Biological quantification of murine osteoarticular joints following treatment using stem cell-based gene therapy

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Summary

Cartilage defects induced by osteoarthritis (OA) or rheumatoid arthritis (RA) are currently irreversible. Available immunotherapies are limited since they target only the inflammatory process, which returns once therapy ceases, and do not restore the damaged cartilage or sclerotic bone. A new approach proposed inducing chondrocyte differentiation through genetic engineering of adult mesenchymal stem cells (MSCs). This cell-mediated gene therapy approach would for the first time directly address pathogenic processes and allow for regulated expression of engineered genes. Thus, the aim of this project was to provide quantitative endpoints to assess microstructural and architectural changes in cartilage, bone and tissue-engineered (TE) constructs associated with various MSC treatment therapies.

In order to develop these tools, three specific aims to be addressed in this work were formulated, namely;

1. Development and validation of imaging technologies for biological tissues,
2. Development and calibration of mechanical testing methods for biological tissues, and
3. Applications of biological quantification following stem cell-based gene therapy.

In response to these aims, this thesis discloses novel imaging tools designed for murine joints, as well as mechanical testing of TE constructs. The importance of the tools is their value in characterising TE constructs, cartilage and bone according to functional composition, the presence of structure-altering disease, and the efficacy of treatment therapies in restoring healthy structure.

Specifically in this work, for the first time, a combined technique using confocal laser scanning microscopy (CLSM) and micro-computed tomography (µCT) was developed and validated for volumetric and topographical imaging of articular surfaces and underlying bone. Successful investigation of the reproducibility of CLSM for
imaging tibial cartilage was performed, as well as the subsequent image process-
ing and analysis; resulting in intraclass correlations for the different compartments
between 0.918 and 0.991. The bias of the system was also assessed, where mea-
surements were corrected for by scaling the CLSM images to 32% of their width to
match histological sections. Secondly, an algorithm for automatic generation of lo-
cal femoral and tibial volumes of interest was developed, resulting in an easy-to-use
procedure for bone analysis.

Additionally, method development and commissioning of a material testing appa-
ratus for classical stress-relaxation testing of viscoelastic materials is included; par-
ticularly for TE constructs. Human MSCs were seeded in hyaluronic acid scaffolds
and grown over a three week period. Their mechanical competency was compared
to native, intact articular cartilage, and at their highest - Day 21 - maximum stress,
$\sigma_{\text{MAX}}$ (mean ± SD: 193.3 ± 35.6 kPa) and equilibrium modulus, $E_{\text{EQ}}$ (395.3 ± 68.6
kPa) were ten times lower than human tibial articular cartilage ($\sigma_{\text{MAX}}$: 2120 ±
580 kPa, $E_{\text{EQ}}$: 3492 ± 1061 kPa), and instantaneous modulus, $E_{\text{IN}}$ (601.6 ± 108.4
kPa) was 20 times less ($E_{\text{IN}}$: 11095 ± 2608 kPa). Thus, this methodology was able
to demonstrate increasing functional competency of the constructs with time, even
though they did not achieve the same levels as native tissue. Furthermore, it was
recommended that transfer to a dynamic culture system or in vivo transplantation
would be prudent from 14 days after seeding, since this would allow for further
improvement in functional capacity.

Finally, the imaging tools were combined in two separate studies to assess sub-
chondral bone and articular cartilage changes. The first study considered a murine
OA model and attempted to chart progressive architectural changes associated with
the disease. The $\mu$CT analysis showed that trabecular and cortical bone changes
occurred depending on the volume of interest studied, as well as the strain and age of
the animal. These changes were primarily seen as bone densification of the epiphysis
in the medial condyles. CLSM results showed that cartilage lesions occurred first in
the medial tibial plateau; a result which was objectively measured from the specific
cartilage surface of the affected tissue. Aspects of the disease became evident in
both the cartilage and bone between 4 and 7 months, making it difficult to discern
a separate timeline of events.

The second study considered a murine RA model, where the mice were treated
with an anti-inflammatory therapy using an established system for efficient non viral
gene transfer of the therapeutic product. Comparison was drawn between the ther-
apeutic efficacy of systemic versus local gene delivery, where CLSM and $\mu$CT were
employed as quantitative technologies for evaluation of cartilage and bone repair.
Summary

Three weeks after electric pulse-mediated gene transfer of a plasmid encoding a dimeric TNF receptor II in the SCID/syno-TNF mouse model, TNFα levels were decreased in the groups treated with the anti-inflammatory therapy and a tetracycline promotor. Concomitantly, increases in cartilage thickness, and decreases in specific cartilage surface were observed, matching histological scores, as well as a significant decrease in subchondral cortical bone destruction.
Zusammenfassung


1. Entwicklung und Validierung von bildgebenden Verfahren für biologisches Gewebe,

2. Entwicklung und Kalibrierung von biomechanischen Prüfmethoden für biologisches Gewebe und

3. Anwendungen biologischer Quantifizierung in Anlehnung an stammzellen-basierte Gentherapie

Zusammenfassung

strukturverändernden Krankheiten und der Wirksamkeit von Behandlungstherapien zur Wiederherstellung gesunder Gewebestruktur.

Speziell in dieser Arbeit wurde das erste Mal eine kombinierte Technik aus confocal laser scanning microscopy (CLSM) und micro-computed tomography (µCT) entwickelt und für volumetrische und topographische Bildgebung von Gelenksoberflächen und angrenzendem Knochen ausgewertet. Es wurden erfolgreiche Untersuchungen zur Reproduzierbarkeit der konfokalen Laser-Raster-Mikroskopie als Werkzeug zur Abbildung von Gelenkknorpeln in der Tibia sowie zur anschliessenden Bildverarbeitung und Analyse durchgeführt, die in intraklassischen Korrelationen der verschiedenen Bereiche zwischen 0.918 und 0.991 resultieren. Der Fehler des Gesamtsystems wurde ebenfalls bestimmt, wobei die Messungen mittels einer Skalierung der konfokalen Bilder auf 32% ihrer Breite korrigiert wurden, um mit den histologischen Abschnitten übereinzustimmen. Weiter wurde ein automatischer Algorithmus zur Bestimmung der Analysevolumen im Femur und der Tibia entwickelt, welcher eine einfach zu bedienende Prozedur zur Knochenanalyse darstellt.

Zusätzlich beinhaltet diese Arbeit Methodenentwicklung und Inbetriebnahme eines Materialprüfsystems für klassische Spannungs-Relaxations-Prüfungen von viskoelastischen Materialien; im Speziellen für künstlich erzeugte Gewebekonstrukte. Humane MSZ wurden in Hyaluronsäure-Gewebekonstrukte gesät und wuchsen über eine Zeitspanne von drei Wochen. Die mechanische Kompetenz wurde mittels einer Spannungs-Relaxations-Einbuchtungsprüfung bestimmt und mit den Werten von intakten Gelenkknorpeln verglichen. Zum Zeitpunkt der höchsten Belastung - am Tag 21 - waren Maximalspannung, \(\sigma_{MAX}\) (Mittelwert ± SD: 193.3 ± 35.6 kPa) und Gleichgewichtsmodul, \(E_{EQ}\) (395.3 ± 68.6 kPa) zehn Mal niedriger als bei menschlichem Knorpelgewebe des tibialen Gelenks (\(\sigma_{MAX}: 2120 \pm 580\) kPa, \(E_{EQ}: 3492 \pm 1061\) kPa), und der Momentanmodulus, \(E_{IN}\) (601.6 ± 108.4 kPa) war 20 Mal geringer (\(E_{IN}: 11095 \pm 2608\) kPa). Daher war diese Methode in der Lage, zu zeigen, dass die funktionale Kompetenz der Konstrukte mit der Zeit zunahm, obwohl sie das Niveau des nativen Gewebes nicht erreichten.

Letztlich wurden die entwickelten Werkzeuge zur Erfüllung des dritten Ziels in zwei verschiedenen Studien kombiniert, um die Veränderungen des Gelenkknorpels und des angrenzenden Knochens zu ermitteln. Die erste Studie beschäftigte sich mit einem Osteoarthritis-Modell in der Maus und versuchte, die progressiven strukturellen Veränderungen der Krankheit aufzuzeigen. Es wurde erwiesen, dass trabekuläre und kortikale Knochenänderungen abhängig vom untersuchten Volumen sowie vom Stamm und dem Alter der Tiere auftreten; Änderungen, die primär als