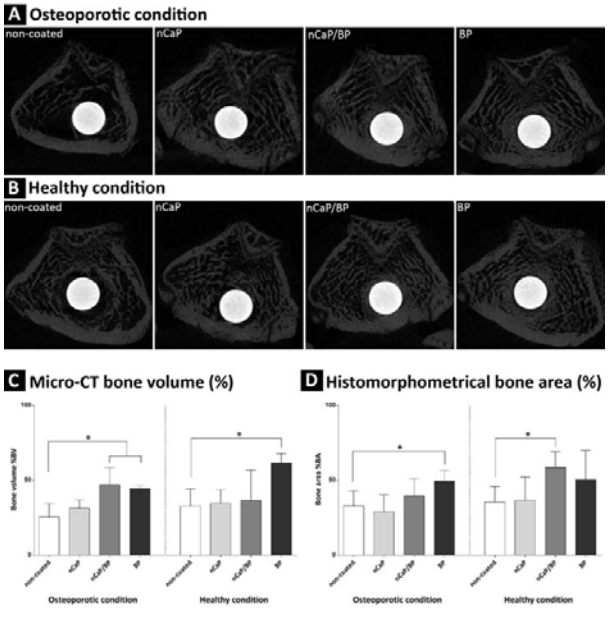

Fig.1: Visualizing and quantifying peri-implant bone formation after 4-weeks.

Fig.2: Histology sections of implants with various coating demonstrating the morphology of the bone tissue in the peri-implant interfaces at 4-weeks.

Furthermore, the deposition of nCaP/BP coatings onto implant surfaces increased bone-to-implant contact (%BIC) compared to non-coated implants in osteoporotic and healthy conditions.

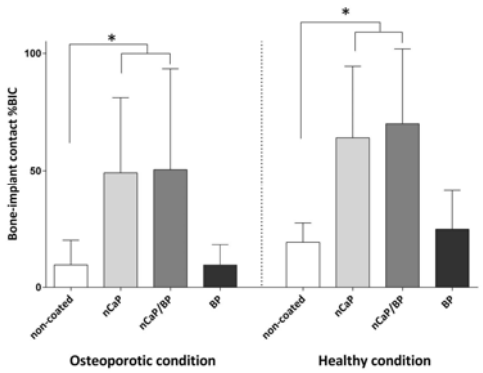

Fig.3: Representative histomorphometrical analysis showing bone-to-implant contact (%BIC).

The results of real-time PCR revealed similar osteogenic gene expression levels to all implant surfaces at 4-weeks post-implantation.

Conclusions

In conclusion, simultaneous targeting of bone formation (by nCaP) and bone resorption (by BP) using nCaP/BP surface coatings represents an effective strategy for improving bone-implant integration, especially in osteoporotic conditions.

Human Rib Perichondrial Transplantation: a 20-year follow-up

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Introduction

Hyaline cartilage defects have a poor regenerative capacity and may require surgery. Although the first reports of perichondrial transplantation (PT) from the rib to the knee showed good results [1], 5-10 year follow-up reports showed poor results in 55% of the treated patients. On the other hand, in 91% of patients with a single defect good results were found [2]. The aim of this study is to present the 20-year follow-up results of this worldwide unique population of 92 patients.

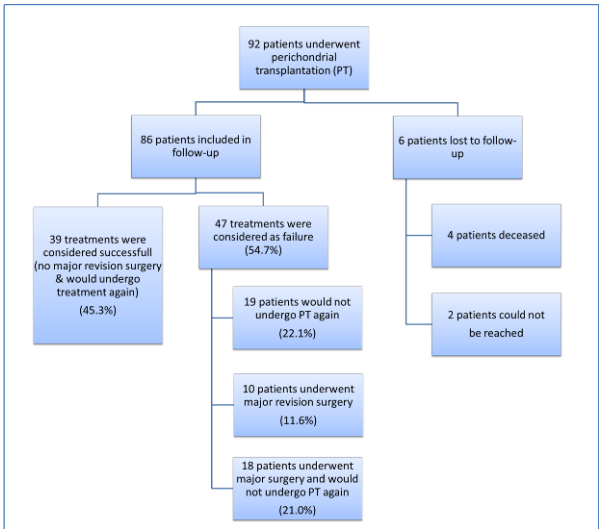

Figure 1: Patient flow chart.

Materials and Methods

Between 1986 and 1993, 92 patients with knee articular cartilage defects were treated in the Maastricht University Medical Center by autologous perichondrial arthroplasty harvested from the rib. We evaluated the results of this treatment at an average follow-up period of 24.4 (19.9-26.9) years.

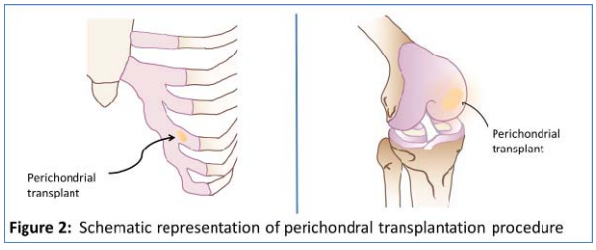

Figure 2: Schematic representation of perichondral transplantation procedure

Results

Of the 92 treated patients 86 were evaluated, 6 were lost to follow-up. Mean age at surgery was 31.8 (15-54) years. Sites of surgery were lateral femoral condyle (2), medial femoral condyle (28), patella (36), trochlea (4) and multiple locations (16).
Twenty-eight of the 86 patients underwent major revision surgery (total/hemi knee prosthesis or patellectomy) in the follow-up period. Thirty-seven patients would not undergo PT again. Either one of these factors was considered as a failure of the procedure and accounted for 47 patients (54.7%).
Best results were observed in n=17 patients younger than 40 years who were treated for a single cartilage lesion and did not have preceding cartilage repair procedures. Thirteen of the 17 procedures were successful (76.5%). Failed procedures in this group were because patients would not undergo the treatment again, no revision surgery was performed in any of these 17 patients.

Conclusion

The long-term results of autologous rib perichondrial transplantation did not show a favourable outcome compared to the long-term reports of other techniques e.g., autologous chondrocyte implantation (ACI). However good results were observed after PT of single articular cartilage defects in young patients that were not treated before; a patient profile that is in line with other studies as reported by Vanlauwe [3].

1. Homminga, G.N., et al., Perichondral grafting for cartilage lesions of the knee. The Journal of bone and joint surgery. British volume, 1990. 72(6): p. 1003-7.
2. Bouwmeester, S.J., et al., Long-term results of rib perichondrial grafts for repair of cartilage defects in the human knee. International orthopaedics, 1997. 21(5): p. 313-7.
3. Vanlauwe, J., et al., Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. The American journal of sports medicine, 2011. 39(12): p. 2566-74.

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Development of a New Therapeutic Technique to Direct Adipose Derived Stem Cells to the Infarcted Area using Targeted Microbubbles: StemBells.

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Stem cell therapy is a promising tool to restore contractile function after myocardial infarction. Unfortunately, clinical trials still show disappointing results with only minor improvements in cardiac function. The major problem of cellular therapy is lack of persistence of sufficient stem cells at the site of injury, independent of administration route. We designed a novel technique to overcome this problem by directing stem cells to the infarcted area using targeted microbubbles (MB) and ultrasound (US). For this we coupled adipose-derived stem cells (ASC) to MB using an antibody against CD90 via biotin-avidin bridging. This stem cell-bubble complex was named 'StemBell'. StemBells were targeted to the infarcted area via a second antibody on the MB: anti-CD54. US (1 MHz) was applied to exert acoustic radiation force on the StemBells. *In vitro* we demonstrated that the procedure to create StemBells, as well as exposure to US had no negative effect on cell viability, using flow cytometry. In a flow system we showed the ability of US to push StemBells from the main flow (0.2 dyne/cm^2) to the side.

In vivo, acute myocardial infarction was mimicked in a rat by ligating the left anterior descending coronary artery for 40 min, followed by reperfusion. 7 Days post-infarction 1 million DAPI-labeled StemBells ('StB') or ASC were injected intravenously. 3 Hours post-injection hearts were excised, stored in liquid N_2 and cryo-sections were made. By performing fluorescence microscopy we found significantly more cells (6-fold increase) specifically in the infarcted area in the StB group compared with 'ASC alone' group (n=6; $p<0.01$). Applying US ('StB+US') lead to an 8-fold increase (n=6; $p<0.01$ vs 'ASC alone', $p<0.05$ vs 'StB'). Notably, retrieved stem cells coincided with CD54 positive areas. In a functional long-term study, we found that StemBells significantly decreased infarct size 42 days post-AMI by 60% ($p<0.01$). Most importantly, the number of retrieved stem cells in infarcted area strongly correlated with improved cardiac function, measured by echocardiography. In conclusion, we successfully demonstrated proof-of-principle of this novel stem cell targeting technique.

Prolonged Presence of VEGF Promotes Regional Vascularization in 3D Bioprinted Scaffolds with Defined Architecture

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INTRODUCTION

Timely vascularization is essential for optimal performance of bone regenerative constructs [1]. Vascularization is efficiently stimulated by vascular endothelial growth factor (VEGF), a substance with a short half-life time [2]. This study investigates the controlled release of VEGF from gelatin microparticles (GMPs) as a means to prolong VEGF activity at the preferred location within 3D bioprinted scaffolds, and the effects on subsequent vascularization.

MATERIALS AND METHODS

Release of VEGF from GMPs was measured with ELISA and bioactivity was assessed using human endothelial progenitor cells (EPC) in Transwell and real-time migration assays. Matrigel scaffolds containing EPCs and VEGF, which was released either in a fast or sustained fashion by application of GMPs, were investigated for their in vivo vasculogenic capacity. In addition, regional differences with respect to VEGF release were introduced in 3D-printed EPC-laden scaffolds. Scaffolds were implanted in subcutaneous pockets in mice for 1 week and analyzed for vessel formation.

RESULTS AND DISCUSSION

Release of VEGF from GMPs was continuous for 3 weeks. VEGF bioactivity was confirmed, EPC migration in the presence of GMP-released VEGF was indistinguishable from VEGF added to the medium. Implantation in subcutaneous pockets in mice demonstrated that vessel formation was significantly higher in the VEGF sustained release group when compared to fast release or control groups. In addition, the different regions in the bioprinted scaffolds were retained and vessel formation occurred analogous with the results seen in the Matrigel plugs.

CONCLUSION

We conclude that GMPs are suitable to generate sustained release profiles of bioactive VEGF, and that they can be used to generate defined differentiation regions in 3D printed heterogeneous constructs. The prolonged presence of VEGF led to a significant increase in scaffold vascularization when applied in vivo.

ACKNOWLEDGEMENTS

This research forms part of the Project P2.04 BONE-IP of the research program of the BioMedical Materials Institute, co-founded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

REFERENCES

[1] Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, et al. Engineering vascularized skeletal muscle tissue. *Nat Biotechnol.* 2005;23:879-84.

[2] Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999;18:3964-72.

Cell-Mediated Retraction Versus Hemodynamic Loading – a Delicate Balance in Tissue-Engineered Heart Valves

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Introduction A major failure of function mechanism found in preclinical studies of tissue-engineered heart valves (TEHVs) is retraction of the valve leaflets. This retraction (tissue shrinkage) is caused by both passive and active cell stress and passive matrix stress. Tissue shrinkage may be counteracted by the stress imposed on the leaflets by hemodynamic loading during diastole, which causes the leaflets to extend. If this loading is large enough, the leaflet extension and shrinkage may counterbalance each other. To get insight into the stress balance within a TEHV leaflet, the amount of stress generation in engineered heart valve tissue and the stress imposed by physiological hemodynamic loading are quantified via an experimental and a computational approach, respectively.

Methods Stress generation by human vena saphena cells was measured using an earlier described in vitro model system, mimicking the constrained culture process of TEHVs. Valvular tissues were cultured for four weeks (triplicate, n=6 per experiment) in the model system. After four weeks constraints were released, subsequently imposed, and stress generation was measured for 77 hours. The experimental data were fit using a biexponential fit. The stress imposed on the TEHV by the hemodynamic loading was determined using a finite element model. The valvular tissue was modeled as an incompressible fiber-reinforced material. Pulmonary pressure of 3 kPa (25 mmHg) and systemic pressure of 12 kPa (90 mmHg) were applied in the finite element simulation. In both cases, radial and circumferential stresses were evaluated.

Results According to the fit parameters, stress generated almost instantly during the first two hours after which it slowly kept on generating to a maximum of 23 kPa. When pulmonary pressure was applied in the finite element model the maximum stress in the radial direction was 18 to 20 kPa. When systemic pressure was applied this was 45 to 50 kPa. In both cases, negative stresses were found along the edge of the leaflet.

Discussion The maximum stress generated by the cells is comparable with that imposed by the pulmonary blood pressure, therefore it is likely that the imposed stress cannot counteract the generated stress. The stress imposed by the systemic blood pressure was two times higher than the cell stress, and might be better able to counteract the cellular traction forces. Though, as the hemodynamic loading is only imposed during diastole, generated and imposed stress may still be too similar to enable shape conservation. Next to this, the negative stresses found along the edge of the leaflet will result in compression of the tissue and will add up to the cell-generated stress, increasing leaflet retraction.

Conclusion This study provides a rational explanation for the retraction found in preclinical studies of TEHVs and represents an important step towards understanding the retraction process seen in TEHVs by a combined experimental and computational approach.

A New Tissue-Engineered Urinary Conduit In A Porcine Model.

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Introduction: Incontinent urinary diversion according to Bricker is the primary choice of treatment for patients after radical cystectomy. Although this approach provides a satisfactory outcome in most cases, the use of gastrointestinal (GI) tissue in this procedure can lead to severe complications. Tissue Engineering and Regenerative Medicine (TERM) may provide an alternative solution through the development of tissue engineered constructs that can replace GI tissue in urinary diversion. In this study we investigated the applicability of a reinforced collagen-based tubular scaffold as an incontinent urostomy in a porcine model.

Materials & Methods: Long, large diameter tubular constructs (l=12 cm, Ø=15 mm) were prepared from 0.7% bovine type I collagen and combined with a fast-biodegradable Vicryl polymer mesh (Ethicon). The constructs were freeze-dried, cross-linked, sterilized by 25 kGray of γ-irradiation and characterized using scanning electron microscopy (SEM). Urothelial cells and smooth muscle cells were isolated from bladder biopsies of female Landrace pigs (+/- 50 kg). After 1 month, an incontinent urostomy was formed using the right ureter with a skin outlet on the right flank with Ø 2.5 cm (Figure 1). Five pigs received a cellular construct, five pigs received an acellulair construct. The pigs were evaluated after approximately 1 month macroscopically and by loopogram.

Preliminary results: Analysis by SEM showed integration of the Vicryl polymer mesh in the collagen scaffold. Successful implantation was accomplished in all pigs, with no drop-outs. The first results show that the collagen-Vicryl construct was resorbed after one month, and that this novel construct is able to form a extraperitoneal tunnel *in vivo*. Loopograms suggested that urinary flow is allowed in both the acellular and cellular constructs up until 1 month (Figure 2). One pig with a cellular construct showed lethargy, fever and stop of urinary flow from the urostomy. At necropsy, hydronephrosis and hydroureters were observed in all pigs, most likely the consequence of urinary reflux. No major differences between the cellular and acellular group were observed, although the preliminary results suggest that cellular constructs resulted in excessive tissue response and a blockade of the stoma.

Conclusions: Implantation of a novel tissue-engineered urinary conduit resulted in a extraperitoneal tunnel which was able to allow urinary flow. The preliminary results suggest that the combination with cells does not provide additional value for tissue regeneration in urinary diversion.

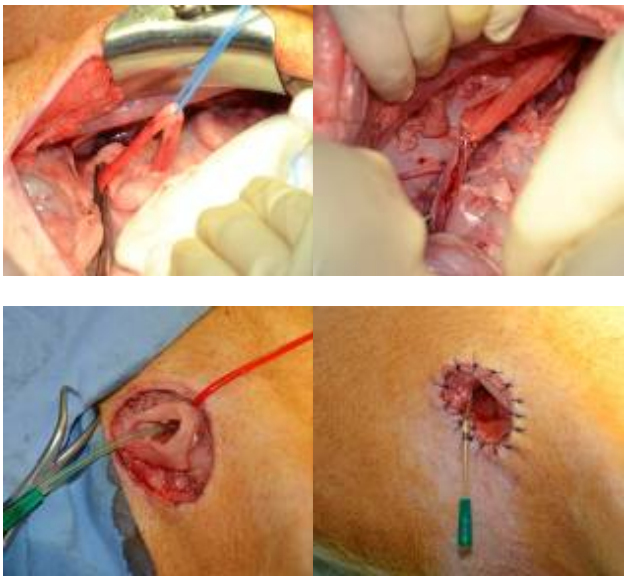

Figure 1: Implantation procedure. *(A) localization of ureter, (B) anastomosis ureter-scaffold, (C) Preparation of skin outlet, (D) skin outlet Ø 2,5cm.*

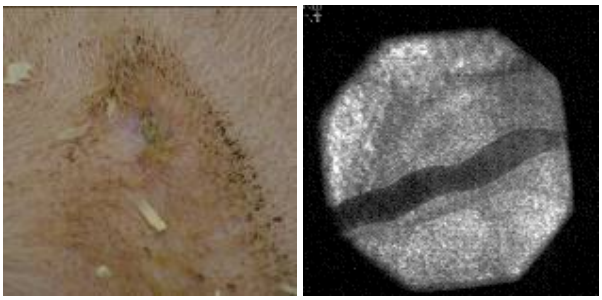

Figure 2: Evaluation. *(A) skin outlet, (B) loopogram.*

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Biodegradable hyper-branched isocyanate-terminated tissue adhesives for meniscus repair

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Introduction

Meniscus tears are very common knee injuries. They result in pain, swelling and locking of the knee joint, and eventually may lead to osteoarthritis. Currently, the best technique to deal with a torn meniscus is suturing. However, this method is complicated, time consuming and expensive. Furthermore, the success rate in the avascular inner zone of the meniscus is low. Therefore, there is an urgent need for a new, innovative solution to repair a torn meniscus. Tissue adhesives are considered to be a promising solution, since they are easy to apply and cause minimal tissue trauma. It has been previously reported that isocyanate-terminated copolymers based on poly(ethylene glycol) (PEG) and biodegradable trimethylene carbonate (TMC) can be successfully used as such tissue adhesives [1]. In this study we advance this work by designing novel hyper-branched copolymers. The mechanical and adhesive properties of the networks can be tuned by varying the composition and the degree of branching of the structures.

Experimental

Oligomers were synthesized by ring opening polymerization of TMC using PEG as initiator. Subsequently, these oligomers (and PEG itself) were reacted with different amounts of citric acid (CA) to introduce diverse degrees of branching in the system. The hydrophilicity can be adjusted by changing both the molecular weight of the PEG and the amount of TMC coupled to it. Reactive copolymers were synthesized by functionalizing the terminal hydroxyl groups of the oligomers with hexamethylene diisocyanate (HDMI). The resulting materials were evaluated as tissue adhesives in lap shear adhesion tests using dermal sheep collagen (chamois leather) as a model for the tissue (see Figure 1). The compositions with the best adhesive properties were subjected to detailed mechanical analysis, and their adhesive properties to bovine meniscus tissue were evaluated.

Figure 1. Testing of the lap shear adhesion strength after curing of the different tissue adhesives.

Results and Discussion

The experimental results showed that the shear adhesive strength of the networks to chamois leather varied from approx. 50 to approx. 600 kPa depending on the composition and design of the molecules. The adhesion to bovine meniscus was significantly lower, approx. 20 to 100 kPa. This shows the strong influence of the used tissue model on the adhesive strength of the glue. The elastic modulus of the networks varied from approx. 1.5 to approx. 45 MPa, this was in the same range of values as that of soft tissues.

Conclusions

We showed that the proposed network structures are favorable candidates to be used use as resorbable tissue adhesives for meniscus repair. They have satisfactory mechanical and adhesive properties that can be adjusted by varying the composition and design of the copolymers. However, more extensive studies are necessary to confirm their compatibility with cells.

References

[1] SBG Blanquer *et al*., J Appl Biomater Function Mater, **10**, 177 (2013)

Acknowledgements

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Priming human Mesenchymal Stem Cells in culture to increase chemotaxis to osteoarthritic cartilage and synovium

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INTRODUCTION: Mesenchymal stem cells (MSCs) are promising candidates for cell therapeutic application in osteoarthritis (OA) because they are the body's natural healers. They can differentiate to generate repair cartilage tissue and they can have immunomodulatory and anti-inflammatory effects on synovium and cartilage. To be therapeutic in OA, MSCs need to move to (affected) intra-articular tissues and remain active. Hypothetically this can be achieved by engraftment of MSCs. Factors influencing MSC survival and engraftment in the joint are currently not known. Higher expression of adhesion/migration factors in MSCs will very likely be in favour of their engraftment potential which will result in longer cell activity intra-articular. We studied the effect of priming of MSCs in culture on the expression of adhesion/migration factors in MSCs that promote engrafment in osteoarthritic cartilage or synovium.

METHODS: Chemoattractant factors fractalkine, MCP-3, PDGF-BB, MIP-1a, MIP-1b, MDC, PDGF-AA, IP10, IL-8 and MCP-1 in OA synovial fluid (SF) and in conditioned medium (CM) of OA cartilage and synovium of six donors each were measured with a 10-plex milliplex assay. MSCs were cultured in monolayer in serum-free medium with 1% ITS at 20% O2 with(out) 50% SF of OA patients or healthy donors for 24 hour or at 20% or 1% O2 with 50ng/ml, 20ng/ml, 1 ng/ml IFNγ and TNFα for 48h. mRNA expression of adhesion/migration factors CD44, CXCR1, CXCR3, CXCR4, CCR1, CCR4, CCR5, PDGFRa, PDGFRb, CX3CR1, ITGb1 and ITGb2 were analysed and related to the housekeeper: HPRT.

RESULTS: All soluble chemoattractant factors were present in OA SF. PDGF-BB, MDC, IP10 and MCP-1 were highly present in OA SF. Cartilage and synovium both secreted chemoattractant factors. Both CM contained a high amount of MDC, IL-8 and MCP-1. Additionally synovium CM contains a high amount of MIP-1a and MIP-1b and cartilage CM contains a high amount of PDGF-AA and IP10.

During "normal culture conditions" all migration/ adhesion factors were expressed in MSCs except for CXCR1. Exposure to OA SF had a minor effect on the expression of adhesion/migration factors in MSCs. It only slightly increased CCR1 and PDGFRa mRNA expression. Both OA and healthy SF decreased the expression of PDGFRb.

Priming MSCs with inflammatory factors significantly increased the mRNA expression of CCR1 and CCR4 and significantly decreased the expressions of PDGFRa and PDGFRb. There was a clear dose response effect of IFNγ and TNFα on the expression of CCR1 and PDGFRa. Oxygen tension did not influence the expression of the adhesion/ migration factors.

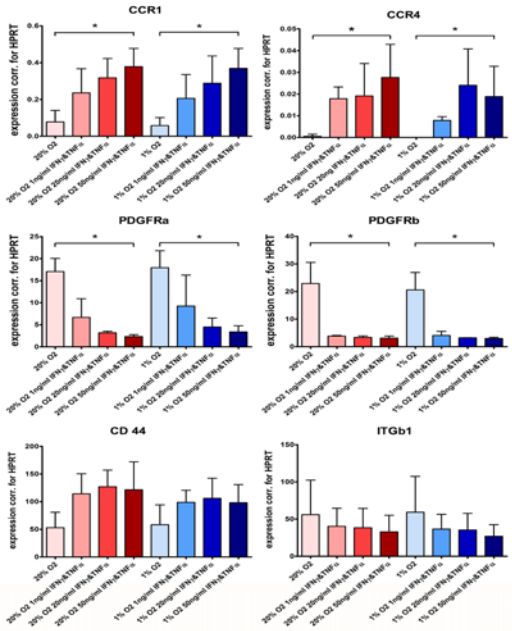

*Fig 1. mRNA Expression of adhesion/migration factors CCR1, CCR4, PDGFRa, PDGFRb, CD44 and ITGb1 in MSC when cultured with inflammatory factors.*P≤ 0.05*

DISCUSSION & CONCLUSIONS: Up-regulation of adhesion/migration proteins on MSCs would hypothetically increase engraftment in the joint after injection. Under "normal culture conditions" MSCs express mRNA for adhesion/migration receptors for soluble chemoattractant factors secreted by OA cartilage and synovium. However, we found a great decrease of injected MSCs in rat knees within two weeks and only a small effect on OA. Priming MSCs with inflammatory factors increases the anti-inflammatory activities of MSCs on osteoarthritic synovium[1]. It also increases gene-expressions of the adhesion/migration receptors CCR1 and CCR4. The receptors bind respectively to chemoattractant factors MIP-1a and MIP-1b which are highly secreted by synovium. On the other hand, gene-expressions of the PDGFRs in MSCs were down-regulated by priming with inflammatory factors. These adhesion/migration factors bind to PDGF-AA, which is highly secreted by cartilage. These data suggest that priming with inflammatory factors can influence chemotaxis of MSCs towards osteoarthritic synovium and this is currently tested using in vitro chemotaxis assays.

ACKNOWLEDGEMENTS: The Netherlands Institute for Regenerative Medicine (NIRM, grant No. FES0908).

REFERENCE:

1. van Buul, G.M., et al., *Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture.* Osteoarthritis Cartilage, 2012. **20**(10): p. 1186-96.

Exploring Transcriptional Differences Induced by Varying Material Surface Properties in Osteoblasts

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Introduction

In improving the biological response of bone graft substitute materials, not only the biological performance but also the handling and mechanical properties are still part of current research activities. This appears challenging because *in vitro* models which truthfully reconcile the behavior of these materials *in vivo* are largely lacking.
Our approach in this biomaterial research focusses on discovering biological differential responses induced by virtue of specific biomaterial properties and henceforth applying this biological knowledge for improving biomaterial properties.
As a model system we analyze the behavior of the osteoblastic cell line MG-63 cultured on well-studied calcium phosphate based ceramic materials [1, 2].

Results

First, we clustered a set of calcium phosphate based biomaterials on their physico-chemical properties on the one hand and their bone inducing capacity at ectopic sites *in vivo* on the other hand. We specifically compared the structural surface properties that are hypothesized to play a role in *in vivo* bone formation capacity (Fig 1). Then, we determined the gene expression profiles these materials elicited in MG-63 with DNA microarray technology. Using bioinformatics tools we analyzed the transcriptional profiles on the different materials with varying surface topographies. Correlating material properties with *in vivo* bone forming capacity and *in vitro* transcriptional profiles revealed genes whose expression may eventually be used as marker for *in vivo* bone formation.
These differences and similarities were further confirmed using qPCR and analyzed in a different but defined set of calcium phosphate based materials with distinct variations in structural surface properties.

This research forms part of the Project **P2.04 BONE-IP** of the research program of the **BioMedical Materials** institute, co-funded by the **Dutch Ministry of Economic Affairs.**

Conclusion

We identified and confirmed the expression of specific genes which correlate to material properties of interest for improving the performance of bone graft substitute materials. Thereafter, we aim at further validating the expression and exploring the biological functionality of these uncovered transcriptional differences. For this purpose we designed artificial materials with the surface properties of interest.
Eventually these discovered hits may be used to further develop, screen and improve materials for bone regeneration purposes.

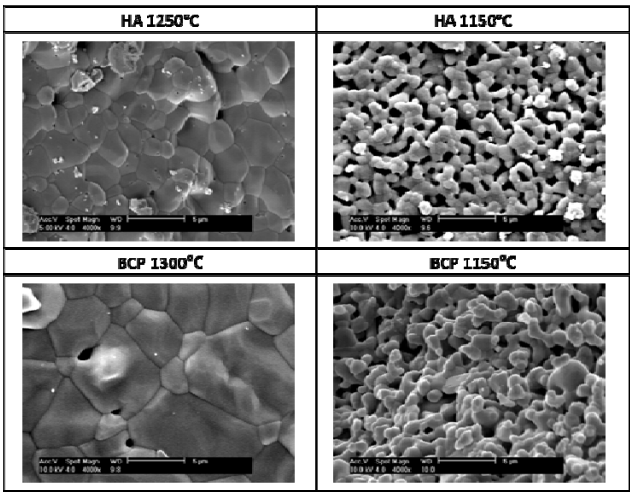

Figure 1 Two hydroxyapatite calcium phosphates (HA) (top row) and two biphasic calcium phosphates (BCP) (bottom row) sintered at different temperatures exhibit different surface topographies, as represented by their SEM images.

References

[1] Yuan H, Fernandes H, Habibovic P, de Boer J, Barradas AMC, de Ruiter A, et al. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. Proceedings of the National Academy of Sciences 2010; 107 (31): 13614-9.

[2] Groen N, van de Peppel J, Yuan H, van Leeuwen JP, van Blitterswijk CA, de Boer J. Bioinformatics-based selection of a model cell type for in vitro biomaterial testing. Biomaterials 2013; 34 (22): 5552-61.

Icariin: is it an efficient osteoinductive promoter to enhance the effectiveness of BMP-2?

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Backgroud : To effectively treat bone disease using bone regenerative medicine, there has been a strong interest in searching for safe and cheap drugs that can potently induce bone formation, and reducing the dose of BMP and so raise the efficiency. Here, we demonstrate the osteogenic effects of icariin, the main active compound of Herba Epimedii.

Objective: To evaluate the biofunctional characteristics of combination of icariin and BMP-2 in inducing osteoblastogenesis of MC3T3-E1 through in vitro time-course and dose-response studies.

Methods: The optimal concentration of icariin in stimulation proliferation of MC3T3-E1 which was chosen at first. And this concentration of icariin was applied in the next studies. The effects of combination of icariin and different concentration of BMP-2(50ng/ml, 100ng/ml and 200ng/ml) stimulation on the proliferation of MC3T3-E1 were examined. Afterwards, alkaline phosphatase activity, OCN expression, and ALP, BMP-2, Col1, OCN, Runx2 mRNA expression were determined.

Results: The optimal concentration of icariin in stimulation proliferation of MC3T3-E1 is 10^{-5M}. Not only adding icariin increased the osteoinductive effects of BMP-2, but also the combination of icariin and lower concentration of BMP-2 achieved higher osteoinductive properties than the higher concentration of BMP-2 alone both in the proliferation, protein secretion, mineralization and mRNA expression.

Conclusion: The results indicated that considering the inexpensiveness plus safety of icariin, it should be a good choice as an osteoinductive drug for clinical use to assistant BMP-2 or substitute it in the bone regenerative occasions.

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IN VITRO TISSUE REGENERATION BY HUMAN DEGENERATED NUCLEUS PULPOSUS CELLS IN HYPEROSMOTIC CULTURE MEDIUM

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Intervertebral disc (IVD) cells normally reside in a high osmolality environment of 450 to 550 mOsm/kg. It is not known how human degenerated disc cells are affected by osmolality, nor what osmolyte would be most effective. The aim of the current study was to determine the optimal medium osmolality in terms of tissue regeneration by human degenerated NP cells.

The osmolality of standard culture medium was adjusted with NaCl and sucrose from 340 to 400, 450 and 500 mOsm/kg. IVD cells from two human donors (Thompson grade III) were cultured for 28 days in high density (1*10^6 cells/cm^2) on collagen II coated filters. Samples were stained with Safranin-O for glycosaminoglycans (GAGs) and with immunohistochemistry for collagen II. Gene expression was evaluated by qPCR and a collagen low density array. Matrix content as reflected by glycosaminoglycan (GAG) production was measured with a dimethylmethylene blue (DMMB) assay and DNA content with a Picogreen assay. Univariate analysis of variance with randomized block design and post-hoc Dunnet *t*-test were performed.

Gene expression shows an increase in aggrecan and collagen II for higher osmolality with NaCl, but not for sucrose. No clear effect at the protein level was found when increasing osmolality using NaCl or sucrose. Effects on collagen deposition appear to be similar to the effects on GAG production.

In conclusion, it does not seem to be necessary to adjust osmolality for matrix production by nucleus pulposus cells in the current model.

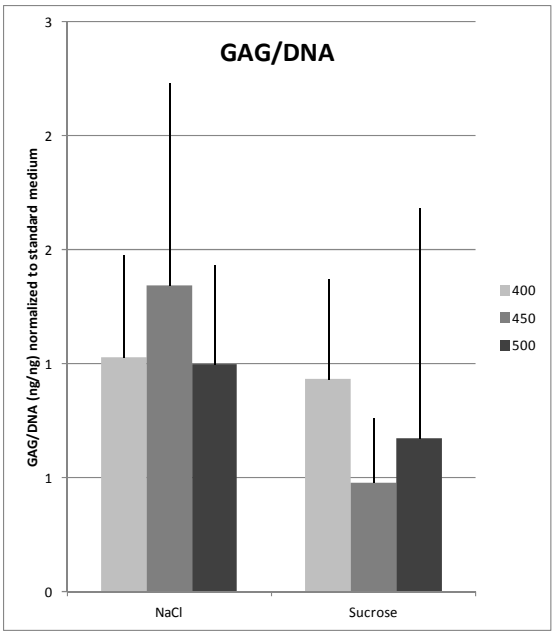

Figure 1 – *GAG per DNA (ng/ng) compared to standard redifferentiation medium (set to 1). Cells cultured with sucrose adjusted medium seem to have produced lower amounts of GAGs compared to standard redifferentiation medium, but this difference is not significant.*

Towards Understanding the Molecular Basis of the Foreign Body Response and Biomaterial-Associated Infections

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Infection of medical devices can have disastrous consequences, including removal of the device. *Staphylococcus epidermidis* is the major cause of biomaterial associated infection (BAI), which in absence of a foreign body hardly ever causes infection. Here, we assess the gene expression underlying the foreign body response to titanium over time and the influence of *S. epidermidis* on this response.

Four experimental groups were compared in the biomaterial-associated infection mouse model: a) sham surgery (no implantation of a biomaterial), b) implantation of a titanium biomaterial, c) sham surgery with an *S. epidermidis* infection, and d) implantation of a titanium biomaterial combined with an *S. epidermidis* infection. At 1 and 6 hours and 2, 4, 9, 14 and 21 days, bacterial colonization, histology and gene expression were analyzed. Histology was performed by multispectral imaging and gene expression was recorded using Affimetrix Mouse Gene-ST microarrays.

The histology and gene expression patterns showed distinct differences between sham and biomaterial groups possibly related to the foreign body response, and between biomaterial without and with infection. These results are a powerful start towards understanding the molecular basis of the foreign body response and biomaterial-associated infection.

This research forms part of the Project P4.01 NANTICO of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs.

In vitro degradation rate of injectable calcium phosphate cement with incorporated dense PLGA microspheres

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Introduction: Calcium phosphate cements (CPCs) are widely used as bone substitute. They exhibit several advantages such as injectability, biocompatibility and osteo-conductivity. However, the drawbacks of CPCs are mainly related to the slow degradation both *in vitro* and *in vivo*. To increase degradation rate, maroporosity was introduced into the cement. A method to introduce macroporosity can be achieved by the hydrolytic degradation of Poly (D,L-lactic-co-glycolic) acid (PLGA) microspheres. Nevertheless, no consensus has been reached in the evaluation of balancing the macroporosity and mechanical strength in CPCs. The objective of this study is to investigate the effect different PLGA formulation (wt%) on the degradation *in vitro*.

Materials and Methods:
CPC powder consisted of a mixture of 85% α-TCP, 10% DCPA and 5% precipitated HA.
PLGA (MW=17kDa, acid terminated, L:G=50:50) was used to prepare dense microspheres.
PLGA/CPC formulations were prepared by adding 30% wt, 40%wt and 50%wt PLGA microspheres. (mean size 94±29 µm).
CPC cement was injected into Teflon molds (cylinders 4.5*9mm).
For degradation the CPCs were incubated in phosphate buffer saline (PBS) at 37˚C. for 1,2,4,6 and 8 weeks. At each time point the samples were subjected to analysis. After removal the pH of the PBS was measured. The compressive strength of was tested by using a testing bench with a cross speed of 0.5 mm/min. X-Ray diffraction (XRD) was used to determine the crystal structure. The morphology of the different formulation was determined with scanning electron microscopy (SEM).

Results and Discussion: In table 1 the porosity and macroporosity of the different formulations is given. It is clear that with increasing PLGA microspheres content, there is a significant increase in porosity. After soaking samples in PBS, the pH started to decrease within 2 weeks (3.4~3.8), and further remained constant (figure 1). At all time periods a significant difference in compressive strength was observed between the 30%, 40% and 50% CPC/PLGA formulation (figure 2). The compressive strength is most likely dependent on the amount of PLGA, which is related to the amount of acidic lactic and glycolic monomers produced in time by the the hydrolysis of PLGA. After one week of incubation XRD results showed the α-TCP phase was changed into an apatite phase. At the other time periods the XRD patterns demonstrated the formation of brushite.

Conclusion: The combination of dense PLGA microspheres with CPC can result in apatitic CPC degradation. Higher amounts of PLGA microspheres result in a lower pH, lower mechanical properties and faster transformation of the apatitic CPC into a brushite containing cement.

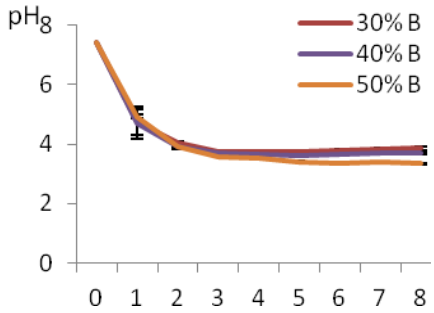

Fig 1: pH change of the PBS as a function of the degradation time in weeks.

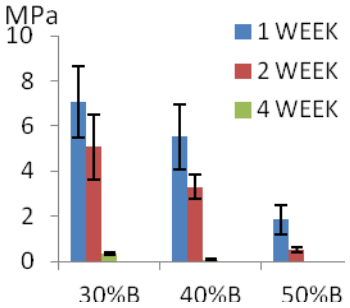

Fig 2: Compressive strength as a function of the degradation time in weeks.

Table 1: Results of the porosity measurements

	30/70 P/C	40/50 P/C	50/50 P/C
Porosity (%)	71.5	76.9	83.1
Macroporosity(%)	31.0	40.8	53.0

Microfluidic strategies to study interactions between cells and biomaterials for bone regeneration

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Introduction

Combining tools from micro-engineering and tissue regeneration fields offers new possibilities to simulate biomaterial/cell and biomaterial/tissue interactions *in vitro*.

One of the objectives of our research group is to develop synthetic alternatives to autologous bone grafts, that suffer from a number of disadvantages, limited availability being the most critical one. In order to be considered a comprehensive alternative to natural bone grafts, synthetic biomaterials need to meet various requirements, including mechanical stability, and bioactivity in terms of osteoconduction and osteoinduction. While in the past decades a great number of synthetic bone graft substitutes, including calcium-phosphate ceramic-based ones has been developed [1, 2], the majority still needs further improvement to be accepted as a true alternative to natural bone grafts. Fundamental understanding of interactions between materials and cells and/or tissues is of great value when it comes to improvement of synthetic bone graft substitutes. Platforms based on microfluidics offer possibilities to increase the throughput of testing of cell/material interactions. In addition, they allow for recreating the biological microenvironment surrounding an implanted bone graft substitute *in vitro* [3].

Materials and methods

In order to mimic the microenvironment of biomaterials relevant for bone repair and regeneration, two microfabricated systems have been developed:

A) A wet etched glass microfluidic cell culture chamber, assembled with a glass cover, and fed by 4 independent diffusive side-channels for nutrient and oxygen supply;

B) Polydimethylsiloxane (PDMS) multi-chamber microfluidic device, assembled over glass/polymeric layers, and fed by 2 independent diffusive side-channel for nutrient and oxygen supply.

Functional studies are performed by culturing of MG-63 human osteosarcoma cells.

Results and discussion

To make the developed microfluidic systems suitable for studies of interactions between cells and biomaterials for bone regeneration, surface properties of the cell culture chambers were modified following two different strategies,

In strategy A, the microfluidic culture chamber was coated by using a sputtering technique. This allowed for deposition of a titanium film with a thickness of few nanometers, that was fully oxidized post-deposition (Figure 1). This technique is now being employed to deposit a range of different biomaterials, including bioactive calcium-phosphates.

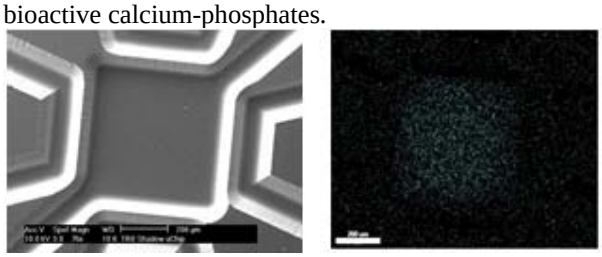

Figure 1: left, scanning electron microscopy image of glass chip; right, X-ray map analysis of sputtered titanium inside the culture chamber of glass chip .

In strategy B, the surface of the microfluidic cell culture chamber was covered by a layer of polymer or polymer/ceramic composite by spin-coating before assembling the system with the support glass and the fluidics in PDMS (Figure 2).

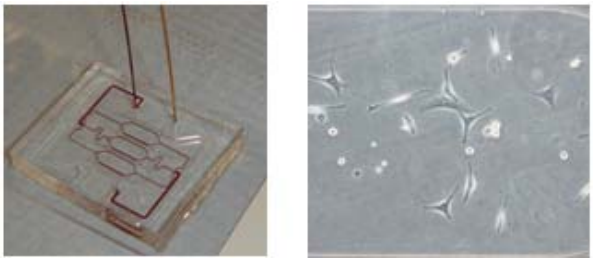

Figure 2: left, image of PDMS multi-chamber device; right, MG62 osteosarcoma cells on the glass surface of the chip(control).

Future experiments will focus on functionalizing the systems with thin films of other materials relevant to bone repair and regeneration, and performing studies on cell-material interactions under flow regimes which more closely resemble the *in vivo* cell microenvironment.

References

[1] Dinopoulos H *et al.* (2012) Bone graft substitutes – what are the options?. *Surgeon* 10(4): 230-239.

[2] Barradas MC *et al.* (2011) Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. *European Cells and Materials*, 21: 407-429

[3] Whitesides GM (2006) The origins and the future of microfluidics. *Nature* 442: 368-373.

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High-throughput Biological screening of Algorithm Generated Biomaterial surfaces.

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Introduction

Small changes in surface structure topology on which cells grow can have a dramatic impact on cellular parameters such as proliferation, orientation, morphology, gene expression and differentiation.[1] Understanding the underlying mechanisms has widespread implications for the development of the biomaterials field.

We developed the TopoChip platform for high-throughput screening of material surfaces, consisting of an *in silico* library of over 150 million unique multiparametric features. From this library, 2,178 structures were randomly selected and arrayed in duplicate as 300×300 μm 'TopoUnits' upon a 2×2 cm polymer 'TopoChip'.[2] In this previous work, we showed that the topographies indeed have striking effects on cell morphology and orientation. Using enlarged surface areas of the found 'hit topographies' allowed us to further investigate the underlying mechanisms that caused these cellular responses.

Materials & Methods

The TopoChips and enlarged hit surfaces were fabricated by hot embossing of polystyrene tissue culture plastic with a photolithographically-produced silicon mold. Human mesenchymal stem cells (hMSC) from multiple donors, were cultured on the TopoChips in α-MEM (Gibco) with 10% FBS before fixing and staining for microscopy. The TopoChips were imaged by automated fluorescent microscopy (BD Pathway 435) and image analysis was performed using a custom-designed CellProfiler[3] pipeline.

Results & Discussion

Quantitative image analysis shows that the different features on the TopoChip can have a significant impact on hMSC morphology, such as cell shape and orientation (Figure 1). Further analysis, using the enlarged surfaces, indicated that cell morphology was reproducible for a given feature. We were able to cluster the cell morphologies based on measurements performed on images of the actin cytoskeleton. This morphological based clustering showed correlation with the levels of ALP expression as well as the localization of YAP/TAZ, co-transcriptional factors in the Hippo-pathway that were recently found to be a regulators of the cellular response on mechanical stimuli.

Conclusion

The surfaces consisting of the multiparametric features do induce different cellular responses. The TopoChip platform can be used to perform high-throughput screening for desired, topographical guidance, cell phenotype and orientation. Furthermore, the enlarged surface areas can be used for validation of the screening results as well as a set-up to further investigate the underlying mechanism of mechanotransduction of physical stimuli from the cell its micro-environment.

Future outlook

Future experiments will focus on the effects of surface topography on cell behavior and more specific, the molecular mechanism behind it. To start this study, the effects of the different surface structures on gene expression will be assessed.

Furthermore, we are interested in the influence of topological features on the cellular responses and if the provided guidance is based on individual parameters or groups of them.

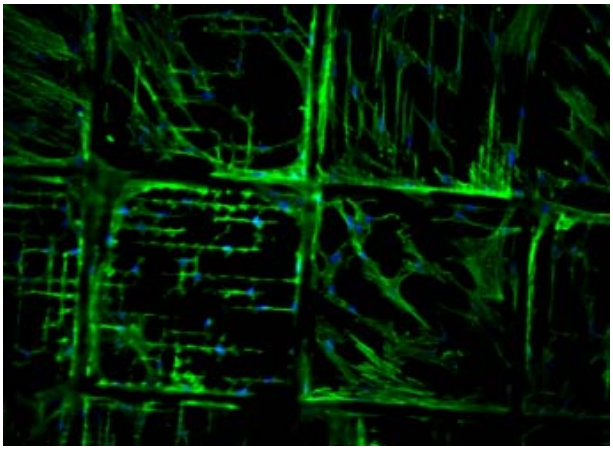

Fig. 1. The effect of surface topography on cell shape. hMSC's were cultured on a Poly(lactic acid) TopoChip, fluorescently labelled with phalloidin Alexa-594™ (pseudocoloured green) and 4',6-diamidino-2-phenylindole (DAPI) (pseudocoloured blue).

1. Dalby, M. J. *et al.* The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature materials* **6,** 997–1003 (2007).
2. Unadkat, H. V *et al.* An algorithm-based topographical biomaterials library to instruct cell fate. *Proceedings of the National Academy of Sciences* **109,** 5905–5905 (2012).
3. Carpenter, A. E. *et al.* CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome biology* **7,** R100 (2006).

An Anatomically Shaped Meniscus Implant Built by Stereolithography Using a Poly(trimethylene carbonate)-based Resin

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Introduction

Menisci are a vital part of the knee joint. Their unique shape and tribological properties allow the distribution of mechanical loads acting on the femur and the tibia over the cartilage surfaces. Meniscal injuries occur frequently in young patients and are the result of trauma, such as sports injuries. Meniscus tears are often situated in the avascular inner part of the meniscus and therefore do not heal spontaneously[1]. In the past the meniscus was then often removed completely to treat short term problems. However, removal of the entire meniscus was found to lead to severe osteoarthritis. To replace damaged meniscus tissue, resorbable porous implants that allow meniscal tissue ingrowth were developed. However, an optimal scaffold is not yet available.

Our overall aim is to build a defined, biodegradable and biocompatible functional meniscus scaffolding implant using stereolithography (SLA). SLA is a rapid-prototyping technique that allows the building of designed 3D structures using photo-crosslinkable macromers[2]. Here, we build a solid implant using MRI imaging data of goat menisci. The photo-reactive resin is based on poly(trimethylene carbonate) (PTMC) macromers, which crosslink into biodegradable, flexible and rubber-like materials with excellent mechanical properties[3].

Materials and Methods

Three-armed trimethylene carbonate (TMC) oligomers were synthesized by ring-opening polymerization using trimethylol propane as initiator. The oligomers were functionalized with methacrylic anhydride to obtain macromers with methacrylate end groups. The molar mass and degree of functionalization (DF) were determined by NMR.

The macromers were dissolved in dichloromethane containing 1 wt% Irgacure® 369 as photo-initiator. Networks were prepared by photo-crosslinking upon UV irradiation, their gel content, volume degree of swelling in chloroform and tensile properties were determined.

The macromers were also used to prepare an SLA resin which further contained 30 or 40 wt% propylene carbonate as a non-reactive diluent, 10 wt% TPO-L as photo-initiator and 0.15 wt% Orasol Orange as a dye. An average geometry of a goat meniscus was determined using MRI. This geometry was used to prepare a goat meniscus implant by SLA (EnvisionTec Perfactory).

Results and discussion

The Mn of the oligomers was 4.6, 10.0 and 19.6 kg/mol and the DF of the macromers was between 90 and 100% as determined by NMR. Table 1 shows that the resulting PTMC networks have excellent mechanical properties.

Table 1*. Properties of PTMC networks prepared by photo-crosslinking PTMC macromers.*

Mn of macromer	Gel content	Degree of swelling	Tensile strength	Tensile modulus	Elongation at break
kg/mol	%		MPa	MPa	%
4.6	97% ± 1.7	2.5 ± 0.3	2.1 ± 0.4	10 ± 0.7	24.4 ± 6.8
10.0	93% ± 0.1	3.2 ± 0.2	2.9 ± 0.7	8.0 ± 0.3	63.2 ± 20.1
19.6	99% ± 0.8	3.7 ± 0.1	3.4 ± 0.4	6.3 ± 0.4	117 ± 7.2

An anatomically shaped goat meniscus was defined from the imaging data of goat menisci and built by SLA with a resin containing PTMC macromers with Mn=10 kg/mol. As shown in Figure 1, the built structure closely matches the anatomical shape and dimensions of a goat meniscus.

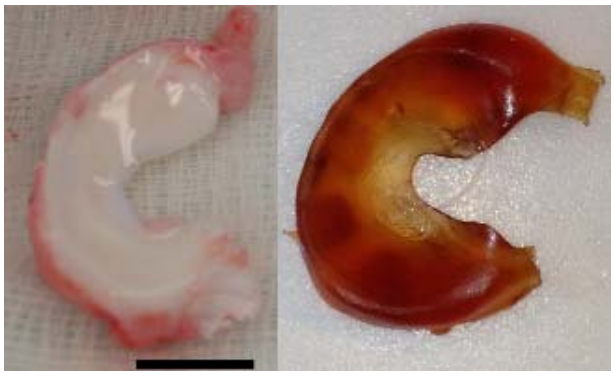

Figure 1. *Comparison of a goat meniscus (left) with an implant built by SLA using a PTMC-based resin (right).*

Conclusions

An anatomically correct biodegradable goat meniscus implant was designed using MRI imaging data and successfully built by SLA using a PTMC-based resin.

Future experiments will focus on building a meniscus implant (or part of it) with a predetermined pore network geometry allowing tissue ingrowth and matrix formation.

References

1) P. Buma, N. N. Ramrattan, T. G. van Tienen, and R. P. Veth, Tissue Engineering of the Meniscus, *Biomaterials*, **25**, 1523 (2004)

2) F. P.W. Melchels, J. Feijen, and D.W. Grijpma, A Review on Stereolithography and Its Applications in Biomedical Engineering, *Biomaterials*, **31**, 6121 (2010)

3) S. Schuller-Ravoo, J. Feijen, D.W. Grijpma, Flexible, elastic and tear-resistant networks prepared by photo-crosslinking poly(trimethylene carbonate) macromers , *Acta Biomater.*, **8**, 3576 (2012)

Synthesis of activated poly(2-oxazoline) polymers for bone tissue engineering

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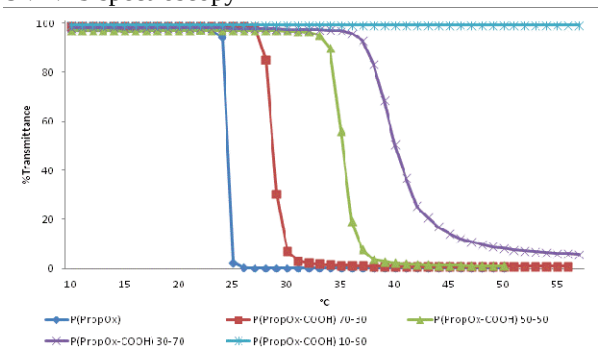
Introduction

Recently, poly(2-oxazolines) (POx) have found their way into biomedical applications[1]. POx-polymers have PEG-like properties regarding 'stealth' and clearance behavior[2]. Moreover, POx-polymers exhibit a glass transition temperature (generally above room temperature) and freely functionalizable side chains. Interestingly, poly(2-oxazolines) bearing propyl side chains exhibit a lower critical solution temperature (LCST) (Figure 1) which can be utilized for purification of polymers . Poly(2-oxazoline) polymers are synthesized by living cationic ring opening polymerization (LCROP) and further postmodified towards amine and NHS-functionalized polymers. One aim of the current project is to exploit the amine and NHS-functionalized poly(2-oxazolines) for the formation of hydrogels for bone tissue engineering. The material properties of the resulting polymers can be tuned by postmodifications, resulting in tailor made polymers for biomedical applications.

Results

-Polymers bearing different functional side chains have been synthesized by living cationic ring opening polymerization and postmodification strategies

-LCST determination of P(PropOx-COOH) polymers by UV-VIS spectroscopy



-Figure 1: LCST-determination of synthesized P(PropOx-COOH) co-polymers by UV-VIS spectroscopy

Conclusions

Well defined polymers are synthesized by a combination of LCROP and postmodification strategies. The defined architecture of both amine functionalized and NHS-functionalized poly(2-oxazolines) makes them suitable for gel formation and allows introduction of various functional molecules (for example proteins).

Indomethacin Differentially Changes Chondrogencic Outcome Depending on Chondrocyte Differentiation Stage

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Introduction: Heterotopic ossification (HO) is the abnormal formation of bone in soft tissues and is a frequent complication of orthopaedic surgery with large exposure of bone marrow (e.g. hip replacement surgery) and traumatic tissue damage. The standard treatment to prevent HO is administration of the non-steroidal anti-inflammatory drug (NSAID) indomethacin. In contrast to its widely usage for this indication, it is not understood how indomethacin affects the heterotopic bone formation process. HOs are described to develop via endochondral ossification and we aimed to address how indomethacin influences the chondrogenic phase of endochondral ossification.

Materials: ATDC5, human bone marrow stem cells (hBMSCs) and rabbit periosteal agarose cultures were employed as chondrogenic progenitor cell models. SW1353, human articular chondrocytes and differentiated ATDC5 cells were used as matured chondrocyte cell models. The inhibitory action of indomethacin on COX-activity was assessed using a specific PGE_2 ELISA. Gene- and protein expression analyses were employed to determine indomethacin action on chondrogenic extracellular matrix formation. Immunohistochemistry was performed on murine growth plates to determine COX-1 and -2 expression.

Results:

To examine the involvement of COX-1 and COX-2 during chondrogenic differentiation, spatiotemporal expression of these enzymes was determined in murine growth plates and during differentiation of ATDC5 cells. COX-1 expression is only increased during late chondrogenic differentiation, whereas COX-2 is briefly expressed early in differentiation and increases again late in differentiation.

To determine the role of the COX enzymes during the different time-points in differentiation (early vs. late), COX enzyme activity was inhibited by the non-specific COX inhibitor indomethacin.

Inhibition of COX-activity was confirmed by significantly decreased PGE_2 levels in all experiments. An indomethacin dose-dependent decrease in expression of Col2a1 and Col10a1 mRNAs, as well as decreased GAG content was observed when progenitor ATDC5 cells differentiated in the chondrogenic lineage in the presence of increasing concentrations of indomethacin. These results were confirmed using primary hBMSCs and *ex vivo* periosteal agarose cultures. Even when hypertrophic differentiation of ATDC5 cells was provoked by BMP-2 (30 ng/ml), the presence of indomethacin resulted in decreased hypertrophic marker expression. In sharp contrast with data derived from chondroprogenitor cells, when mature chondrocytes (primary human articular chondrocytes and SW1353 cells) were treated with indomethacin, a significant increase in Col2a1 expression was observed while no significant differences in Col10a1 expression were measured. These results were confirmed when ATDC5 cells were pre-differentiated for 10 days to first obtain a chondrocyte phenotype and subsequently indomethacin was added from this time point onwards. In these conditions low concentrations of indomethacin also resulted in increased Col2a1 expression.

Conclusions: Indomethacin inhibits chondrogenic differentiation of progenitor cells and elicits differential effects on chondrogenic differentiation depending on the chondrocyte's differentiation stage. Our observations point towards differential role for the COX enzymes during different phases on chondrogenic differentiation during endochondral ossification. Ongoing research is focussing on further elucidating the functional partition of the cyclooxygenases and possible specific prostaglandin production during the different phases on chondrogenic differentiation.

Amyloid Scaffolds for Cartilage Tissue Engineering: The Effect of Amyloid Structures on Cell Viability and Proliferation.

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Damaged cartilage cannot heal itself due to its low self-repair capacity. Current treatment options do not result in high quality cartilage. Therefore, new methods are required to obtain artificial cartilage that has mechanical properties comparable to those of native cartilage.

This project consists of two parts. Firstly, amyloid structures will be investigated as scaffold material for cartilage tissue engineering. Amyloid structures consist of proteins or peptides that self-assemble into β-sheet rich fibrils with a diameter of tens of nanometres. These structures are interesting since their mechanical properties resemble collagen fibres and they can be functionalized.

Secondly, the quality of the produced cartilage will be investigated by imaging proteoglycans. Proteoglycans consist of protein backbones with sugar side chains and are a part of the extracellular matrix of cartilage. The length of the protein and sugar chains influences the mechanical properties of cartilage. Proteoglycans isolated from artificial cartilage will be imaged using atomic force microscopy.

Before amyloid structures can be used as scaffolds and the quality of the produced cartilage can be investigated, their effect on cell viability has to be investigated. Amyloid structures are natural occurring, but also observed in several diseases although their role remains unclear. Preliminary results indicate that amyloid aggregates increase cell proliferation with no decrease in cell viability.

Effect of Pore Shape Gradient on human Mesenchymal Stem Cell Behavior in 3D scaffolds

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Introduction

The combination of porous biomaterials and stem cells is a field in expansion because of the possibility to develop three-dimensional (3D) structures that create a suitable environment for cell growth and the ability of stem cells to differentiate into different cell phenotypes in response to defined stimuli. During tissue development, stem cells fate is controlled by different signals based on gradients. The concept of gradient structures has been applied in different studies in two dimensional (2D) systems, such as microfluidics chambers and gels, to control or analyze cell migration. In tissues, cells grow in a 3D enviroment. Thus, 3D scaffolds presenting a gradient structure can provide cues similar to the native enviroment and stimulate stem cells to differentiate toward the targeted tissue to be regenerated. Among currently available scaffold fabrication technologies, rapid prototyping (RP) emerged as it allows controlling pore structure and architecture. RP scaffolds with an in-built porosity gradient was already proven to enhance cell seeding efficiency for already differentiated cells, when compared to non gradient scaffolds [1]. The aim of this study was to create 3D scaffolds presenting an axial gradient in pore shape by varying the plotting pattern, and evaluate its influence on human mesenchymal stem cells (hMSCs) growth and differentiation.

Materials and Methods

Polyactive 300/55/45 (PolyVation) scaffolds with or without gradient were fabricated by RP (Bioscaffolder, SysENG) at a pressure of 5 bars and a speed of 200 mm/s. Fiber deposition pattern was kept constant at 0-90 and 0-15 for non gradient (NG) scaffolds and varied from 0-90 to 0-15 for gradient (G) scaffolds. Bone marrow derived hMSCs were isolated from a patient after informed consent, expanded and seeded (passage 2) at a density of 500'000 cells/scaffold. After 7 days in proliferation medium, constructs were cultured in the same condition or in mineralization and chondrogenic media for oher 4 weeks. Cell growth and differentiation were evaluated by DNA, GAG and ALP assays after 8 and 35 days of culture. Cell adhesion and morphology as well as extra cellular matrix (ECM) formation were also evaluated by scanning electron microscopy (SEM).

Reults and Discussion

Gradient scaffolds (G) were prepared by changing the fiber deposition pattern every 7 layers (0-90, 0-45, 0-30, 0-15). The resulting scaffold presented pores with a squared shape on the bottom, which change into a more and more rhomboid structure when moving along the Z axis. This corresponded to an increase in major diagonal and a decrease in the minor diagonal length (Table 1). The non-gradient scaffolds (NG) were prepared by keeping the angle between the layers constant at 15° and 90° (0-15 and 0-90 respectively).

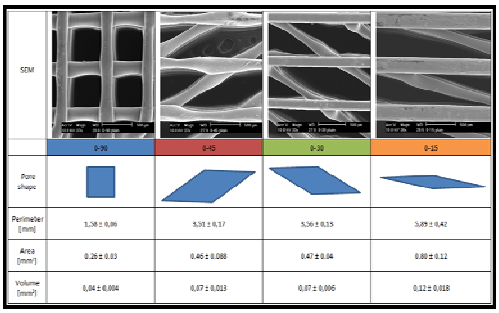

Table 1: SEM micrograph showing the pore shape changing in the different scaffold sections. The pores ranged from a square to a rhomboidal shape.

The pore perimeter and area increased along the Z axis, even though this difference was negligible between the pores when the pattern was 0-45 and 0-30 (Table 1).
hMSCs osteogenic and chondrogenic differentiation seems not to be affected by the gradient. Though a trend in osteogenic and chondrogenic differentiation was observed in relation with the pore shape. Squared pores supported a better chondrogenic differentiation whereas rhomboidal pores showed higher ALP levels.

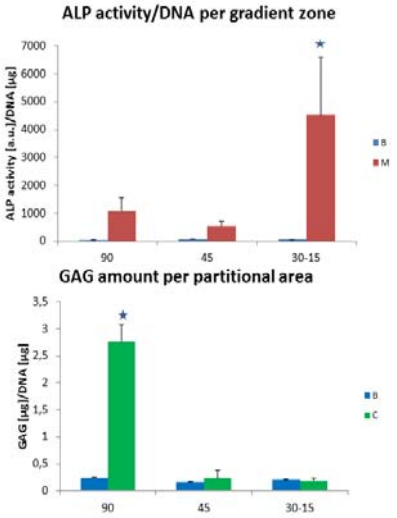

Figure 1: Plots showing the opposite differentiation trend. Rhomboidal pores determines higher ALP levels, whereas squared pores support higher GAG deposition.

Conclusions

Pore geometry seemed to enhance hMSCs diffrentiation toward the osteogenic (rhomboidal) and chondrogenic (squared) lineage. Further studies are needed to confirm the data with difference cell donors.

References

1 J. M. Sobral, S. G. Caridade, R. A. Sousa, J. F. Mano and R. L. Reis, *Acta Biomaterialia*, 2011, 7, 3, 1009-1018

Acknowledgements

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Poly(Amido Amine) Nanogels for Multi-Modal Imaging Purposes

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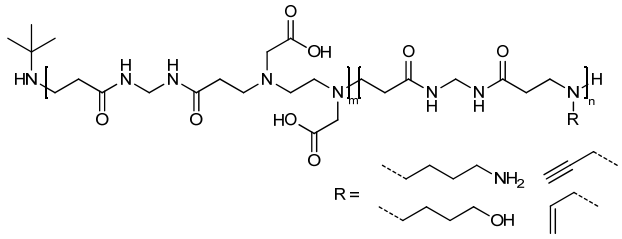
Introduction: The combination of Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) into PET/MR appends the highly sensitive tissue-specific information provided by PET to the anatomical information provided by MRI. The general aim of this collaborative project is to develop a targeted bimodal PET/MR nanoparticle to improve prostate cancer diagnosis.

The work presented here focuses on the preparation of nanogels based on Poly(Amido Amine)s (PAAs, structure depicted in Figure below), which can be modified for imaging applications due to the presence of carboxylic acid groups on the PAA nanogel surface. The same system is however applicable for other imaging techniques (e.g. fluorescence or SPECT) and drug delivery applications.



General structure of the synthesized PAAs

Methods: Carboxylic acid bearing PAAs were synthesized by a Michael addition and analyzed by GPC and NMR. A number co-polymers were synthesized as indicated above, containing either alkenes, alcohols, alkynes or primary amines.

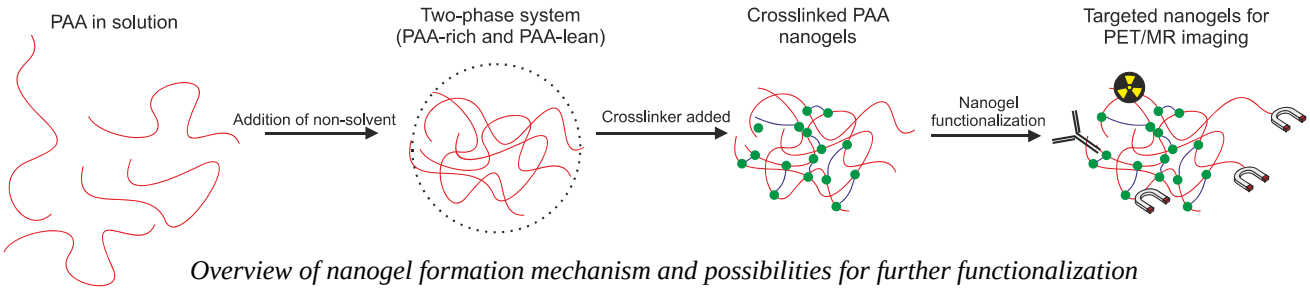
The nanogels were prepared according to the Scheme shown below: a PAA was dissolved in a suitable solvent and carefully adding a nonsolvent until nano-sized polymer aggregates were formed. The aggregates were then crosslinked by EDC coupling with a bis-amine. After the reaction was completed the nanogels were purified and re-suspended in water.

Results: The synthesis of a new set of PAAs with carboxylic acid groups attached to the polymer backbone was successful. New functional groups were introduced into the polymer, which allow addition of imaging or targeting moieties via e.g. epoxide coupling or click chemistry. The structures of the polymers were confirmed by NMR and the molecular weight was determined to be around 5 kDa by GPC as compared to PEG standards.

The nanogels were successfully prepared by employing a surfactant-free nanoprecipitation method[1,2] and subsequent crosslinking by EDC coupling with a bis-amine. Particle size was determined by DLS

The free carboxylic acid groups on the nanogel surface allows the attachment of imaging moieties and antibodies for targeting purposes. Finally the swelling behavior of nanogels under acidic conditions is promising for applications in drug delivery and MRI contrast enhancement.

Conclusions: Preparation of poly(amido amine) nanogels was successful and show great potential for further modification. Modification for imaging purposes and the toxicity in vitro of the synthesized nanogels will be further investigated.



Overview of nanogel formation mechanism and possibilities for further functionalization

(1) Urakami, H.; Hentschel, J.; Seetho, K.; Zeng, H.; Chawla, K.; Guan, Z. *Biomacromolecules* **2013**, *14*, 3682–8.

(2) Steinhilber, D.; Witting, M.; Zhang, X.; Staegemann, M.; Paulus, F.; Friess, W.; Küchler, S.; Haag, R. *J. Control. Release* **2013**, *169*, 289–95.

Bioinspired Supramolecular Biomaterials for *in-situ* Cardiovascular Tissue Engineering

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Cardiovascular and valvular diseases are responsible for a significant cause of death worldwide. Although surgical interventions to replace heart valves or arteries provide a significant improve in quality of life, long-term failure of the implants often leads to a decreased life expectancy. To this extend, *in-situ* cardiovascular tissue engineering has evolved as a potential alternative in order to create an instructive, biodegradable scaffold. This scaffold aims at using the natural regenerative potential of the human body to engineer a replacement tissue *in vivo*. In our group, we aim at the synthesis and development of cell-free vascular graft materials using supramolecular chemistry. These graft materials should be indistinguishable from nature, requiring control over both their mechanical properties and their bioactive adaptation capacity. Tailor-made materials are developed based on supramolecular polymers end-capped with ureido-pyrimidinone (UPy) moieties, able to dimerize upon quadruple hydrogen-bond formation giving rise to a dynamic character, that are spaced with polycaprolactone blocks to meet mechanical requirements of the material. Bioactive UPy-modified peptides are incorporated via a modular approach in order to provide necessary biological signals to attract and stimulate cells inside the scaffold. In the design presented here, the supramolecular scaffold consists of two layers. The first, luminal layer targets endothelial progenitor cells (EPC) that will be recruited from the blood stream by bioactive factors such as SDF-1 α (stromal cell-derived factor 1 alpha) derived peptides incorporated in the scaffold. These EPCs subsequently are proposed to differentiate into endothelial cells. In the second layer another bioactivity is introduced, for example TGFβ (transforming growth factor beta) derived peptide sequences or heparin binding peptide motifs that can bind heparin. It is proposed that the bioactivity in the second layer is able to induce endothelial-to-mesenchymal transition (EndMT) yielding the formation of a layer of smooth muscle cells. This approach is a step forward in the design of a cell-free instructive synthetic biomaterial for future *in-situ* cardiovascular tissue engineering applications.

Immuno-protective Membrane Based Scaffolds For Extra Hepatic Islet Transplantation

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Introduction

Type 1 Diabetes Mellitus is a chronic disease and is characterized by destruction of the β cells due to an autoimmune reaction. It is known for its severe acute and long-term complications due to micro- and macroangiopathic lesions and has a significant social and economic impact. Diabetes Type 1 patients, with severe glyceamic lability, recurrent hypoglycaemia, and hypoglycaemia unawareness are in need of alternative therapies. Current alternatives for insulin injections are total pancreas transplantation or islet of Langerhans transplantation. Although transplantation of islets of Langerhans has proven to be an effective treatment, more than 60-70% of the islets are lost immediately after transplantation. In addition, the life-long use of immunosuppressive drugs, necessary to decrease the chance of rejection, is associated with numerous complications. Therefore, the goal of this project is to find a new islet encapsulation method to overcome the need for immunosuppressive drugs and to improve islet transplantation for type 1 Diabetes patients. We propose the use of membrane based scaffolds as an immunoprotective encapsulation method for islet of Langerhans transplantation. During this study, different membrane encapsulation methods will be tested.

Materials and Methods

The first membrane based encapsulation method, is a modified poly(ether sulfone) (PESM) Multibore® hollow fiber from Inge AG (Greifenberg, Germany). The 4 mm single fiber consists of 7 bores with a diameter of approximately 0.9 mm each. The membrane has pores of 20 nm. These multibore hollow fibers are commercially available and used for water treatment. In literature they are described as a hepatocyte bioreactor and a medium perfusion system for three dimensional tissue engineered constructs. In these studies it was shown that they were biocompatible and do not foul. Glucose and insulin convection was determined by dead-end permeation. Islets were seeded and cultured statically and dynamically to assess islet viability and functionality via histology and glucose induced insulin secretion test. In addition, as aggregation of islets is detrimental for islet function, due to long diffusion distances, agarose beads were used to physically separate islets.

Results

Results showed that glucose and insulin pass through the membrane, as the permeance of glucose and insulin were not significantly different from the water permeance. In addition, a TUNEL assay showed hardly any apoptotic cells after 7 days of culture. First trials of glucose induced insulin secretion tests show a moderate response. With the use of the agarose beads, aggregation of islets was prevented.

Conclusion

These preliminary results indicate the possibility of islet encapsulation in a multibore hollow fiber.

ACKNOWLEDGEMENT

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Multilayered Thin Films from Poly(amido amine)s, Poly(vinyl alcohol) and Chondroitin Sulfate

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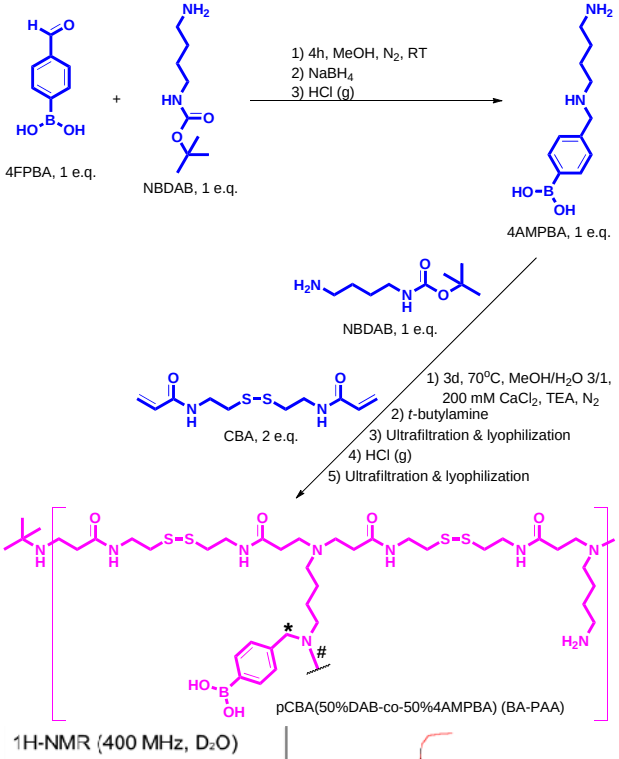
Introduction: Biodegradable multilayered thin films (MTF) based on boronic ester formation may serve as functional scaffolds for cell growth and/or differentiation. Multi-functionality may be introduced by incorporation and subsequent controlled release of bioactive agents, utilizing the inherent sugar- and pH-responsive properties.

Materials and methods:

Boronic acid (BA) functional monomer and polymer (BA-PAA) Syntheses. Syntheses were carried out as described in Scheme 1. BA functionality was set to 50% of total number of repeating unit to facilitate better solubility and deposition.



Scheme 1 Synthesis and ^{1}H-NMR spectrum of BA-PAA

Layer by layer (LbL) Assembly. MTFs were prepared by alternately dipping piranha-activated substrates in BA-PAA solution and PVA or chondroitin sulfate (ChS) solutions, with washing solutions in between (Figure 1). The deposition cycle was repeated to reach typically 10 or 10.5 (BA-PAA#PVA or ChS) layer pairs, indicated as (BA-PAA#PVA or ChS)$_{10.5}$. Increases in the amount of incorporated BA-PAA with each deposition cycle were followed using UV-Vis spectroscopy (λ_{max} obtained through the phenyl-moieties of the polymer).

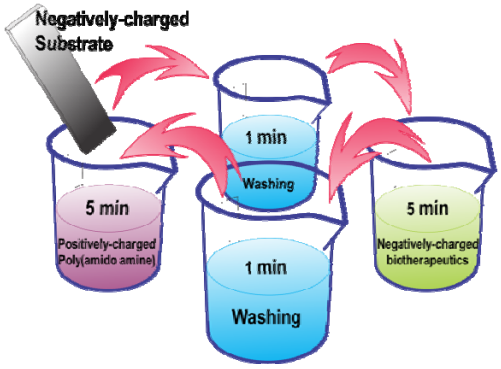


Figure 1 Schematic illustration of LbL assembly

Stability of the MTFs in various reducing environments and in the presence of glucose was investigated by incubation of the films in respective PBS solutions, pH 7.4 at 37 °C. As model bioactive/therapeutic agent, alizarin red S (ARS; a dye containing vicinal diols) was added into the deposition solutions of PVA and ChS for incorporation during layer build-up and the release was studied under physiological conditions.

Results, discussion and conclusion: PVA and ChS were used as possible counterparts for the BA-PAA. MTFs formed from PVA are much thinner (~150 nm thick) as compared to those formed from ChS (>700 nm thick), based on AFM scratch tests. Both systems showed linear build-up profiles.

All MTFs are responsive to different concentrations of glutathione and DTT, but only MTFs prepared using PVA are responsive to different glucose concentrations, indicating the dependence of the PVA MTF formation on boronic ester formation. On the other hand, build-up with ChS relies primarily on electrostatic interactions between the two macromolecules.

All MTFs were able to incorporate ARS through boronic-ester formation between the dye and the BA functionality. ARS is released from the two systems in the same manner under physiological conditions, displaying a burst release profile with fast release for the first 12 h, which is followed by prolonged release over a period of several days.

Producing Artificial Chondrons for Improved Cartilage Repair

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Introduction

Articular cartilage has limited self-repairing capacity and untreated damage often leads to osteoarthritis or joint failure. Currently, autologous chondrocyte implantation is the most successful method for repairing focal cartilage defects. This therapy involves the isolation of chondrocytes by enzymatic degradation of autologous cartilage, which strips the cells from their matrix. Consequently, the chondrocytes are separated from their highly specialized microenvironments, called chondrons (Fig1). This results in dedifferentiation, *i.e.* the isolated chondrocyte progressively lose their chondrogenic phenotype. We aim to develop a strategy to encapsulate single chondrocytes in artificial chondrons. When successful, this approach represents a cost-effective method to prevent the dedifferentiation, while simultaneously allows for optimal and custom designed stimulation of the encapsulated chondrocytes. Here, we present a microfluidic device able to encapsulate single chondrocytes in chondron-sized microgels in an efficient and cell-friendly manner (Fig1).

Method

The master mold for the optimized microfluidic droplet generator was made by micropatterning ~25µm thick SU8-50 (MicroChem) on a silicon wafer using standard photolithography techniques. PDMS (Sylgard 184, Dow Corning) was thermally cured on the master and bonded to glass after plasma treatment. Aquapel (Vulcavite) was introduced in the chip before usage to ensure hydrophobic channels walls. Chondron-sized droplets were produced by emulsifying a chondrocyte-laden hydrogel precursor solution of 10% (w/v) polyethylene glycol diacrylate (PEGDA, Laysan Bio, Inc.) and 0.1% (w/v) photoinitiator (Irgacure 2959, Ciba Specialty Chemicals) in a surfactant and photoinitiator containing oil. Subsequently, the microgels were formed in an on-chip delay channel by curing the emulsion with 365nm UV-light (Hamamatsu LC8). We compared various combinations of oils, surfactants, photoinitiators and UV dosages, as these are of paramount importance to obtain chondron-sized microgels.

Results

Chondrocytes were encapsulated in PEGDA microgels using droplet microfluidics (Fig1). The number of encapsulated cells followed a Poisson-distribution that was dependent on the cell concentration. We aimed to minimize the microgels' dimensions by comparing different combinations of oils, surfactants, photoinitiators and UV dosages. Fluorinated oils (Novec 7500 and Fluorinert FC-40, 3M) in combination with surfactants Pico-Surf 1 and 2 (Dolomite) resulted in the most stable emulsions and thus allowed for the lowest photoinitiator concentration and UV dose for cell-laden microgel production. However, the use of fluorinated oils resulted in relatively large gels, which is a consequence of their low viscosity compared to the hydrogel precursor solution. In contrast, the use of a more viscous hydrocarbon oil hexadecane (Sigma-Aldrich) with 1% (v/v) Span 80 (Sigma-Aldrich) surfactant resulted in smaller, chondron-sized microgels. However, emulsions with this oil/surfactant combination were less stable off-chip. To prevent droplet coalescence, the on-chip crosslinking of the cell-laden gel precursor droplets was improved. A dual photoinitiator system was used to ensure fast and solid on-chip crosslinking while minimizing the amounts of cytotoxic photoinitiator and UV-dose. Finally, by using 0.1% Irgacure 2959 in the disperse and 0.1% Irgacure 651 in the continuous phase, we were able to encapsulate single chondrocytes in stable PEGDA microgels with a diameter below 50µm in a cell-friendly manner (Fig1).

Conclusion & Discussion

We have developed a microfluidic platform and optimized the production process for the encapsulation of single chondrocytes in chondron-sized microgels. Future work will focus on the functionalization of the microenvironment within the biomaterial by means of incorporation of growth factors and extracellular matrix molecules to create biomimetic and chondrogenic artificial chondrons.

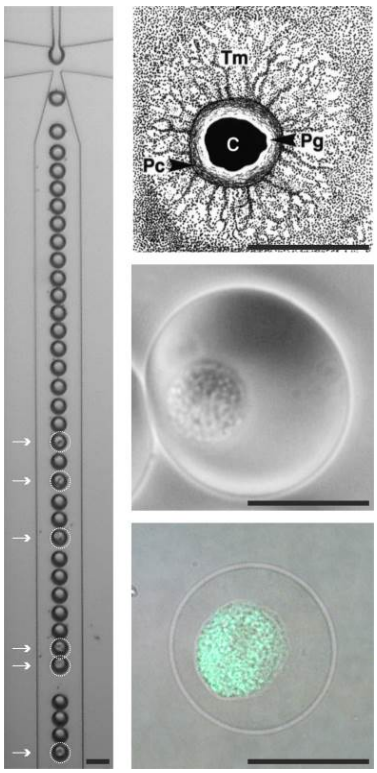

Fig1. left: microfluidic droplet generator producing monodisperse cell-laden hydrogel precursor droplets (arrows); right-top: Transmission electron microscopy image of a chondron within extracellular cartilage matrix, containing a chondrocyte (c) and its pericellular matrix (Tm); right-middle and right-bottom: PEGDA microgels encapsulating single viable chondrocytes. Scale-bar left: 50µm. Scale-bar right: 25µm.

Rat Bone Marrow-derived Cell Population features selective Migration Behavior on a submicro- and nano-dimensional multi-patterned Substrates

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Introduction

Recently bone-implant optimization is mainly focused on modifications of material-surfaces. Most commonly, modern titanium implants are modified by grit blasting in combination with acid etching, laser machining and oxidation. These industrially utilized methods introduce (among other modifications of surface properties) substantial changes to the topography. The increasing surface-complexity in form of roughness consists of isolated features with sizes down to the nano scale and has been shown to positively influence bone integration in clinics. Interestingly, topography seems not only to be effective on the organ level, but stimulates various types of cell-behavior by isotropic and anisotropic macro-, micro- and nano-meter-sized features. As has been shown by multiple in vitro studies, manipulation of processes like cell differentiation, proliferation and migration can be achieved by utilizing different topographies.

It is interesting that not only cell behavior like differentiation can be provoked, but also inhibited by use of topography. It has been shown that stem cells cultured on a specific nano-pit surface can maintain their self-renewing and differentiation capacity, an effect that can usually only be seen in nature for stem-cells being in direct contact with their niche, which is known to have specific bio-chemical properties. The question arises whether other cellular phenomena, like repopulation of stem-cell niches after chemotherapy – known as homing, could also be a result of cell-surface pattern recognition. Therefore the cells would need to have the ability of recognition of, distinction between and preference for different patterns.

Material and Methods

To evaluate the possibility of a cellular-migration selectivity based on the experienced surfaces, we made use of a multi-patterned culturing platform that contained 36 different sub-micro and nano-sized topographies, namely fields of 500x500 µm² filled with squares and grooves between 10 and 500 nano-metersin size, each being surrounded by a smooth surface. These so called “biochips” were used for cell culture experiments using rat bone marrow derived cells, which were allowed to migrate on the substrates (4, 24, 48, 72 hours) while “choosing” between patterns. We reasoned that cell-surface interactions during the first hours after cell seeding would be determined mainly by cell attachment to the substrate, while numbers of later timepoints would be defined by cell migration and proliferation. Randomly seeded cells should accumulate in time on patterns of preference, showing higher numbers when compared to the smooth surrounding area, while cell-repelling patterns should lead to a reduced cell retaining and smaller numbers compared to the surrounding area and to visually less dense fields in late phases of proliferation. The obtained data from DAPI stained cells, was interpreted by using a regression analysis model leading to a selection of 6 topographies with strongest differences in cell attraction or repelling, which have been further analyzed for additional properties, such as proliferation by utilizing a fluorescence-based EdU method.

Results and Discussion

We observed clear differences in cellular migration behavior on the different topographies. Especially the cell repelling effect on cells was significantly strong starting at 24 hours after seeding and increasing in time until the last timepoint of 72 hours. The strongest repelling effect was observed on squared patterns (cell numbers down to 60% compared to smooth surrounding surface), followed by grooved patterns with a ridge to groove ratio of 1:3 (down to 70% compared to smooth surrounding surface). Micrographs (Light, Fluorescence and SEM) showed cells on smooth surrounding rather framing the topographical fields than being in direct contact. That effect could be observed even after 9 days of culturing.

Additionally, we have tested whether the differences in cell numbers could be explained by differences in proliferative capacity of the used cells on the topographies. Interestingly, cells proliferated stronger on the patterned surfaces in comparison to the smooth surrounding.

Conclusion

The experiment showed that the setup of a multi-patterned substrate could be utilized for preferential cell migration studies. Strong evidence has been found for the ability of surface patterning to influence cell migration behavior in a way that could be explained by cells featuring a preference mechanism, allowing cells to distinguish between different topographical features. This finding could be applied for the development of cell-repelling materials.

Tissue-engineered hypertrophic cartilage: a model to study endochondral ossification

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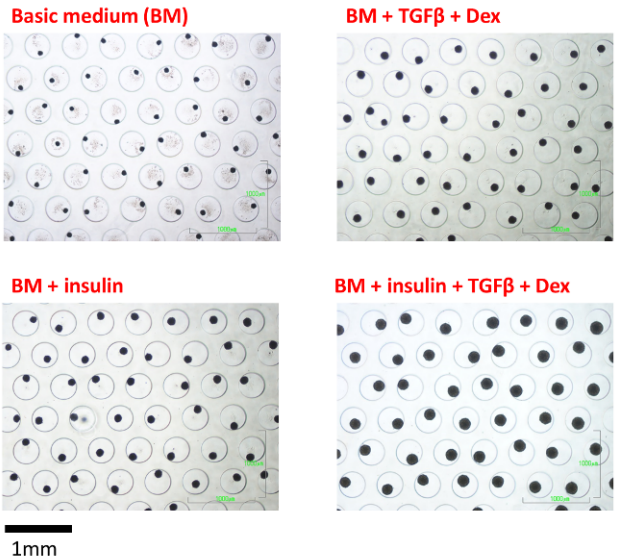
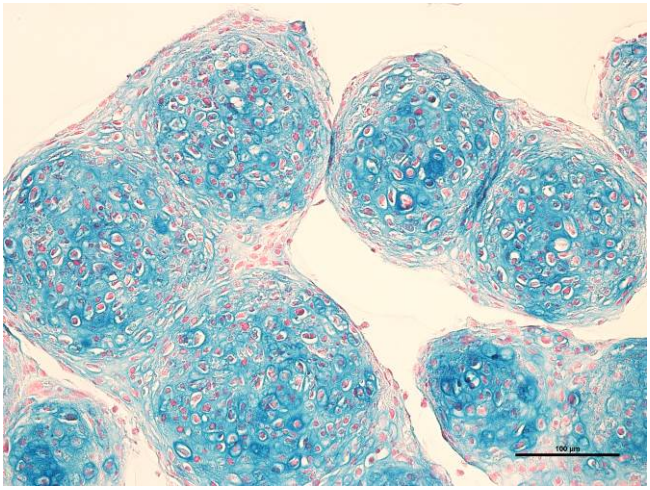
Introduction: Recapitulation of the endochondral ossification process in tissue-engineered (TE) constructs *in vivo* has been a partial success. Although the proof of principle has been achieved, reproducibility of the methods and up-scaling for clinical applications remain a big challenge. In order to solve the key problems in creating, optimizing and up-scaling this type of TE constructs, we develop a novel hypertrophic cartilage model using the chondrogenic cell line ATDC5 and a micro-aggregate cell culture platform.

Method: Micro-aggregates of ATDC5 cells (approximately 1000 cells per aggregate) are cultured in an agarose mould consisting of hundreds of microwells, each measured 400µM in diameter. To optimize chondrogenic differentiation, medium compositions made of different combinations of growth factors e.g. insulin, transforming growth factor beta (TGFβ) and dexamethasone (Dex) were used. To enhance vascularization at the end stage of hypertrophy, we used small molecules capable of inducing a hypoxia mimic response, resulting in up-regulation of vascular endothelia growth factor alpha (VEGFa). To induce *in vitro* mineralization, we supply the culture medium with either inorganic phosphate or β-glycerophosphate. After 2 or 3 week culture *in vitro*, the TE hypertrophic cartilage will be implanted in nude mice subcutaneously to access bone forming capacity.

Result: Medium composition consisting of insulin, TGFβ and Dex is most effective in promoting chondrogenic differentiation in this model, inducing high up-regulation of collagen II, collagen X, SOX9, HIF1a. Histological analysis shows uniform hypertrophic morphology at week 2-3 in aggregates cultured with both insulin and TGFβ. Phenalthroline can induce 3 times higher VEGF expression after just 3 days in the culture medium.

Discussion and conclusion: This ATDC5 micro-aggregate model offers a 3D culture environment without compromising nutrient delivery to the cells. Instead of ATDC5 cell line, other clinical relevant cells can also be used. High throughput screening can be performed to search for molecules that further improve the hypertrophic characteristic of the aggregates. This model can also be readily upscaled in order to provide sufficient material for potential clinical applications.





BM + insulin + TGFβ + Dex, alcian blue staining at week 2.

Bioprintable hydrogels for heterogeneous constructs with osteoprogenitors and endothelial progenitors

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Introduction

In the field of bone tissue engineering, one of the major processes is timely vascularization, in order to ensure survival of large, clinically relevant-sized cell-based bone tissue engineered constructs.

Current strategies often use a single cell population and matrix type for production of mineralized bone. In contrast, natural bone tissue is composed of multiple cell types, profiting from cell interaction. Adding endothelial progenitors cells (EPCs) to multipotent stromal cell (MSC)-based tissue engineered constructs leads to endothelial vessel networks *in vitro* and is beneficial to the performance of the bone-forming cells and stimulates neovascularization when implanted.

A well know strategy in the field of regenerative medicine is bio-mimicking, in which the anatomical organization of cells, matrix, and bioactive molecules of the native tissue is imitated.

Organ- or tissue-printing is one of the preferred technologies in this approach, as it can be used to investigate whether imposed cell organization is necessary for obtaining fully functional newly formed tissues. With the 3D fiber deposition system, layers of cell-laden hydrogel strands are deposited according to a computer design. The resulting 3D scaffolds are accurate and reproducible in size, shape, porosity and geometry. By using different printed heads containing distinct matrix materials, cell populations can be printed at defined locations resulting in a heterogeneous construct.

The general aim of this project is to investigate whether a construct with heterogeneous cell organization can lead to specific tissue formation in the defined locations.

to this end, different available bioprintable hydrogels were selected based on their capacity to support progenitor cell differentiation.

Materials & Methods

Different hydrogels were used at concentrations suitable for bioprinting. Hydrogels consisted of methacrylated gelatin (GelMA), methacrylated hyaluronic acid (HAMA), Matrigel, alginate, gellan gum, chitosan, and mixtures of these gels.

Cell viability, osteogenic differentiation of MSCs, and network formation of EPCs were assessed, by seeding the cells in small plugs of previously mentioned hydrogels.

Goat MSCs were resuspended with $5*10^6$ cells/ml in plugs and cultured in expansion medium or osteogenic medium. Viability of the seeded cells was assessed after 24 hours and after 7 days. Alkaline phosphatase activity and free calcium were measured to quantify osteogenic differentiation at day 7 and 14. Matrix deposition/mineralisation were investigated on paraffin embedded samples cultured for 14 and 21 days using von Kossa and alizarin red.

Network formation was evaluated in a co-culture model. Goat MSCs and EPCs (ratio 4/1) were mixed and resuspended at a concentration of $5*10^6$ cells/ml in the different hydrogels and cultured in combined EPC-osteogenic medium for 10 and 20 days. Network formation was assessed on whole gel immunocytochemistry and on paraffin embedded sections.

The different hydrogels were printed in a single design. By comparing the actual printed construct to the design, printability was assessed. Several designs were printed with different hydrogels.

Results

Viability of the seeded cells after 7 days was >60% in all gels except those containing chitosan. Constructs composed of GelMA and Matrigel with MSCs resulted in the highest ALP and Ca^{2+} concentrations, which was confirmed by immunocytochemistry.

Also in the co-culture constructs we observed abundant calcium phosphate depositions in particular in preparations based on GelMA and Matrigel. Network formation was only seen in the constructs containing Matrigel.

Overall, GelMA and alginate performed best on printability, based on stability of the structure.

Conclusion

From the different natural hydrogels selected to optimize the printing of heterogenic bone tissue engineered constructs, GelMA, alginate and Matrigel appeared to be most promising. The results give an insight in both biological and mechanical performance of these gels.

Acknowledgements:
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Development of a gelMA-based Bioink for Additive Tissue Manufacturing

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Introduction

Additive manufacturing forms a potential route towards economically viable production of cellular constructs for tissue engineering[1]. Hydrogels are a suitable class of materials for cell delivery and 3D culture, but are generally unsuitable as construction materials. Gelatin-methacrylamide (gelMA) is an example of such a hydrogel system widely used in the field of tissue engineering, e.g. for cartilage[2] and blood vessels[3]. Here, we obtain suitable rheological properties for gelMA-based biofabrication by the addition of gellan gum and tailoring salt concentration. Its potential for additive tissue manufacturing purpose is demonstrated.

Methods

GelMA (10 wt%), supplemented with 0-1.0 wt% gellan gum and varying salt concentrations (up to physiological level) were characterised by rheometry and extruded with a custom-made bioprinter (34°C). MC3T3 fibroblasts were encapsulated in printable gels by UV-curing. Metabolic activity was assessed using an Alamar Blue assay after 1 day culture.

Results and Discussion

Gellan gum can be reversibly crosslinked to weak gels by cations, such as protonated lysine residues on gelMA. The viscosity of gelMA solutions (>32°C) is therefore increased by orders of magnitude upon addition of gellan gum, which together with strong pseudo-plastic behavior greatly enhances the ability to extrude gel strands. The ionic interaction also introduces a yield stress, which provides shape stability to plotted gel structures (Fig. 1). This yield stress, which was measured by compression testing on uncured gels, increased with increasing salt concentration up to 80 mM NaCl, above which phase separation occurred. Optimal results for plotting gel structures were obtained at 16-24 mM NaCl, resulting in solid and porous gel structures which could be further stabilised by UV curing (Fig. 2).

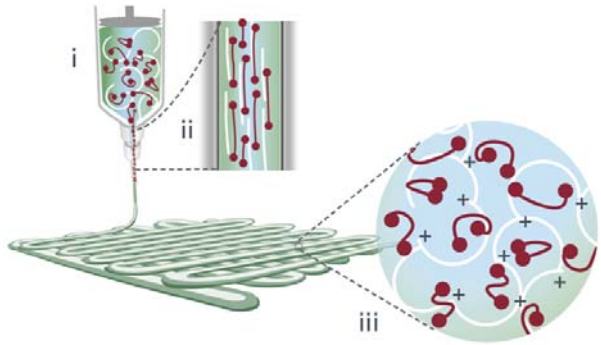

Figure 1: schematic of rheological phenomena governing the plotting of gelMA/gellan hydrogels

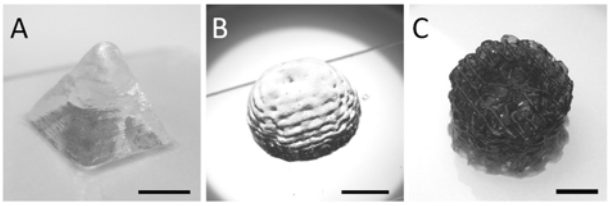

Figure 2: gel structures plotted using 10% gelMA + 1% gellan gum and 24 mM NaCl (34°C).

Interestingly, the addition of gellan and substitution of ions (PBS) by the sugar mannose (in order to maintain hypotonic conditions) caused no significant decrease in viability of encapsulated cells. The viability after 1 day culture was approximately 90% in gelMA/gellan (10/0.5%) gels with isotonic mannose as well as in 10% gelMA alone in PBS. In contrast, all cells died when encapsulated in gelMA gels in demineralised water.

Summary/Conclusions

GelMA/gellan gum mixtures with tailored salt concentrations are attractive candidates for bioprinting, because they exhibit desirable rheological behavior while maintaining high cell viability.

References

1. Melchels FPW *et al.*, Prog Polym Sci (2012), 37:1079-1104
2. Schuurman W *et al.*, Macromol Biosci (2013), 13:551-61
3. Chen YC *et al.*, Adv Funct Mater (2012), 22:2027-39

Acknowledgments

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BIOMATERIALS FOR TISSUE REGENERATION: STUDY OF CELL-MATERIAL AND CELL-TISSUE INTERACTION USING PROTEIN ANALYSES

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Introduction

Synthetic biomaterials are becoming increasingly important in the field of orthopedics and cranio-maxillo-facial surgery, since application of natural bone grafts (i.e patient's own bone, bone from a donor) is associated with important drawbacks of donor site morbidity, infections and limited availability. Synthetic bone graft substitutes, which are relatively inexpensive, and available in large quantities and off-the-shelf, present an interesting alternative to natural bone grafts, but their biological performance, in terms of osteoconductivity and osteoinductivity is generally considered inferior to that of their natural counterparts[1]. In order to be able to improve bioactivity of synthetic bone graft substitutes, it is imperative to understand mechanisms of their interaction with the biological environment. Here we aim to use advanced proteomics techniques to study interactions between synthetic biomaterials and cells/tissues.

Materials and Methods

Human mesenchymal stromal cells (hMSCs) were cultured for 8 hours, 2 days and 7 days respectively on four different types of calcium phosphate ceramics: hydroxyhapatite (HA), two biphasic calcium phosphate ceramics sintered at different temperatures (BCP1150 and BCP 1300) and β-tricalcium phosphate (TCP). These materials have previously been demonstrated to possess distinct levels of bioactivity in terms of osteoinductivity and bone regenerative potential[3]. Mass spectrometry (MS) was applied to determine expression of protein profiles of cells on different materials. Validation of protein expression was performed by using Western blot. In addition, qPCR was used to study expression of relevant markers on mRNA level (figure 1).

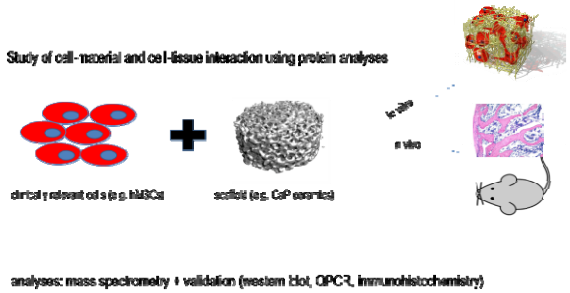

***Figure 1**. Experimental set-up*

Results

MS analysis resulted in identification of 661 proteins. The identified proteins were categorized based on their function (figure 2A). The group with proteins categorized as "others", i.e. with poorly identified functions was the largest (53%), followed by enzymes (24%). Based on their role in process related to bone regeneration, 6 proteins were selected for further analyses. Expression of these selected proteins was shown to be influenced, to a great extent, by the type of calcium-phosphate ceramic used. The level of their expression was also dependent on culture time. As an example, figure 2 shows the spectrum counts (2B) and protein expression (2C) of Ectonucleotide pyrophosphatase /phosphodiesterase-1 (Ennp1) obtained by MS and Western blot respectively. The expression of Enpp1 was higher by cells cultured on BCP1150 and TCP after 7 days of culture, as compared to the other two ceramics used.

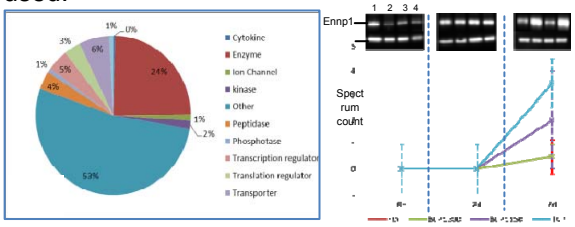

***Figure 2**. (**A**) Pie chart of the identified proteins categorized based on functionality. (**B**) spectrum count of Enpp1 of MSCs. The cells (n=2) were cultured on HA, BCP1500, BCP1300 and TCP for 8 hours, 2 days and 7 days, respectively. (**C**) Black bar shows Western blot for Enpp1 and GAPDH (loading control). 1,5,9=HA, 2,6,10=BCP1500, 3,4,11=BCP1300 and 4,8,12 =TCP.*

Discussion

Enpp1 is an establish mediator for mineralization initiation in tissue and has an important role in osteoblast differentiation[2]. In this study Enpp1 was differentially expressed on the four materials, as analyzed by MS and confirmed by Western blot. The trend of its expression was in accordance with the trend of in vivo bioactivity of the four materials tested[3].

Acknowledgement

This project is supported by NWO TA-COAST grant # 05321104.

References

1. P Habibovic and K de Groot, *J Tissue Eng Reg Med* 2007
2. HK Nam et al. J Biol Chem 2011.
3. H Yuan et al. Proc Natl Acad Sci USA 2010

A Pharmacokinetic Modeling Approach of ^{18}F-fluoride PET/CT scans To Assess Bone Graft Incorporation In Posterior Lumbar Interbody Fusion

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Introduction

Posterior Lumbar Interbody Fusion (PLIF) is a surgical procedure to treat patients with symptomatic spondylolisthesis when conservative measures have failed. The desired outcome of this procedure is stabilization through bony bridging, by the insertion of intervertebral cages filled with autologous bone. The clinical success may be limited by pseudarthrosis, leading to recurring back problems. Presently, no non-invasive technique is available to reliably assess bone graft incorporation early after PLIF. A PET-scan with a bone-seeking tracer such as ^{18}F-fluoride could be a promising modality for early detection of pseudarthrosis since it is able to quantify bone metabolism by measuring osteoblast activity, reflecting the process of bone graft incorporation. The addition of a co-registered CT-scan adds anatomical information to the functional PET information. To date, the most widely used parameter for quantification of radioactivity in PET-scans is the standardized uptake value (SUV). SUV is a semi-quantitative measure obtained from a static scan. Using static ^{18}F-fluoride PET/CT imaging, an increased bone metabolism in patients with persisting symptoms after PLIF surgery has been shown. When a dynamic PET-scan is made, additional parameters that describe the tracer uptake are obtained through a pharmacokinetic modeling approach. Hypothesized is that the dynamic analysis method will provide a more accurate and more complete representation of the bone graft incorporation. In this study, a pharmacokinetic modeling approach using dynamic ^{18}F-fluoride PET/CT scanning to assess bone graft incorporation after PLIF is evaluated.

Materials and Methods

Twenty patients who underwent PLIF were included. Following a low-dose CT scan, a 30 minutes dynamic PET acquisition (Philips, Gemini TF PET/CT) covering the PLIF region was started directly after intravenous injection of ^{18}F-fluoride. One hour post-injection also a static PET/CT scan was acquired. The dynamic data was analyzed using two different pharmacokinetic models: the irreversible two-tissue-compartment model (2TCM) and the Patlak model. For each patient, six regions of interest (ROIs) were defined, including the intervertebral disc space and upper and lower endplates of both the operated segment and a normal reference segment. For each region three parameters reflecting overall bone metabolism were determined: $SUV_{mean,stat}$, $K_{bone,2TCM}$, $K_{bone,Patlak}$. For the 2TCM the rate constants, $K_{1,2TCM}$, $k_{2,2TCM}$ and $k_{3,2TCM}$ were determined as well. Using the 2TCM rate constants the $SUV_{mean,2TCM}$, at one hour after administration, was estimated from the dynamic scan.

Results

As expected, $K_{bone,2TCM}$ and $K_{bone,Patlak}$ were highly correlated (R=0.99). Furthermore, a high correlation (R=0.90) was observed between $SUV_{mean,stat}$ and $SUV_{mean,2TCM}$. SUV_{mean} and $K_{bone,2TCM}$ and SUV_{mean} and $K_{bone,Patlak}$ were correlated to a lesser degree (R=0.84 respectively R=0.85) which could be explained by the fact that the SUV is affected by patient-specific arterial blood clearance of the tracer, whereas this effect is taken into account in the K_{bone} measures. Accounting for this effect increased the strength of correlation (R=0.90). All investigated parameters except $k_{3,2TCM}$ ($p=0.112$) showed a significant increase for the PLIF segment compared to the control segment ($p<0.05$).

Conclusion

The dynamic scan approach using K_{bone} may be preferable for bone metabolism quantification as it is able to distinguish between the amount of activity delivered to the tissue by the blood and the actual activity of the tissue, while SUV contains a mix of both effects. Our study shows that the one hour post-injection $SUV_{mean,stat}$ can be reliably calculated from the 30 minutes dynamic PET scan, allowing a substantial shortening of the total PET/CT procedure. Moreover, the 2TCM provides additional information concerning the individual rate constants. The clinical relevance of dynamic ^{18}F-fluoride PET/CT for bone graft incorporation is currently under evaluation in a prospective study.

The role of macrophages in the foreign body reaction

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Macrophages are a pivotal cell type during the foreign body reaction (FBR). They orchestrate the pro-inflammatory microenvironment inside and around biomaterials by secretion of mediators such as chemokines, cytokines and growth factors. Outside the biomaterial these factors may instruct the fibroblasts that produce a fibrous capsule around the biomaterial. Inside the biomaterial these factors attract and activate more inflammatory cells. Additionally, macrophages mediate the degradation of biomaterials through secretion of matrix-degrading proteinases and phagocytosis. In this study we investigated what happens during the FBR when macrophages are not present. Hexamethylene diisocyanate-crosslinked collagen scaffolds were implanted in "Macrophage Fas-Induced Apoptosis" (MaFIA) mice, which allow induction of macrophage depletion.

We observed that macrophage depletion completely inhibited ingrowth into the scaffolds and resulted in an increased capsule size. QPCR analysis revealed decreased expression levels of pro-inflammatory mediators such as TNFα and IL1β, and increased expression levels of collagens and fibroblast-stimulating growth factors such as EGF, FGF1, FGF2 and TGFα. Our results indicate that macrophages are indeed crucial for the generation of a pro-inflammatory microenvironment inside implanted biomaterials, leading to inflammatory ingrowth. In contrast, macrophages do not appear to be important for the generation of a fibrous capsule around implanted biomaterials. In fact, our data suggest that the macrophages present in the capsule might instruct the surrounding fibroblasts to produce less fibroblast-stimulating factors and less collagens.

Substrate surface alter mechano-responses of vaginal fibroblasts from prolapsed tissues in premenopausal women with pelvic organ prolapse

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Introduction

Pelvic organ prolapse (POP) is a disease characterised by the weakening of the pelvic floor and subsequent prolapse of the vagina, bladder or rectum outside the body. POP is also a multifactorial disease with unclear pathogenesis affecting almost 50% of elderly women worldwide. Current treatments using synthetic meshes to restore support are far from optimal. New therapies such as cell-based tissue engineering may provide promising alternatives to current treatments.

Fibroblasts (FB) are the mechanosensitive cells responsible for the remodelling of the extracellular matrix (ECM) and tissue maintenance, but little is known about their role in the pathogenesis and current treatments of POP. Therefore, this study aimed to identify the effects of *in vitro* cyclic mechanical loading (CML) on two different substrate surfaces on ECM remodelling by FB from the anterior vaginal wall of premenopausal women with different degrees of prolapse.

Methods

Biopsies were taken from the peri-cervical region (non-POP site) of 8 healthy controls, and 10 POP cases (4 mild and 6 severe POP patients). Biopsies were also collected from the vaginal wall (POP-site) of women with POP and were used as patient's own control. A total of 28 different vaginal primary fibroblast isolates were cultured up to passage 3 and seeded on collagen-coated or uncoated bioflex® plates ± CML (Flexercell; 0,2Hz; 48h). Cell attachment and proliferation were analysed by total DNA and KI67, respectively. Certain matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) were also evaluated at protein (by zymograms or ELISA) and gene expression (as measured by real time PCR) levels. Statistical analysis was performed using paired or unpaired t-test accordingly.

Results

No differences were seen between healthy controls and the non-POP site from POP cases. Cell attachment was facilitated by collagen-coated surfaces, and proliferation was up-regulated on uncoated plates. Mechanical loading inhibited cell proliferation and decreased cell attachment on uncoated but not on collagen plates. No apparent differences on cell attachment and proliferation were seen between control and POP cases. Fibroblasts from the POP site secreted lower MMP-2 than the non-POP site and healthy controls on collagen-coated plates. CML induced MMP-2 activation in uncoated plates. This effect was more pronounced in FB from non-prolapsed tissues. Secreted TIMP-2 was not affected by CML. Gene expression of Col 3α1, MMP-9 and TIMP-2 was up-regulated in collagen-coated plates. In uncoated plates, CML increased gene expression levels of MMP-14 in all the cells and MMP-2 and TIMP-2 only in fibroblasts from the prolapsed site.

Discussion and conclusion: Mechanoresponses of vaginal FB to matrix components and mechanical loading are different in prolapsed and non-prolapsed tissues. This was implicated by lower secretion and activation of MMP-2, and by differential expression of MMP-2 and TIMP-2 in POP vs. non-POP site FB. This implies an acquired rather than an intrinsic defect.
We speculate that fibroblast responses to mechanical loading in the presence of synthetic polymeric substrates, may lead to an imbalance in synthesis/degradation of ECM proteins affecting the mechanical properties of the pelvic floor in women with POP.

In conclusion, fibroblasts derived from prolapsed tissues of patients with POP, display altered *in vitro* functional characteristics depending on the matrix substrate and compared to non-prolapsed site or healthy controls. This provides new clues for tissue engineering-based concepts for treatment of pelvic organ prolapse.

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Lab-on-a-chip Device to Screen for the effects of Mechanical Signals on Cells

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Introduction: One of the major bottlenecks in tissue engineering is the lack of a vascular network shortly after implantation, resulting in a lack of nutrients and oxygen for the cells in the engineered tissue (1). We have pioneered a promising strategy to overcome this problem, by including endothelial cells that form a (pre)vascular network (2) that can connect to the host vasculature shortly after implantation (3). Since mechanical signals are important for the development of both bone and vascular networks, we hypothesize that prevascularized bone tissue engineering can be further optimized by the application of mechanical signals.

The role of mechanical signals in affecting cell behavior is well established. There is however limited quantitative data on this correlation, making it hard to be effectively utilized in tissue engineering approaches. To obtain the required mechanical information we have designed a semi high throughput screening system. With this system, we will study the effect of combinaions of (i) strains in the surface to which they are attached and (ii) fluid shear on their free surfaces on the cells that are used in prevascularized bone.

Materials & Methods: The device design consists of an array of units generating different strains overlayed with areas experiencing varying fluid shear. Strains are generated by deforming a PDMS membrane over NOA-81 pillars using negative pessure and varied between units by varying the area to which negative pressure is applied. Fluid shear is applied by flowing fluid in chambers over the strain units and varied by varying the flow channel width along the way. (fig. 1a).

Finite element structural and fluid dynamics modeling have been done using ANSYS to assess the feasibility of the design. A prototype (fig. 1b) has been built to look at 5 strain x 5 shear combinations each in 4 replicates (100 units on a chip). The chip has the dimesnions of regular 96 well plates making possible automated imaging by a bioimager like the BD Pathway. Strains (fig. 1e) and fluid flows (fig. 1f) have been determined empirically by tracking beads. Fibronectin patterning to limit cells to areas of uniform strain is being optimized (fig. 1g). Next, the cells (hMSCs, HUVECs and co-cultures of these cell types) will be included in the experiments.

Results & Discussion: Modeling showed that by varying (i) pressure or (ii) the area to which the pressure is applied, different strains can be achieved in the membrane (fig. 1c,e). Fluid dynamics modeling showed that by using a varying width fluid flow channel, different shear domains can be achieved (fig. 1d). Fluid structure interaction (FSI) modeling showed that design parameters can be adjusted so that the membrane deformation has minimal effect on the fluid flow. Empirical strain (fig. 1e) and fluid flow (fig. 1f) measurements showed good agreement with the models.

Conclusion: A device to expose cells to combinations of surface strains and fluid flow shears has been designed. A working prototype is currently being validated.

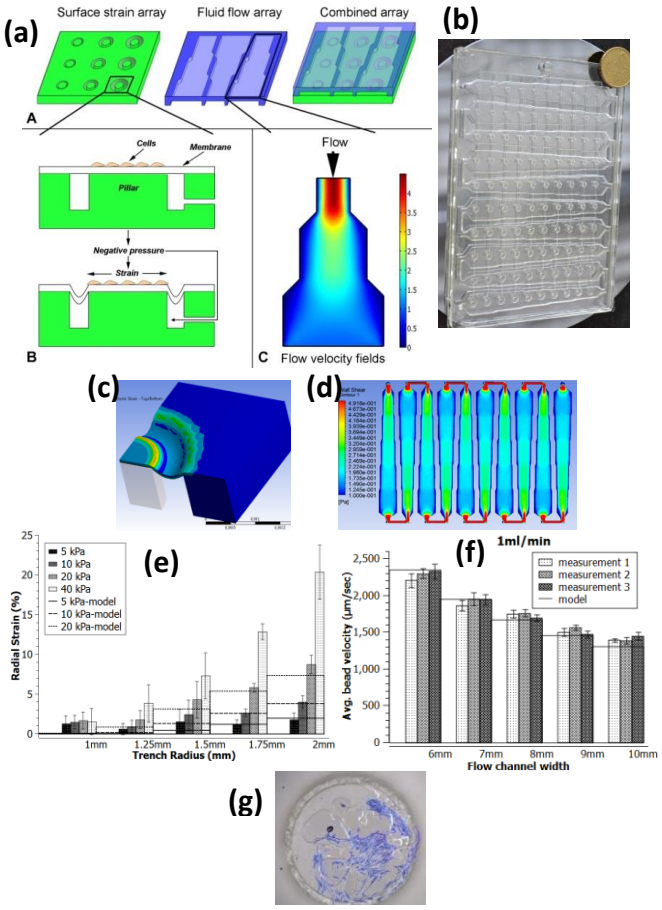

Fig. 1.(a)device design schematic; **(b)**device prototype; Finite element structural**(c)** and fluid dynamics**(d)** models; empirical strain **(e)** and fluid flow **(f)** measurements; **(g)**fibronectin patterning**.**

References:

1 J. Rouwkema, N.C,Rivron, C.A.van Blitterswijk, *Trends in Biotechnology*, 2008, **26**, 434.
2 J.Rouwkema, J.de Boer and C.A.van Blitterswijk, *Tissue Engineering*, 2006, **12**, 2685.
3 S. Levenberg, J.Rouwkema, M.Macdonald, E.S.Garfein, D.S.Kohane, D.C.Darland, R.Marini,C.A.van Blitterswijk, R.C.Mulligan, P.A. D'Amore and R.Langer, *Nature Biotechnology*, 2005, **23**, 879.

Nano-spun Scaffolds for Tissue Engineering, Characterization and Cytotoxicity

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Introduction:

Electrospinning is a straightforward and inexpensive method for producing scaffolds with high surface area-to-volume ratio and ultra-thin fibrous structure. Due to nano-fibrous architecture and isotropic orientation, non-woven meshes are well-known for their superior roperties in resembling natural ECM, cellular adhesion and proliferation. Here we show the biocompatibility of two electospun meshes produced from organic solvents nd their characteristics.

Experiments:

Polymeric solutions of 15% PCL (Purac, M_w=124 kD) and PLGACl (Sigma, M_w=50 kD) in CHCL3 and THF (Sigma) were prepared respectively and spun with the set-up parameters represented in table 1.

Condition	Flow rate	Voltage	Distance	Solvent	Collector
PCL	1 ml/h	15 K	15 cm	CHCL3	Aluminum, horizontal
PLGACL	1 ml/h	20 K	18-20 cm	THF	

Table1. Processing conditions

Meshes were sterilized by 2hours soaking in 70% ethanol followed by an overnight incubation in 1% antibacterial medium. Human vaginal fibroblasts (Passage6) were seeded on sterile samples at a density of 150,000 cell/ cm^2 and cultured in DMEM supplemented with 10% FCS and 1% antibiotic. At days 1 and 3 a live/dead staining with calcein AM and ethidium homodimer-1 was performed on all samples after washing with DPBS and cells were pictured with florescent microscopy. Scanning electron microscopy (SEM) and uni-axial tensile test were performed on meshes to characterize structure and mechanical properties of the samples, as shown in table 2 (n=2).

Characteristics	PCL15%,CHCL3	PLGACL15%,THF
Fibers diameter	8-9.5 micron	5-7 micron
Pore size	14 micron	12-13 micron
Porosity	65-70%	65%
Thickness	50 micron	60 micron
Tensile strength	2.6 MPa	4 MPa
Elongation	120%	85%

Table2. Characteristics of electrospun meshes

Results & discussion:

Live/dead staining showed great biocompatibility > 95% for PLGACL meshes. From day 1 to 3 fibroblasts increased in number by looking at their population on PLGACL. Cells on PCL meshes migrated out from the mesh, which happened more from day 1 to 3, so on day 3 less cells were visible on the mesh live or dead.

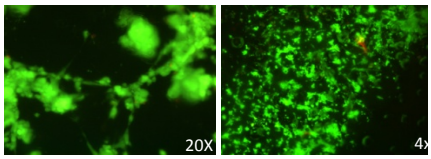

Figure1. L/D stain of PLGACL mesh, left at day 1, right at day 3.

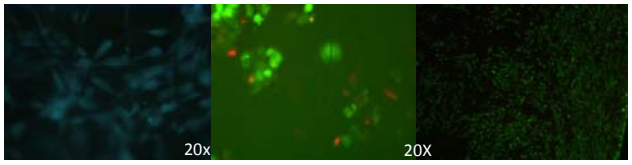

Figure2. L/D stain of PCL mesh, left at day 1, middle at day 3, right the flask bottom showing cells migration from mesh into the plate.

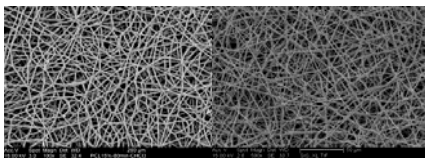

Figure3.SEM pictures of spun meshes, left PCL, right PLGACL.

Those cells that attach to the flask bottom showed non-toxic extract of the PCL otherwise cells would have died out of toxicity. Due to hydrophobic of PCL, cells could not adhere well, and since fibroblasts, like most other cells, are anchorage dependent they either died or migrated to the flask bottom. Porosity and pore sizes of the meshes are proper for cells interaction and nutrition, as well as mechanical strength which provide support for cells ingrowth.

Conclusion and future directions:

Although non-woven electrospun scaffolds seem suitable for tissue engineering purposes, more of investigations are needed to have better insight into ECM deposition as well as mechano-biological responses on them.

Refrences: 1) Pham Q.P. *et al*, Biomacromolecules 2006, 7. **2)**Hiep N.T. *et al*, J Mater Sci Mater Med 2010,21(6).

IN-SITU FORMING HYDROGELS BY CO-CROSSLINKING OF POLYSACCHARIDE TYRAMINE CONJUGATES FOR CARTILAGE TISSUE ENGINEERING

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Introduction

An ideal scaffold for cartilage regeneration is expected to have a controlled degradability, provides adequate mechanical strength, promotes cell survival and differentiation and allows nutrient diffusion, adhesion and integration with the surrounding native cartilage tissue.

Hydrogels, elastic three-dimensional networks, mimic hydrated native cartilage tissue and are considered suitable scaffolds for cartilage tissue engineering. In situ forming hydrogels offer additional advantages, such as the possibility of implantation by injection and homogeneous incorporation of bioactive molecules and/or cells, allowing an appropriate alignment with the surrounding tissue and promoting cell growth. A large number of specialized hydrogels have been developed and optimized for cartilage regeneration applications. In our labs we have showed that fast in-situ forming hydrogels can be obtained via enzymatic crosslinking of dextran-tyramine conjugates (Dex-TA) or chitosan-phloretic acid conjugates in the presence of HRP and hydrogen peroxide[1]. This project consists in the evaluation of the mechanical and biological properties of novel biomimetic hybrid hydrogels based on dextran, heparin and hyaluronic acid tyramine.

Methods

Synthesis. Dextran-tyramine conjugates with a degree of substitution, defined as the number of tyramine units per 100 anhydroglucose rings of 15 were prepared by activation dextran with p-nitrophenyl chloroformate and subsequent reaction with tyramine. Heparin and sodium hyaluronate were activated by N-ethyl-N'-(3-dimethyl -aminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxysuccinimide (NHS) and conjugated by the reaction with tyramine.

Gelation. In-situ forming hydrogels were prepared by horseradish peroxidase-mediated co-crosslinking of Dex-TA, Hep-TA and HA-TA conjugates (Figure 1).

Cytotoxicity assay. Hydrogels containing bovine chondrocytes were prepared under sterile condition by mixing Dex-TA/Hep-TA/HA-TA hydrogels/cell suspension with PBS solutions of HRP/H_2O_2. Chondrocytes were cultured inside the hydrogels with a density of $5x10^6$ cells/ml and viability studies were performed after culturing for 14 and 28 days with a Live/Dead assay Kit. Agarose gels (0.5%) were used as a control. Samples were visualized using fluorescence microscopy.

Results

In this study, injectable hydrogels were prepared by the HRP mediated co-crosslinking of Dex-TA and Hep-TA and/or HA-TA conjugates and used as scaffolds for cartilage tissue engineering (Figure 1). The gelation time of 10 wt% polysaccharide solutions with different compositions in PBS were all less than 20 seconds and the gel contents were approximately 80%. The mechanical properties of these gels as determined by rheology measurement shown the storage moduli ranging from 18 to 44 kPa depending on the compositions of the polysaccharide gels.

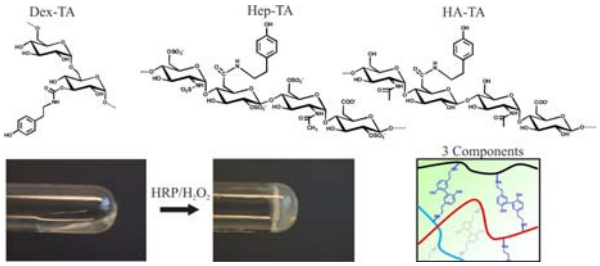

Figure 1. Structure and crosslinking of polysaccharide tyramine.

Dex-TA/Hep-TA/HA-TA hydrogels were fully disintegrated in the presence of hyaluronidase at a concentration of 20 units/mL and the degradation times decreased with increasing HA-TA content. Furthermore, increasing the HA-TA content of the gels led to increasing porosity of dried gels, important for nutrient transport and cell mobility.

Chondrocytes incorporated in 10 wt% polysaccharide hydrogels showed good viability after incubation in chondrocyte differentiation medium for 28 days. Moreover, Dex-TA/Hep-TA/HA-TA hydrogels containing chondrocytes showed improved production of collagen type II, compared to e.g. Dex-TA and Dex-TA/Hep-TA gels.

Conclusions

The fast gelation of the polysaccharide tyramine conjugates by HRP/H_2O_2, and easy incorporation of chondrocytes in the precursor solution make these injectable hydrogels highly promising for cartilage repair

References

1. Jin, R.; Hiemstra, C.; Zhong, Z.; Feijen, J., Enzyme-mediated fast in situ formation of hydrogels from dextran-tyramine conjugates. Biomaterials 2007, 28, (18), 2791-2800.

Influence Of Substrate Stiffness On Macrophage Behavior

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During inflammatory processes, such as fibrosis and the foreign body reaction, macrophages encounter relatively stiff matrices ranging from 100 kPa in fibrotic environments to several orders of GPa on implanted biomaterials. Since macrophages sense and respond to changes in the mechanical properties of their extracellular environment, we question how matrix stiffness affects macrophage behavior in terms of their polarization capacity and their capacity to form giant cells. Murine macrophages were seeded on polyacrylamide gels with an elastic modulus ranging from 6 kPa to 105 kPa and stimulated with LPS/IFNγ or IL4/dexamethasone to induce polarization. Gene expression analysis showed that the stiffness of fibrotic tissue (105 kPa) increased the polarization capacity to both M1 and M2 phenotypes. Giant cell formation was induced by prolonged stimulation with LPS. On gels with a stiffness mimicking fibrotic tissue, giant cell formation was increased. Taken together, our data indicates that the stiffness of the matrix encountered by macrophages during fibrosis and the foreign body reaction may favor their polarization and their ability to form giant cells.

The zebrafish embryo as a novel vertebrate model for the in *vivo* analysis of biomaterial associated infection and immune responses

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Introduction

Failure of indwelling or implanted medical devices is mainly due to adverse immune reactions (bio-incompatibility) and biomaterial-associated infection (BAI), predominantly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*.

To prevent infection, biomaterials with high biocompatibility and low infection-susceptibility are required. In order to test the *in vivo* biocompatibility of biomaterials in a high throughput fashion a novel vertebrate animal model is desirable. Recently the zebrafish (embryo) has emerged as a versatile vertebrate animal model to study immune processes and infectious diseases. It combines genetic tractability and the ability to study host-biomaterial interactions *in vivo* and real time with the possibility to perform high throughput analysis.

Materials and Methods

In order to set up our zebrafish embryo model, poly (ε-caprolactone) (PCL) microspheres with desired size distribution for injection and loaded with blue fluorescent dye were prepared using O/W emulsion and solvent evaporation method. Fluorescent poly styrene (PS) microspheres were purchased from Lift technology. Fluorescent dye loaded microspheres and/or mCherry expressing *S. aureus* were injected into embryos using a microinjector. Injections were performed in transgenic zebrafish embryos, expressing either GFP or mCherry in neutrophils and macrophages respectively. All following up images were captured by stereo fluorescence microscopy.

Results and Discussion

Implantation of PCL microspheres to the tissue of embryo of transgenic zebrafish embryos (fmsGal4-UNM x mpo:EGFP) at 3 days post fertilization led to a strong influx of both macrophages (green) and neutrophils (red) within 2 hours post implantation (2hpi) (Fig 1-a). However, numbers of neutrophils (red) strongly decreased and macrophages (green) resided at the implantation site at 1dpi (Fig 1-b). At 2dpi and 3dpi, macrophages (green) were still present in close proximity to implanted microspheres but not at control injection site, suggesting different cellular immune responses to PCL and PBS.

Co-injection of fluorescent polystyrene microspheres (red) with *Staphylococcus epidermidis* (*S.epi*, green) caused a more severe infection at the tissue of embryo at 2dpi comparing to the control group with only *S.epi* infection (Fig 2-b, 2dpi). The infection progressions (green) were further more distinct at 3dpi and 4dpi (Fig 2-b, 3 and 4 dpi).

The result of scoring percentages of positive embryos based on the infection fluorescence demonstrated that there was a higher percentage in the group with microsphere implantations at 3dpi, indicating an increasing infection susceptibility in the presence of biomaterials in embryo model (Fig 2-a).

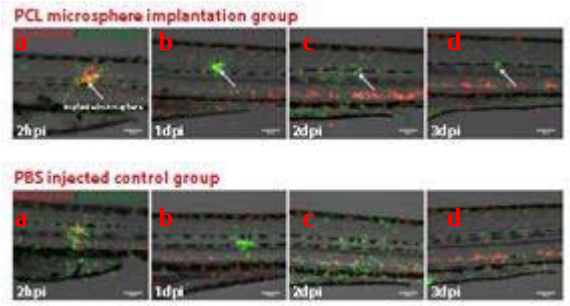

Figure 1. Cellular immune responses to PCL microspheres and PBS (neutrophils in red fluorescence, macrophages in green fluorescence): a). 2hpi, b). 1dpi, c). 2dpi, d). 3dpi

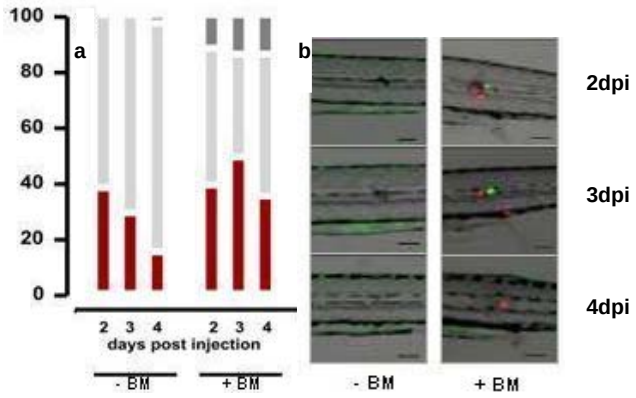

Figure 2. a).Percents of positive embryos in groups with and without PS microspheres (+/- BM) according to infection fluorescence. Red fractions indicate the percents of positive embryos on different days. b). Representative images recorded the infection progression (green) in presence and absence of PS microspheres (red) at different days

Conclusions

Transgenic zebrafish embryos permitted the time resolved visual analysis of cellular immune responses to PCL microspheres. Implanted microspheres led to an increasing infection susceptibility to *S.epi* in tissue of zebrafish embryos. Taken preliminary results together, zebrafish embryo model is a promising system to study biocompatibility and biomaterial-associated infection.

Acknowledgement

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