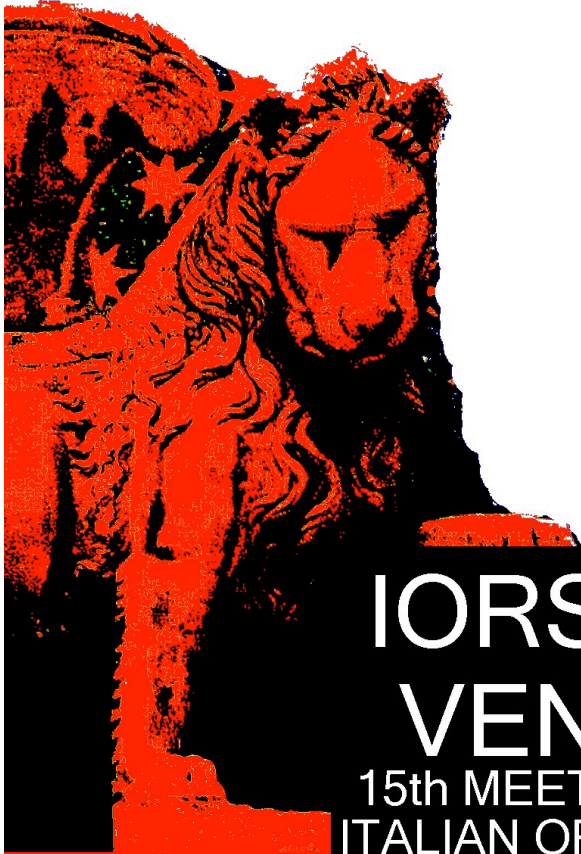




**IORS 2013**  
**VENEZIA**  
15th MEETING OF THE  
ITALIAN ORTHOPAEDIC  
RESEARCH SOCIETY  
12 OTTOBRE 2013  
SAN SERVOLO  
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ITALIAN  
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## **Consiglio Direttivo IORS (2012-2015)**

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Antonio Gigante

Giulio Maccauro

Vincenzo Sollazzo

Gianluca Vadalà

Guido Zarattini             *Segretario Tesoriere*

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## **XV Congresso**

**Società Italiana di Ricerche in Ortopedia e Traumatologia**

**I.O.R.S.**

*Presidente:* Prof. Nicola Baldini

Alma Mater Studiorum - Università di Bologna

Istituto Ortopedico Rizzoli

*Segreteria Scientifica:* Dr.ssa Francesca Perut

SSD Fisiopatologia Ortopedica e Medicina Rigenerativa

Istituto Ortopedico Rizzoli, Bologna

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## PROGRAMMA PRELIMINARE

**Sabato, 12 ottobre 2013**

Centro Congressi Isola di San Servolo, Sala Teatro

- 9:30 Registration
- 10:00 Opening  
**Nicola Baldini** (Bologna), **Michele Attilio Rosa** (Messina)

### Keynote Lecture

Introduced by Nicola Baldini

- 10:05 *Toward the clinical application of intervertebral disc regeneration strategies*  
**Gianluca Vadalà** (Roma)

### Free papers BASIC SCIENCE

Moderators: **Antonio Gigante** (Ancona), **Guido Zarattini** (Brescia)

- 10:20 *In vitro development of bone niche models with graded osteogenicity via tissue engineering*  
**Serena Danti**, Luisa Trombi, Delfo D'Alessandro, Lorenzo Pio Serino, Stefania Moscato, Sabrina Danti, Mario Petrini, Stefano Berrettini, Michele Lisanti (Pisa)
- 10:28 *Mesenchymal stem cells derived from vertebrae (vMSCs) show the best biological properties*  
**Giovanni Barbanti Bròdano**, Silvia Terzi, Giorgia Maroni, Luisa Trombi, Cristiana Griffoni, Stefano Boriani, Maria Cristina Magli (Bologna, Pisa)
- 10:36 *Early effects of Trabecular Titanium (TT) on the in vitro osteogenic differentiation of mesenchymal stem cells in vitro*  
**Vincenzo Sollazzo**, A. Palmieri, A Girardi, F Carinci (Ferrara)
- 10:44 *Genetic effects of tantalum powder on MG-63 human osteoblast-like cells: an in vitro study*  
**Vincenzo Sollazzo**, A Palmieri, A Girardi, F Carinci (Ferrara)
- 10:52 *May bone marrow cell mobilization by Granulocyte-Colony-Stimulating Factor enhance osteointegration? A prospective phase II clinical trial*  
**Antonio Marmotti**, Filippo Castoldi, Roberto Rossi, Matteo Bruzzone, Federico Dettoni, Davide Edoardo Bonasia, Davide Blonna, Marco Assom, Corrado Tarella (Torino)
- 11:00 *Minced umbilical cord fragments: an effective cell source for cartilage and bone tissue engineering?*  
**Antonio Marmotti**, Silvia Mattia, Matteo Bruzzone, Davide Edoardo Bonasia, Luca Mazzucchelli, Filippo Castoldi, Daniele Gioia, Roberto, Giuseppe Peretti (Torino, Milano)
- 11:08 *Comparison of the biologic effects of three different extracorporeal shock waves devices focusing on chondrocytes*

Angela Notarnicola, Giuseppe Maccagnano, Maria Immacolata Forcignanò, Giovanni Vicenti, **Biagio Moretti** (Bari)

- 11:16 *Cartilage fragments for one-step cartilage repair: still a valid cell source in hypoxic conditions as a model of joint microenvironment?*  
**Antonio Marmotti**, Silvia Mattia, Davide Edoardo Bonasia, Matteo Bruzzone, Silvio Terrando, Elena Ponzo, Filippo Castoldi, Roberto Rossi, Giuseppe Peretti (Torino, Milano)
- 11.24 *Utilization of platelet rich plasma (PRP) in orthopaedic practice: clinical and biological analysis of therapeutic effectiveness*  
**Rocco Papalia**, Francesco Franceschi, Biagio Zampogna, Sebastiano Vasta, Lorenzo Diaz Balzani, Vincenzo Denaro (Roma)
- 11.32 *Effect of human PRP treated with shock wave therapy when added on mouse osteoblast cultures*  
Giovanni Vicenti, Vito Pesce, Lorenzo Moretti, **Giuseppe Maccagnano**, Biagio Moretti (Bari)
- 11.40 *Biomaterials: a good alternative to bone grafts for spine fusion*  
**Giovanni Barbanti Bròdano**, F. Lolli, G. Giavaresi, F. Salamanna, Milena Fini, Lucia Martini, Tiziana Greggì, Stefano Boriani (Bologna)
- 11.48 *Nanostructured materials: a new approach to design innovative scaffolds for the treatment of bone defects*  
**Paolo Domenico Parchi**, Marco Cecchini, Sara Antonini, Nicola Piolanti, Lorenzo Andreani, Andrea Poggetti, Michele Lisanti (Pisa)
- 11:56 Ghrelin is involved in osteoblast-osteoclast interaction through WNT/b-catenin signalling pathway  
Emanuela Mrak, Francesca Pagani, Elisa Dieci, Guido Zarattini, **Valeria Sibilìa** (Milano, Brescia)
- 12.04 *Diabetic foot disease: involvement of autoimmunity in the pathogenesis of Charcot neuroarthropathy*  
**M Galli**, M Breccia, M Peruzzi, D Pitocco, G Scavone, P Rizzo, S Caputo, P Pozzilli, G Ghirlanda, A Nissim (Roma)
- 12.12 *Association between osteoprotegerin G1181C and T245G polymorphisms and Charcot neuroarthropathy of the foot in diabetic patients*  
**M Galli**, M Breccia, M Peruzzi, D Pitocco, G Scavone, S Caputo, F Martini, F Zaccardi, G Ghirlanda (Roma)
- 12.20 *An autologous and allogenic nerve graft versus pure autologous and pure allogenic graft alone*  
**Filippo Boriani**, Nicola Fazio, Caterina Fotia, Zrinka Bulj, Nicoletta Zini, Nicola Baldini (Bologna)
- 12.30 **IORS General Assembly**
- 13:00 **Lunch**

## Keynote Lecture

Introduced by **Michele Attilio Rosa**

14.00      *The architecture of cortical bone and the nutrition of its cells*  
**Ugo Ernesto Pazzaglia** (Brescia)

## Keynote Lecture

Introduced by **Ugo Ernesto Pazzaglia**

14:20      *Developmental engineering of a bone organ through endochondral ossification*  
**Celeste Scotti** (Milano)

## Free papers, CLINICAL SCIENCE

Moderators: **Giulio Maccauro** (Roma), **Vincenzo Sollazzo** (Ferrara)

- 14:35      *Patient-specific pin guides in total knee arthroplasty: a prospective randomized study*  
Aaron Burrofato, Ivan De Martino, Rocco D'Apolito, Carmine De Ieso, Fabio Pascarella,  
Domenico Alessandro Santagada, Vincenzo **De Santis** (Roma)
- 14:43      *Evaluation of the effects of the Metha short stem on periprosthetic bone remodelling in total hip arthroplasties: results at 3 years and literature review*  
**Paolo Domenico Parchi**, Gianluca Ciapini, Iacopo Castellini, Nicola Piolanti, Silvia Maffei,  
Michele Lisanti (Pisa)
- 14:51      *Total hip arthroplasty by using a cementless ultrashort stem: a subject-specific finite element analysis of a young patient clinical case*  
**G Epasto**, A Foti, E Guglielmino, Michele Attilio Rosa (Messina)
- 15:59      *Failure analysis of a modular knee prosthesis*  
**G Epasto**, L Fisichella, E Guglielmino, Michele Attilio Rosa (Messina)
- 15:07      *Two stage protocol in septic knee arthroplasty revision: articulating vs static antibiotic-loaded spacers*  
Ivan De Martino, Rocco D'Apolito, Domenico Alessandro Santagada, Aaron Burrofato,  
**Vincenzo De Santis**, Giorgio Gasparini, Carlo Fabbriani (Roma, Catanzaro)
- 15.15      *Standard and custom-augmented antibiotic-loaded spacers in septic knee arthroplasty revision*  
Ivan De Martino, Rocco D'Apolito, Aaron Burrofato, Vincenzo De Santis, Giorgio Gasparini, Carlo Fabbriani (Roma, Catanzaro)
- 15.23      *Inserti a stabilizzazione posteriore a confronto con inserti ad alta congruenza nell'artroprotesi totale di ginocchio. Studio clinico prospettico randomizzato*  
Ivan De Martino, Rocco D'Apolito, Aaron Burrofato, Vincenzo De Santis, Carlo Fabbriani, Giorgio Gasparini (Roma, Catanzaro)
- 15.31      *The application of the external circular fixator in bone transport of tibial septic nonunions: clinical and biomechanical aspects*  
L Fisichella, D Fenga, E Guglielmino, G Epasto, Michele Attilio Rosa (Messina)
- 15.39      *Degenerative facet joint changes after lumbar percutaneous pedicle screw fixation*

Luca Proietti, Laura Scaramuzzo, Giuseppe Schirò, Sergio Sessa, Gabriella D'Aurizio, Carlo Fabbriciani (Roma, Milano)

- 15:47 *A new structural method permits evaluation of bone quality and fracture risk in a case of rapid and progressive osteoporosis of the hand (CRPS type II)*  
Francesca Cosmi, Marco Attanasio, Nathalie Steimberg, **Giovanna Mazzoleni** (Trieste, Brescia)
- 16:55 *Percutaneous technique for incisional biopsy of soft tissue tumors: preliminary evaluation of an accurate alternative to open biopsy*  
**Barbara Rossi**, Carmine Zoccali, Umberto Prencipe, Nicola Salducca, Leonardo Favale, Fabio Erba, Roberto Biagini (Roma)
- 16:03 *Carbon fiber-reinforced PEEK intramedullary nailing for the treatment of pathologic fractures due to metastases: short term results*  
**Barbara Rossi**, Carmine Zoccali, Umberto Prencipe, Nicola Salducca, Virginia Ferraresi (Roma)
- 16:11 *Patient-specific templates for pedicle spine screws placement: literature review and proposal of a new design*  
**Paolo Domenico Pardi**, Vincenzo Ferrari, Sara Condino, Marina Carbone, Lorenzo Andreani, Michele Novi, Michele Lisanti (Pisa)
- 16:19 *Plexur M™ in the reconstruction of subchondral bone: a new perspective in oncological orthopedics*  
**Carmine Zoccali**, Barbara Rossi, Umberto Prencipe, Giulio Marolda, Nicola Salducca, Leonardo Favale, Fabio Erba, Roberto Biagini (Roma)
- 16:27 *The Mutars X-pand prosthesis for the treatment of primary malignancies: surgical technique and lengthening mechanism*  
M.S. Spinelli, Giulio Maccauro, Michele Attilio Rosa (Messina, Roma)
- 16:35 *Intrarticular injections for degenerative cartilage lesions of the knee: platelet rich plasma vs hyaluronic acid*  
Rocco Papalia, Francesco Franceschi, Biagio Zampogna, Sebastiano Vasta, Lorenzo Diaz Balzani, Vincenzo Denaro (Roma)
- 16:43 *Comparison of clinical results between microfracture technique with the use of PRF intraoperative injection, PRP postoperative injection and microfracture only for osteochondral lesions of the knee: a preliminary study*  
Rocco Papalia, Francesco Franceschi, Biagio Zampogna, Sebastiano Vasta, Lorenzo Diaz Balzani, Vincenzo Denaro (Roma)
- 16:51 *Prospective multicentric study*  
**Filippo Boriani**, Umraz Khan, Ata Ul Haq, Moazzam Tarar, Riccardo Cipriani, Nicola Baldini (Bristol, Lahore, Bologna)
- 17:00 **Closing remarks**  
**Nicola Baldini** (Bologna)

The 15th IORS Congress will take place immediately before the Combined Meeting of Orthopaedic Research Societies, CORS 2013 (<http://www.cors2013.org>), which will be held in Venice, at the Congress Centre Isola di San Servolo, from 13 to 16 October.

CORS 2013 is the triannual meeting of Associations that are active within the CORS consortium (Orthopaedic Research Society, Australian & New Zealand ORS, British ORS, Chinese ORS, European ORS, Indian ORG, Japanese Orthopaedic Association, Korean ORS, Taiwanese ORS), and for the first time this important event is hosted in Italy.

The Italian Orthopaedic Research Society proudly supports this unique opportunity of scientific exchange and global interaction and warmly welcomes all scientists and clinicians from all over the World!

## TOWARD THE CLINICAL APPLICATION OF INTERVERTEBRAL DISC REGENERATION STRATEGIES

Gianluca Vadalà e Vincenzo Denaro  
Università Campus Bio-Medico di Roma

La lombalgia è una condizione clinica molto frequente associata ad elevata morbidità. Essa è la principale causa di disabilità nella popolazione al di sotto dei 45 anni, e rappresenta una delle principali spese economiche nei paesi sviluppati. La causa principale della lombalgia è la degenerazione del disco intervertebrale. I recenti avanzamenti nella conoscenza della biologia del disco intervertebrale hanno portato ad un maggiore interesse per lo sviluppo di nuove terapie biologiche. La terapia con cellule staminali ha la potenzialità di favorire la rigenerazione del disco intervertebrale superando le limitate capacità auto-rigenerative del tessuto discale. L'utilizzo di diversi tipi di cellule staminali dell'adulto, quali le cellule staminali mesenchimali (MSC – *Mesenchymal Stem/Stromal Cells*) del midollo osseo è stato descritto con prospettive promettenti. Recenti evidenze scientifiche hanno dimostrato che l'iniezione intradiscale di MSC modifica positivamente il corso della degenerazione discale. Studi *in vitro* suggeriscono che il potenziale rigenerativo delle MSC deriva dalle interazioni tra MSC e cellule del nucleo polposo, che regolano la sintesi di proteine della matrice extracellulare. Tale effetto sinergico è legato al differenziamento delle MSC verso cellule simil-nucleo polposo e all'effetto trofico delle MSC sulle cellule del nucleo polposo. Studi *in vivo* hanno dimostrato l'efficacia del trapianto di MSC in modelli animali riproducibili in termini di aumento del contenuto di proteoglicani, altezza del disco ed idratazione.

Nel tentativo di introdurre questo nuovo trattamento biologico nella pratica clinica, il nostro gruppo sta continua ad effettuare esperimenti preclinici per risolvere alcune problematiche irrisolte nel processo di trasferimento di questi risultati alla clinica, come la scelta del carrier ideale e la dose cellulare ottimale. Questa nuova terapia cellulare ha la potenzialità di diventare un potente strumento terapeutico per il futuro trattamento della degenerazione del disco intervertebrale.

## IN VITRO DEVELOPMENT OF BONE NICHE MODELS WITH GRADED OSTEOGENICITY VIA TISSUE ENGINEERING

Serena Danti<sup>1</sup>, Luisa Trombi<sup>2</sup>, Delfo D'Alessandro<sup>1</sup>, Lorenzo Pio Serino<sup>3</sup>, Stefania Moscato<sup>2</sup>, Sabrina Danti<sup>1</sup>, Mario Petrini<sup>2</sup>, Stefano Berrettini<sup>1</sup>, Michele Lisanti<sup>3</sup>

<sup>1</sup> Dept. of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Pisa, Italy.

<sup>2</sup> Dept. of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

<sup>3</sup> Dept. of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.

**AIMS.** Although the traditional approach was declared "minimalistic" by the tissue engineering community in 2006 [1], the development of complex tissue models *in vitro* remains a challenge. Typically, mesenchymal stromal cells (MSCs) and/or osteoprogenitor cell populations are seeded on 3D scaffolds, cultured and differentiated using appropriate stimuli in the culture medium and resulting in a homogeneous osteoblast population at the endpoint. However, after achieving terminal osteo-differentiation, cell viability is usually markedly compromised. Differently, naturally occurring osteogenesis relies on the coexistence of MSC progenies at distinct differentiative stages in the same niche. This microenvironmental diversification enables long-term viability of the mature tissue. In this study, simple tissue-engineered osteogenic niches were assembled so as to progress towards the *in vitro* regeneration of biomimetic bone substitutes, which can be functional and viable at the time of implantation. The idea lying behind this study is the generation of a 3D niche hosting simultaneously a spectrum of cells at different osteogenic stages, which range from the undifferentiated MSCs to the terminally differentiated osteoblasts.

**METHODS.** We report an easy and tunable *in vitro* method to engineer simple osteogenic cell niches in a biomimetic fashion [2]. The niches were grown via periodic re-seeding of undifferentiated MSCs on MSC/scaffold constructs, the latter undergoing osteogenic commitment. MSCs were cultured on 3D spongy scaffolds based on poly(L-lactic acid) (PLLA) and gelatin. The system was investigated over three seeding groups with multiple cell inoculations (namely "multi-shot") and equal number of total seeded cells (500,000 cells/sample), but with different seeding densities per period: (i) single shot (= traditional method, niche #3); (ii) multiple shots with decreasing cell densities (niche #2); and (iii) multiple shots at equal cell densities (niche #1). In the three cases, the initially seeded cells per scaffolds were 500,000, 250,000 and 125,000, respectively. Specific analyses were performed to evaluate the following parameters: (i) cell viability, (ii) scaffold colonization, (iii) bone-ECM molecule expression.

**RESULTS.** Time-fractioning of the seeded cell number during differentiation time of the constructs allowed graded osteogenic cell populations (not only terminally differentiated osteoblasts) to be grown together on the same scaffolds. In such cell-dynamic systems, the overall differentiative stage of the constructs could be tuned by varying the cell density seeded at each inoculation. In this way, we generated two different biomimetic niche models able to host good reservoirs of pre-osteoblasts and other osteo-progenitors after 21 culture days. At that time, the niche type resulting in 40.8% of immature osteogenic progenies and only 59.2% of mature osteoblasts showed calcium content comparable to the constructs obtained with the traditional culture method ( $100.03 \pm 29.30$  vs.  $78.51 \pm 28.50$  pg/cell, respectively;  $p = n.s.$ ), the latter colonized only by fully differentiated osteoblasts showing exhausted viability.

**CONCLUSIONS.** This assembly method for tissue-engineered constructs enabled a set of important parameters, such as viability, colonization and osteogenic yield of the MSCs to be balanced on 3D scaffolds, thus achieving biomimetic *in vitro* models with graded osteogenicity, which are more complex and reliable than those currently used by tissue engineers.

### REFERENCES

Ingber DE, Mow VC, Butler D, *et al.* Tissue Engineering and Developmental Biology: Going Biomimetic. *Tissue Eng* 12, 3265, 2006.  
2.Danti S, Serino LP, D'Alessandro D, *et al.* Growing Bone Tissue-Engineered Niches with Graded Osteogenicity: An In Vitro Method for Biomimetic Construct Assembly. *Tissue Eng Part C Methods*. 2013 Apr 30. [Epub ahead of print]

## **MESENCHYMAL STEM CELLS DERIVED FROM VERTEBRAE (vMSCs) SHOW BEST BIOLOGICAL PROPERTIES**

Giovanni Barbanti Brodano, Silvia Terzi, Giorgia Maroni<sup>1</sup>, Luisa Trombi<sup>2</sup>, Cristiana Griffoni, Stefano Boriani, Maria Cristina Magli<sup>1</sup>

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### **AIMS**

Due to their properties and characteristics human mesenchymal stem cells (MSCs) appear to have great therapeutic potential, because they can contribute to the tissue regeneration. Many different populations of MSCs that differ for the embryonic origin and the anatomical localization have been described and to understand whether they have equivalent biological properties is a critical issue for their therapeutic application.

Spine fusion is frequently used to treat traumatic, degenerative and oncological spine diseases. Autologous bone graft has been considered the gold standard for spine fusion procedures because of its osteogenic, osteoinductive and osteoconductive ability. However, its use is associated with significant disadvantages including donor site pain, increased operative time, insufficient availability, and nonunion post-lumbar fusion. Allograft bone has been the most widely used substitute to avoid the complications of donor site morbidity, but it has increased risks of infection and rejection and poor osteoinductive properties. Various bone substitutes have been developed to promote spinal fusion. Also MSCs have been successfully tested for spinal fusion in several small and large animal models.

### **METHODS**

In order to define the properties of MSCs for the use in clinical applications, we proposed to analyze the cellular and molecular characteristics of human adult MSCs derived from different body locations, such as bone marrow from iliac crest (Ic-MSCs), sternum (St-MSCs) and vertebrae (V-MSCs), as well as colon (Co-MSCs) and dental pulp (DPSCs), in order to identify the cell population showing better biological properties for spine fusion clinical application.

### **RESULTS**

MSCs from different sources presented very different growth kinetics, even if they shared a common immunophenotype, where CD44, CD90, CD105, CD146 antigens were significantly expressed and Cd34, CD45 antigens were absent. While iliac crest and sternum-derived MSCs could be maintained in culture for about three months, undergoing a limited expansion, mesenchymal cultures derived from dental pulp and colon grew much more rapidly, but could not be maintained longer. Strikingly, MSCs obtained from vertebrae (vMSCs) continued to propagate at high rate even after 3.5 months *ex vivo*.

MSCs from different sources were also induced to osteogenic, adipogenic and chondrogenic differentiation following exposure to specific inducing agents. We observed that vMSCs generate mature cells of all mesenchymal lineages with greater efficiency, thus resulting the best population both in terms of expansion and differentiation.

### **CONCLUSIONS**

This finding could be very interesting and open new perspectives for the improvement of spine fusion, which is a mandatory step for the success of spinal surgical procedures.

In the course of a surgical procedure for spinal fusion, vertebral bone marrow can be harvested in amount proportional to the length of the arthrodesis, simultaneously with the preparation of the site for pedicle screw insertion. Then multipotent MSCs can be isolated from vertebral bone marrow during the progress of the surgical procedure and reintroduced in the fusion site (using homologous or autologous bone tissue or biomaterials as scaffold), without any additional surgical time nor any other donor site involvement.

## **EFFETTI PRECOCI DEL TITANIO TRABECOLARE (TT) SULLA DIFFERENZIAZIONE IN SENSO OSTEOLASTICO DI CELLULE MESENCHIMALI STAMINALI IN VITRO**

Sollazzo V., Palmieri A., Girardi A., Carinci F.

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Il titanio e le sue leghe sono materiali ampiamente utilizzati in chirurgia ortopedica per le loro buone proprietà meccaniche, stabilità chimica e biocompatibilità. Sebbene siano stati ottenuti eccellenti risultati clinici con l'utilizzo del titanio, permangono alcune limitazioni relative alla bassa volumetria della porosità del materiale ed al suo modulo di elasticità relativamente alto. Per superare questi limiti e migliorare l'osteointegrazione dell'impianto è stato prodotto un nuovo titanio altamente poroso (Trabecular Titanium, TT, Lima LTO). Dato che gli eventi molecolari attraverso i quali il TT trabecolare influisce sull'attività osteoblastica sono ancora poco conosciuti, l'obiettivo di questo lavoro è stato quello di indagare gli effetti precoci dell'attività del TT sulla differenziazione osteoblastica di cellule mesenchimali staminali prelevate da midollo osseo (hMSC). Sono stati quindi studiati i livelli di espressione di alcuni geni come RUNX2, SPP1, COL1A1, COL3A1, BGLAP, ALPL, and FOSL1 in cellule mesenchimali staminali mantenute a contatto con dischetti di TT per 7 giorni in RT-PCR. Nelle cellule mantenute a contatto con il TT, questo ha provocato l'induzione del gene dell'osteopontina (SPP1), del gene dell'osteocalcina (BGLAP) e di quello della fosfatasi alcalina (ALPL). I nostri dati sono indicativi di una spinta alla differenziazione in senso osteoblastico delle cellule mesenchimali staminali a contatto con il TT e depongono per la buona osteointegrazione di questo materiale.

**EFFETTI GENETICI DI POLVERE DI TANTALIO IN OSTEOLASTI UMANI MG-63:  
STUDIO IN VITRO**

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Laboratorio di Ortopedia e Chirurgia Maxillofacciale, Sezione di Scienze e Tecniche Applicate alla Medicina ed alla Chirurgia, Università di Ferrara.

Il titanio è largamente impiegato nella pratica clinica per la produzione di protesi articolari e mezzi di sintesi in virtù delle sue buone proprietà meccaniche e di biocompatibilità. Il Tantalio si propone di migliorare le caratteristiche di biocompatibilità del titanio e di favorire l'osteointegrazione degli impianti protesici. Il meccanismo attraverso il quale questo materiale influenza l'attività osteoblastica è ancora poco conosciuto. Abbiamo perciò studiato l'espressione genica in osteoblasti umani esposti a polvere di tantalio con la tecnica dei microarray. Tale metodica consente l'analisi contemporanea dell'espressione di oltre 20,000 geni. Abbiamo trovato significativamente sopra o sottoespressi diversi geni responsabili di un ampio spettro di attività funzionali della cellula come la trasduzione del segnale, la trascrizione, la regolazione del ciclo cellulare, l'adesione cellulare e la produzione di componenti della matrice extracellulare. Il tantalio ha dimostrato di essere ben tollerato dagli osteoblasti e non ha indotto in essi fenomeni apoptotici. I dati che emergono dal nostro studio incoraggiano l'utilizzo clinico di questo materiale.

## **MAY BONE MARROW CELL MOBILIZATION BY GRANULOCYTE-COLONY-STIMULATING-FACTOR MAY ENHANCE OSSEOINTEGRATION? A PROSPECTIVE PHASE II CLINICAL TRIAL**

Marmotti Antonio<sup>1</sup>, Castoldi Filippo<sup>1</sup>, Rossi Roberto<sup>1</sup>, Bruzzone Matteo<sup>1</sup>, Dettoni Federico<sup>1</sup>, Bonasia Davide Edoardo<sup>1</sup>, Blonna Davide<sup>1</sup>, Assom Marco<sup>1</sup>, Tarella Corrado<sup>2</sup>

<sup>1</sup>University of Torino, Department of Orthopaedics and Traumatology, Torino, Italy

<sup>2</sup>Department of Haematology and Cell Therapy, University of Torino, Turin, Italy

### **AIMS**

Preoperative bone-marrow-derived cell mobilization by G-CSF is a safe orthopaedic procedure and allows circulation in the blood of high numbers of CD34+ve cells, promoting osseointegration of a bone substitute.

Granulocyte-colony-stimulating-factor(G-CSF) has been used to improve repair processes in different clinical settings for its role in bone-marrow stem cell(CD34+ and CD34-) mobilization. Recent literature suggests that G-CSF may also play a role in skeletal-tissue repair processes. Aim of the study was to verify the feasibility and safety of preoperative bone-marrow cell (BMC) mobilization by G-CSF in orthopaedic patients and to evaluate G-CSF efficacy in accelerating bone regeneration following opening-wedge high tibial valgus osteotomy(HTVO) for genu varum.

### **METHODS**

24 patients were enrolled in a prospective phase II trial. The osteotomy gap was filled by a hydroxyapatite-tricalciumphosphate bone substitute(HATriC). Patients were randomized to receive (GROUP A) or not receive (GROUP B) preoperatively a daily dose of 10µg/kg of G-CSF for three consecutive days, with an additional dose 4 hours before surgery.

BMC-mobilization was monitored by white blood cell (WBC)-count, flow-cytometry analysis of circulating CD34+cells and Colony-forming cell assays.

Patients were evaluated by: 1) Lysholm and SF-36 scores preoperatively and at 1, 2, 3, 6, and 12 months after surgery; 2) X-ray evaluation preoperatively and at 1, 2, 3, 6, and 12 months after surgery, in order to compare the percentage of osseointegration of the bone-graft junction using a semi-quantitative score (by two blinded observers); 3) CT-scan of the host bone-substitute interface at 2 months, in order to estimate the quality of the newly formed bone at the bone-graft junction by a quantitative measure of bone density (by Hounsfield unit) at the proximal and distal bone-graft junctions.

### **RESULTS**

All patients completed the treatment program without major side effects; G-CSF was well tolerated.

BMC mobilization occurred in all Group A patients, with median peak values of 110/µL (range 29-256) of circulating CD34+ve cells. Circulating clonogenic progenitors paralleled CD34+ve cell levels.

A significant improvement in the SF-36-Role-Physical scale and in the Lysholm score was recorded at follow-up in Group A compared to Group B (p<0.05). At the X-ray evaluation, there was a significant increase in osseointegration at the bone-graft junction in Group A at 1, 2, 3 and 6 months post-surgery compared to Group B (p<0.05). The CT scan of the grafted area at 2 months post-surgery showed no significant difference in the quality of the newly formed bone between the two Groups.

### **DISCUSSION AND CONCLUSIONS**

These results suggest that G-CSF can be safely administered preoperatively in subjects undergoing HTVO. In addition, the clinical, radiographic and CT monitoring indicate that preoperative G-CSF administration promotes bone graft substitute osseointegration. Enhanced osseointegration might be the result of the direct activity of G-CSF on the host bone or a cellular effect mediated by bone marrow-derived progenitors mobilized by G-CSF, or by a combination of all these factors. This study is a proof-of-principle that preoperative G-CSF might be an alternative treatment option to enhance bone regeneration in the field of bone marrow stem cell therapy and reconstructive orthopaedic surgery.

# MINCED UMBILICAL CORD FRAGMENTS: AN EFFECTIVE CELL SOURCE FOR CARTILAGE AND BONE TISSUE ENGINEERING?

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**AIMS.** Mesenchymal stem cells from minced umbilical cord fragments may represent a valuable cell population for tissue engineering. A promising approach for cartilage and bone repair is the use of umbilical cord (UC) as a source of mesenchymal stem cell (MSC). Through a simple and efficient protocol based on mincing the umbilical cord, a consistent number of multipotent UC-MSCs can be obtained. The aim of this in-vitro study is to investigate the pluripotency of UC-MSCs and, in particular, the chondrogenic and osteogenic potential of UC-MSCs grown in tridimensional scaffolds, in order to identify a potential clinical relevance for patients who might benefit from MSCs-therapy.

**METHODS.** Fresh UC samples from women with healthy pregnancies were retrieved at the end of caesarean deliveries. The UC samples were manually minced without any enzymatic digestion into very small fragments (less than 4 mm length) and cultivated in an MSC expansion medium. At day 14, the UC tissue was removed and adherent cells were allowed to expand for 2 additional weeks. At day 28, the adherent cells were collected and replated until confluence was reached (Passage 1 or P1). Immunophenotypic characterization, Fluorescence In Situ Hybridization(FISH), telomere length analysis, Immunosuppression of T lymphocyte cultures, and multilineage differentiation was performed in UC-MSCs at P1 or P2. For chondrogenic differentiation on scaffold, UC-MSCs at P2 were loaded either on a hyaluronic-acid (HA) felt (Hyaff-11) or on a collagen-I/III membrane(Chondro-gide), with a coating of fibrin glue(Tisseel), and grown in chondrogenic medium both in normoxic and hypoxic(10%O<sub>2</sub>) conditions. After 1 month, sections were stained with haematoxylin/eosin and Safranin-O. Expression of chondrocyte markers (sox-9, type II collagen) and hypoxic markers (HIFs) was assessed using immunofluorescence(IF). For osteogenic differentiation on scaffold, UC-MSCs at P2 were loaded into a bone-graft-substitute(Orthoss) and grown in osteogenic medium. After 10, 20,30 days, sections were stained with haematoxylin/eosin and expression of osteocalcin and RunX2 was assessed using immunofluorescence.

## RESULTS

At P1, we obtained a mean value of  $22.8 \times 10^6$  cells(SD 1.7) from each UC, corresponding to  $0.66(\text{SD } 0.14) \times 10^6$  cells/gram of UC. Cells were positive for CD73, CD90, CD105, CD44, CD29 and HLA-I, and negative for CD34 and HLA-class II, with a subpopulation that was negative for both HLA-I and HLA-II. Results from FISH demonstrated that 95-100% of UC-MSCs were of fetal origin. Telomere length of UC-MSCs was similar to that of Bone Marrow(BM) MSC from young donors(aged 20-30 years). At 5 days, the supernatant of UC-MSC cultures had immunosuppressive activity upon T-Lymphocyte cultures. The mixed UC-MSC population was able to differentiate towards osteogenic (monolayer), adipogenic, myogenic and chondrogenic (pellet culture). Chondrogenic differentiation on scaffolds was also confirmed; hypoxic condition improved the expression of chondrogenic markers. Osteogenic differentiation on scaffold was also confirmed after 20 days of culture.

## CONCLUSIONS

These results suggest that the straightforward procedure of collecting UC-MSC at P1 from minced umbilical cord fragments can achieve a valuable cell population that seems to have a potential for orthopaedics tissue engineering such as the on-demand cell delivery using chondrogenic or osteogenic scaffold. The concept of this study may have a clinical relevance as a future hypothetical option for single-stage cartilage repair and bone regeneration.

## **COMPARISON OF THE BIOLOGIC EFFECTS OF THREE DIFFERENT EXTRACORPOREAL SHOCK WAVE DEVICES FOCUSING ON CHONDROCYTES**

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### **AIMS**

The aim of the authors is to evaluate the biological effects of extracorporeal shock wave therapy on the cartilage tissue, to determine if the effects vary between healthy tissue and osteoarthritis tissue and if the cellular behavior varies according to the type of machine used.

### **METHODS**

We cultured the human chondrocytes lineage taken from healthy donors (n=10) and osteoarthritis patients (n=16) in Petri dishes which underwent in a single step 1000 pulses at EDF 0.05 mJ/mm<sup>2</sup> using 3 different shock wave devices, electromagnetic (Minilith SL1-Storz), piezoelectric (WellWaves-Wolf), electrohydraulic (derma-PACE, Sanuwave), and a ballistic wave machine (SwissDolorcast-EMS). Twenty-four hours after the stimulations of the chondrocytes, they were analyzed through immunohistochemistry and cytofluorometry for IL-10, N-caderina and beta-catenina compared to a non stimulated control group.

### **RESULTS**

After shock wave therapy the cells from the healthy and osteoarthritis subjects revealed a statistical reduction of IL-10 expression, however this value was higher in the osteoarthritis patients. At the same time we also found on increased of N-caderina and Beta-catenina expression and regeneration and adhesion cellular markers. We didn't find any statistical differences between the different shock wave devices used.

The shock wave stimulation doesn't cause cellular damage according to literature. This stimulation activates the metabolism and chondrocytes regeneration. The effects between the cells from healthy and pathological subjects don't show any statistical differences.

### **CONCLUSIONS**

Different shock wave machines don't give any statistically significant different results. We believe that it's necessary to start experimenting and monitoring clinical treatment protocols.

# **CARTILAGE FRAGMENTS FOR ONE-STEP CARTILAGE REPAIR: STILL A VALID CELL SOURCE IN HYPOXIC CONDITION AS A MODEL OF JOINT MICROENVIRONMENT?**

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## **AIMS**

Minced cartilage fragments are a viable cell source for one stage cartilage repair, as shown in both in preclinical and clinical studies [1,2,3]. However, the joint microenvironment, in which the repair process takes place, is hypoxic and few evidences are present in literature regarding the behaviour of cartilage fragments in a hypoxic environment. Aim of the study is to verify if hypoxia could influence chondrocyte outgrowth from cartilage fragments into a Hyaluronic-Acid/fibrin scaffold and evaluate its effects on migrating chondrocyte behaviour, compared to normoxic condition. This could be significant in the perspective of a wide clinical application of human chondral fragments for single stage repair.

## **METHODS**

Constructs were prepared with minced cartilage fragments (from 20 human young donors) loaded onto a non-woven esterified Hyaluronic-Acid-derivative felt (Hyaff-11) and retained with a coating of fibrin glue (Tisseel). Constructs were cultured either in normoxic or in hypoxic (10% O<sub>2</sub>) condition. The growth medium contained DMEM-high-glucose (4500mg/l) with 10% fetal-bovine-serum. After 1 month, construct sections were stained with haematoxylin/eosin and Safranin-O and examined for cell counting. Expression of chondrocyte markers (SOX9, collagen-II, collagen-I), hypoxic markers (HIFs) and proliferative markers (beta-catenin, PCNA) was assessed using immunofluorescence

## **RESULTS**

Migrating cells predominantly showed a spindle-like shape when close to the fragments and a more roundish shape when embedded into the scaffold. A slight decrease in chondrocyte migration and proliferation was observed in hypoxic cultures, albeit not statistically significant. Conversely, an increase in the expression of SOX9, beta-catenin, HIFs, collagen-II ( $p < 0.05$ ) in migrating chondrocytes from hypoxic cultures was shown by immunofluorescence.

## **CONCLUSIONS**

Hypoxia seems to improve the chondrocyte phenotype/behaviour of cell outgrowing from cartilage fragments onto a HA/fibrin scaffold. Moreover, hypoxic condition did not hamper the ability and the mechanisms by which chondrocytes migrate from cartilage fragments and proliferate into the surrounding environment.

This is clinically relevant in order to validate one-step repair techniques by means of human cartilage fragments loaded into composite scaffolds.

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## UTILIZATION OF PLATELET RICH PLASMA (PRP) IN ORTHOPAEDIC PRACTICE: CLINICAL AND BIOLOGICAL ANALYSIS OF THERAPEUTIC EFFECTIVENESS

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**AIMS.** PRP is obtained by centrifugation of whole blood with the aim of producing plasma with high concentration of platelets. The literature provides little information about the variability of the clinical efficacy of PRP in relation to the biological characteristics of the product. Objectives of the study in question are: clinical evaluation of effectiveness of PRP "home made" a group of 89 patients, analysis of cytokine profile and concentrations of growth factors in a 10 patients homogeneous group, during treatment with intra-articular and peritendinous injections of PRP; description of possible correlation between changes in cytokine pattern and concentration of growth factors with clinical response to treatment in the same group of patients

**METHODS.** 89 patients were selected and declared fit to receive treatment with PRP "home made". In view of the underlying disease, (Tendinopathy, Epicondylitis, Chondral Lesions, Degenerative lesions of grade I-II-III-IV, Rotator Cuff Lesions) patients underwent to 3-5 cycles of intra-articular or peritendinous injections of PRP, at intervals of 7 or 15 days apart.

Scores of clinical evaluation were administered to each patient before treatment, before any infiltration, and six months after the last injection. The concentration of growth factors, and inflammatory cytokines present in the PRP, were evaluated in a homogeneous group of 10 patients with degenerative lesions of the knee cartilage of grade III-IV sec. Kellgren-Lawrence century. Finally correlation between growth factors, cytokine and haematological parameters of the patient and PRP with clinical parameters was analyzed.

**RESULTS.** Overall Response Rate (ORR) was noted in 69 of 83 patients (84%). In addition, all the scores used have shown a significant rate ( $P < 0.05$ ) in the period immediately after the second injection. Analysis of the growth factors showed a progressive increase in concentration than baseline, after the first application, with a peak level at the second (beginning of the period of clinical improvement statistically significant), and subsequent decrease at the third infiltration. Several more ( $P=0.01$ ) or less ( $P=0.05$ ) significant correlations were identified, both with clinical scores and haematological index. The pattern of inflammatory cytokines, after a stable trend from time 0, showed a decrease after the second clinical application, at the peak of growth factors and beginning of the patient's clinical improvement.

**CONCLUSIONS.** The results reported clinical efficacy in 84% of patients treated. In addition, the biological analysis showed at the beginning of the clinical improvement the increase in growth factors and decrease in inflammatory cytokines. More level I studies are necessary to identify the best production methodology and application of the product.

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## EFFECT OF HUMAN PRP TREATED WITH SHOCK WAVE THERAPY WHEN ADDED ON MICE OSTEOBLAST CULTURE

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### AIMS

We provided an experimental study about osteoblast behaviour when cultured with PRP (platelet rich plasma) previously treated with SW. We want firstly to evaluate the effect of SW on platelets which are able to release growth factors for modulation of bone regeneration; then we want to study the effect of growth factors infiltration when associated with SW exposure to treat various musculoskeletal diseases.

### METHODS

We obtained PRP through venous blood apheresis; we aliquotted PRP with medium DMEM in 1.8 ml test tubes. We treated those with SW fields, using an electromagnetic generator STORZ-Minilith SL1 (energy density of 0.17 mJ/mm<sup>2</sup> and number of pulses 500). We used as control other PRP test tubes not treated with SW field. Osteoblast cells were extracted and purified from skull bones of newborn mice and cultured either with PRP treated with SW fields (experimental group) or with not treated PRP (control group). We finally compared the osteoblasts cellular activity of the two groups. Real-time PCR and Western blot analysis allowed us to evaluate the expression of IGF-1, IGFBP-3, IGFBP-4, IGFBP-5, collagen type I, osteocalcin and RUNX2 at 24, 48 and 72 hours after administration of SW.

### RESULTS

OB culture receiving PRP treated with SW fields was associated with a higher production of IGFs and IGFBP-s among 48 to 72 hours after treatment, acting as a stimulus for proliferation and differentiation of osteoblast.

Also bone matrix proteins release, whose production is modulated by the transcription factor RUNX2, showed an increase in osteoblasts culture receiving PRP treated with SW fields.

### CONCLUSIONS

Bone cell proliferation and differentiation is modulated by different growth factors. GF concentration through venous blood apheresis and subsequent PRP infiltration has many clinical applications in maxillofacial surgery, orthopedics and sport medicine. We studied Insulin-Like Growth Factors (IGFs) secreted by osteoblasts and provided of autocrine regulation function, through direct interaction with IGF-binding proteins (IGFBPs). SW were indirectly able to promote osteogenesis when primarily used to treat PRP after added with osteoblast culture. This validate the issue to associate the two methods of treatment, lithotripsy and infiltration of growth factors, to improve results. Furthermore SW had a synergic effect on platelets already located at the focal field, enhancing osteogenesis produced by direct stimulation of osteoblasts.

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## **BIOMATERIALS: A GOOD ALTERNATIVE TO BONE GRAFTS FOR SPINE FUSION**

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### **AIMS**

Spinal fusion is one of the most common surgical procedures in spine surgery, whose primary objective is the stabilization of the spine for the treatment of many degenerative, traumatic and oncological diseases of the spine. Autologous bone is still considered the "gold standard" technique for spinal fusion. However, the removal of autologous bone can be limited by donor site morbidity and by the limited availability of bone which occurs in some patients, and also the access to bone banks for the homologue grafting may not always be easy and economical.

For this reason biomaterials which are potentially osteogenic, osteoinductive and osteoconductive can be used to increase the process of spinal fusion. We proposed to evaluate two new bone substitutes as an alternative to autologous bone for spinal fusion, using an animal model of large size (adult sheep).

### **METHODS**

A preclinical study was designed to compare the efficacy of SINTlife® Putty and DBSINT® biomaterials with conventional bone autograft in an ovine model of lumbar spine fusion. SINTlife® is a biomaterial made from hydroxyapatite enriched with magnesium ions, resulting to be very similar to natural bone. DBSint® is a paste composite bone, osteo-inductive, pliable and conformable, consisting of demineralized bone matrix (DBM) carried by hydroxyapatite biomimetics.

Eighteen adult female sheep were selected. After anesthetization, a midline skin incision was made followed by a unilateral fascial incision, and the T11-L5 paraspinal muscles were dissected. The cortical-cancellous bone harvested from transverse processes and laminae at T12-T13 and L2-L3 was used as autograft. Pedicle screws were inserted at each fusion level and fixed with a rod. The animals were divided in two groups: in Group A, one fusion level was treated with SINTlife® Putty and the other level received cortical-cancellous bone autograft; in Group B, one fusion level was treated with DBSINT® and the other level received cortical-cancellous bone autograft. At the end of the experimental time, all the animals were euthanized. The lumbar spine segments were subsequently analyzed macroscopically, radiographically, microtomographically, histologically and histomorphometrically.

### **RESULTS**

The SINT-Life® Putty shows a perfect osteointegration in all the histological specimens. A high percentage of newly formed bone tissue is detected, with lots of trabeculae having structure and morphology similar to the pre-existing bone and numerous osteocytes. In all the specimens collected from DBSINT®-treated animals the presence of hydroxyapatite alone is reported but not the demineralized bone matrix. The presence of newly formed bone tissue can be detected in all the specimens but newly formed bone shows very thin and irregular trabeculae next to the cartilage zone, while away from the border of ossification there are thicker trabeculae with structure and morphology similar to the pre-existing bone.

### **CONCLUSIONS**

The use of the experimental biomaterial SINT-Life® Putty in an ovine model of spine fusion leads to the development of newly formed bone tissue without qualitative and quantitative differences with the one formed with autologous bone. The experimental material DBSINT® seems to lead to less deposition of newly formed bone with wider intertrabecular spaces.

## **NANOSTRUCTURED MATERIALS: A NEW APPROACH TO DESIGN INNOVATIVE SCAFFOLDS FOR THE TREATMENT OF BONE DEFECTS**

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### **AIMS**

The wide bone defects, caused by trauma, tumor, infectious, periprosthetic osteolysis, need to be surgically treated because their low potential of repair. Nowadays the bone allograft and autograft represent 80% of all transplantation done in the world. However this technique shows many disadvantages, such as the risk of infections, the immunological rejection, the low bone availability and the high costs. These reasons have motivated extensive research to find alternative strategies. As shown in literature, the future strategies are based on the synergic combination of different methodologies: use of biomimetic scaffold in order to support bone regeneration, use of mesenchymal stromal cells (MSCs) and growth factors. Successful regeneration necessitates the development of tissue-inducing scaffolds that mimic the hierarchical architecture of native tissue extracellular matrix (ECM). Cells in nature recognize and interact with the surface topography they are exposed to via ECM proteins. Here we are going to show the guidelines recently published for the design and development of nanostructured scaffolds for the bone regeneration, and the morphofunctional changing of MSCs interacting with nanogratings.

### **METHODS**

Aim of this study is to design, develop and preclinical test PET nanostructured scaffolds for the transplantation and differentiation of MSCs in the treatment of bone defects. The first step of our study was the extraction of patient's bone marrow and the isolation of MSCs. After characterizing (demonstrating the typical cell surface markers) and isolating the MSCs were cultivated on the PET substrates. The PET nanosubstrates were obtained by a low temperature embossing lithography (HEL) achieving low-damage nanotopographic surface modifications. After MSC cultivation on PET substrates we made a cytotoxicity evaluation, an optic and confocal microscopic evaluation (cells adhesion, cells polarization...) and tests to optimize cell differentiation towards osteogenic fate.

Results. PET is a highly suitable thermo-plastic material, able to sustain the necessary methods to obtain nanostructured substrates. MSCs cultivated on nanostructured PET rapidly align with the direction of the nanostructure itself without any cytotoxic effects. After the cultivation on the nanostructures, MSCs sustained cytoskeleton changes suggesting the activation of intracellular signaling (mechanotransduction) promoting osteogenesis.

### **CONCLUSIONS**

The mechanisms by which nanotopographic cues influence stem cell proliferation and differentiation appear to involve changes in cytoskeletal organization and structure, potentially in response to the geometry and size of the underlying features of the ECM by a process called mechanotransduction. The interaction of cells with nanotopographical features such as pores, ridges, groves, fibers, nodes, and their combinations has proven to be an important signaling modality in controlling cellular processes. Integrating nanotopographical cues is especially important in engineering complex tissues that have multiple cell types and require precisely defined cell-cell and cell-matrix interactions at the nanoscale. Thus, in the next-generation regenerative engineering approaches, nanoscale materials/scaffolds are expected to play a primary role in controlling MSC fate and the consequent regenerative capacity. We believe that the continuous development of nanotechnology and deeply comprehension of how mechanical inputs can affect cell biology is fundamental to design the future scaffold for orthopedic application.

# GHRELIN IS INVOLVED IN OSTEOBLAST-OSTEOCLAST INTERACTION THROUGH WNT/ $\beta$ -CATENIN SIGNALING PATHWAY

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## AIMS

Considerable attention has been focussed on the effect of energy regulating peptides such as leptin and ghrelin on bone metabolism.

Ghrelin, the natural ligand of the growth hormone secretagogue receptor (GHS-R), is considered a positive regulator of bone mass. Ghrelin, in fact, acts directly on osteoblasts and stimulates osteoblast proliferation and differentiation. However, the detailed mechanisms of ghrelin's modulation of osteoblast activity remain unclear and require further studies. The Wnt/ $\beta$ -catenin signalling pathway plays a crucial role in osteoblastic cell differentiation and bone formation. When Wnt receptor binding interactions are absent,  $\beta$ -catenin is phosphorylated by glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ) leading to  $\beta$ -catenin degradation in the proteasome. Following Wnt receptor activation GSK-3 $\beta$  is phosphorylated and inactivated, leading to the stabilization of  $\beta$ -catenin and its accumulation in the cytoplasm.  $\beta$ -catenin then migrates to the nucleus where it regulates transcription, and modulates the expression of target genes responsible for osteoblast proliferation and differentiation.

The aim of the present study was to investigate the involvement of the Wnt/ $\beta$ -catenin signalling in the anabolic effect of ghrelin and the receptors involved in such activity.

## METHODS

We used primary cultures of osteoblast like cells derived from rat calvariae exposed to increasing concentrations of ghrelin ( $10^{-11}$ - $10^{-7}$ M) for 60 min.  $\beta$ -catenin and GSK-3 $\beta$  protein levels were measured by Western Blot in the whole cell lysates.

## RESULTS

Ghrelin significantly increases the phosphorylation and degradation of GSK-3 $\beta$  and therefore it increases  $\beta$ -catenin levels and  $\beta$ -catenin nuclear migration.

These effects are due to a specific interaction of ghrelin with its GHSR1a receptor since pretreatment with the GHS-R1a receptor antagonist D-LYS-GHRP6 ( $10^{-7}$ M for 30min), prevents ghrelin-induced  $\beta$ -catenin stabilization and nuclear migration. Ghrelin-dependent induction of  $\beta$ -catenin increases osteoblast proliferation as shown by MTT assay.

Considering that osteoprotegerin (OPG), a target gene of the Wnt/  $\beta$ -catenin signaling, plays a key role in the regulation of cross-talk between osteoblasts and osteoclasts, we examined the effect of increasing ghrelin concentrations ( $10^{-11}$  -  $10^{-8}$  M) on the expression of OPG mRNA measured by Real Time PCR in osteoblasts. Ghrelin ( $10^{-10}$  M) significantly increases OPG mRNA levels 24 h after treatment. Thus, it is conceivable that ghrelin by increasing the Wnt/ $\beta$ -catenin signaling pathway not only exerts an anabolic action but it is also involved in osteoblast-osteoclast interaction.

## CONCLUSIONS

In conclusion, we have shown that ghrelin stimulates osteoblast proliferation and increases OPG expression at the same time through Wnt/ $\beta$ -catenin signalling pathway. Even if further in vivo studies will be required to confirm this assumption, this study suggests ghrelin as a key candidate to maintain bone homeostasis when there is imbalance between resorption and formation as occurs in osteoporosis.

## **DIABETIC FOOT DISEASE: INVOLVEMENT OF AUTOIMMUNITY IN THE PATHOGENESIS OF CHARCOT NEUROARTHROPATHY**

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### **INTRODUCTION**

Charcot neuroarthropathy is a disabling complication of diabetes with an unknown pathogenesis. The disease is characterised by varying degrees of bone and joint disorganisation with an underlying neuropathy, trauma and perturbations of bone metabolism<sup>1</sup>. It has been proposed that the genetic regulation of bone remodelling is associated with Charcot neuroarthropathy<sup>2</sup>.

### **AIM**

In this project, we use ELISA to establish a potential relationship between Charcot neuroarthropathy (and related neuropathies) and oxidative modifications of collagen type I and II.

### **METHODS**

Sera from patients with diabetic neuropathy (DN) with and without Charcot disease (Ch) and healthy (H) controls were tested for the presence of autoantibodies against native collagen type I and II (CI and CII), and post-translationally modified CI and CII, using reactive oxygen species (ROS).

### **RESULTS**

Autoantibodies to native and ROS modified collagen type I (ROS-CI) and ROS-CII have been detected in patients with Charcot disease and diabetic neuropathy, but not in healthy controls.

### **CONCLUSION**

We propose that autoimmune reactions to native and post-translationally modified self antigens may play a role in the pathogenesis of Charcot and related neuropathies.

## **ASSOCIATION BETWEEN OSTEOPROTEGERIN G1181C AND T245G POLYMORPHISMS AND CHARCOT NEUROARTHROPATHY OF THE FOOT IN DIABETIC PATIENTS**

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### **OBJECTIVE**

Charcot neuroarthropathy is a disabling complication of diabetes. Although its pathogenesis remains unknown, we suppose that genetics may play a relevant role.

### **RESEARCH DESIGN AND METHODS**

We performed a case-control study with 59 subjects with diabetic Charcot neuroarthropathy (Ch group), 41 with diabetic neuropathy without Charcot neuroarthropathy (ND group), and 103 healthy control subjects (H group) to evaluate the impact of two single nucleotide polymorphisms (SNPs) of the osteoprotegerin gene (G1181C and T245G) on the risk of Charcot neuroarthropathy.

### **RESULTS**

Regarding the SNPs of G1181C, we found a significant linkage between the G allele and Charcot neuroarthropathy (Ch vs. ND, odds ratio [OR] 2.32 [95% CI 1.3– 4.1],  $P=0.006$ ; Ch vs. H, 2.10 [1.3–3.3],  $P=0.002$ ; and ND vs. H, 0.90 [0.7–1.9],  $P=0.452$ ); similarly, we found a linkage with the G allele of T245G (Ch vs. ND, 6.25 [2.2–19.7],  $P=0.001$ ; Ch vs. H, 3.56 [1.9 – 6.7],  $P=0.001$ ; and ND vs. H, 0.54 [0.6 –5.7],  $P=0.304$ ), supporting a protective role for the allele C and T, respectively. For this reason we investigated the frequency of the protective double homozygosis CC + TT (7% in Ch) that was significantly lower in Ch compared with H (0.18 [0.06 – 0.5],  $P=0.002$ ) and with ND (0.17 [0.05– 0.58],  $P=0.006$ ), whereas there was no difference between H and ND (1.05 [0.43–2.0],  $P=0.468$ ). In a multivariate logistic backward regression model, only weight and the lack of CC and TT genotypes were independently associated with the presence of Charcot neuroarthropathy.

### **CONCLUSIONS**

This is the first study that shows an association between genetic regulation of bone remodeling and Charcot neuroarthropathy.

# **AN AUTOLOGOUS AND ALLOGENIC COMPOSITE NERVE GRAFTS: A PROJECT TO EVALUATE A NOVEL TECHNIQUE FOR NERVE DECELLULARIZATION**

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## **AIMS**

For unrepairable nerve defects, to date autogenous nerves are considered the golden standard, but donor site morbidity, limited availability and operation time prolongation are relevant problem. Acellular nerves from cadaveric donor, introduced since more than one decade ago, represent a novel promising alternative to bridge unrepairable nerve gaps.

Aim of this study is to provide a new tool to ameliorate the assistance of the numerous patients suffering from traumatic, oncological and iatrogenic nerve lesions. For this purpose, our project is promoting a progress beyond the state of the art of nerve gaps bridging surgery by developing a new technique to obtain acellular nerve allografts (ANAs).

## **METHODS**

Several methods to examine the effect of detergents on nerve tissue morphology and protein composition have been previously reported. Most of them are too expensive and time consuming. The novel decellularization technique is a modification of the Michigan detergent-based organic material removal, to speed up myelin and cellular debris detachment. The previously published Hudson's method has been chosen as control of the decellularization process. To validate the new nerve decellularization method, in terms of histological characteristics, outcomes were estimated through morphological and immunohistochemical studies.

Results: Histological analysis of processed acellular nerve have been performed to evaluate the preservation of the structure and almost complete clearance of donor cells and cellular debris. Immunostaining analysis confirmed absence of Schwann cells and the maintenance of basal lamina.

## **CONCLUSIONS**

The novel method will allow the storing of acellular nerve allografts. First results obtained by morphological analysis and immunofluorescence experiments indicate that the internal structure of native nerve is maintained. It is then possible to decellularize nerves with the novel technique reducing both manufacturing times and costs. The relatively inexpensive method of decellularization will allow to increase the number of SSN assisted patients who will benefit from reconstruction of nerve defects with ANAs.

## DEVELOPMENTAL ENGINEERING OF A BONE ORGAN THROUGH ENDOCHONDRAL OSSIFICATION

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Bone development and repair of most bones share the same formation process, being both based on endochondral ossification, namely through formation of a cartilage intermediate. We previously demonstrated that adult human bone marrow-derived mesenchymal stem/stromal cells (hMSC) can drive an endochondral program and ectopically generate mature bone in immunodeficient mice (1, 2). In the current work, we demonstrate that hMSC pushed along the endochondral route can generate an up-scaled ossicle with features of a “bone organ”, including physiologically remodeled bone, mature vasculature and a fully functional haematopoietic compartment (3). By analogy with the inflammatory environment occurring during bone repair, in our model the engineered hypertrophic cartilage required IL-1 $\beta$  in order to be efficiently remodeled into a bone organ. The remodeling process was paralleled by ingrowth of blood vessels, displaying sinusoid-like structures and stabilized by pericytic cells. One of the most striking features of this model was the full functionality of the host-derived bone marrow engrafted in the engineered ossicles: marrow areas contained phenotypically-defined haematopoietic stem (HSC) and progenitor cells at similar frequencies as native bones, and marrow from ossicles reconstituted multi-lineage long-term hematopoiesis in lethally irradiated mice. The work provides a model useful for fundamental and translational studies of bone development and regeneration, as well as for the establishment of advanced mouse models of normal and malignant hematopoiesis.

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## PATIENT-SPECIFIC PIN GUIDES IN TOTAL KNEE ARTHROPLASTY: OUR PROSPECTIVE RANDOMIZED STUDY

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### AIMS

The purpose of our prospective, randomized study was to compare the advantages and disadvantages of mechanical axis-based patient-specific pin guides system for total knee arthroplasty (TKA) (Patient specific instruments, PSI; Zimmer, Inc) to those of standard TKA instrumentation.

### METHODS

Thirty primary unilateral TKA patients were enrolled and completed surgery. All patients underwent TKR surgery for primary OA and were randomly allocated to group A (PSI-TKA) or group B (conventional TKA), using either PSI or standard instrumentation to implant NexGen LPS-Flex (Zimmer, Inc). All procedures were performed at Agostino Gemelli Hospital (Rome, IT) by the same surgical team between June and October of 2012. The custom made pin guides (group A), were generated using preoperative magnetic resonance imaging (MRI) of hip, knee and ankle. For each patient of group A we also chose preoperative determinations (implant size, resection depth etc.) using a software (PSI planner). For all patients perioperative bleeding and surgical time were recorded. Blood loss was evaluated in terms of pre-operative and post-operative haemoglobin (Hb) and haematocrit (Hct) levels, need for transfusion and amount of the volume in post-operative drains. Surgical time was assessed in terms of tourniquet time (time between inflation and deflation of tourniquet). Furthermore we analysed the cost-effectiveness of this device.

### RESULTS

Mean tourniquet time was reduced in PSI group ( $55.5 \pm 13.2$  vs  $74.8 \pm 8.9$  minutes). This time followed a decreasing trend starting from 78 minutes for the first procedure and reaching 39 minutes for the last one. The mean haemoglobin and haematocrit loss was higher in group B ( $3.5 \pm 0.8$  vs  $2.9 \pm 0.5$  grs/dl and  $10.8 \pm 2.2$  vs  $6.8 \pm 1.2$  %). Patients who needed for transfusion were 3 in PSI-TKA group and 6 in conventional TKA group. Postoperative blood loss was lower in group A ( $968 \pm 225$  cc vs  $1084 \pm 318$  cc).

### CONCLUSIONS

Our data show how patient specific instrumentation aids in reducing time of surgery. The learning curve pointed out favorable trend especially in consideration of time of surgery. This could be due to simplification of surgical technique (fewer instruments and less surgical steps). We also found reduction in blood loss because of lower time of surgery as well as for less invasive procedure (it was not necessary to use intramedullary alignment guide). According to our results cost-effectiveness of patient specific pin guides seems to be advantageous in spite of initial investment (instrumentation, MRI).

# EVALUATION OF THE EFFECTS OF THE METHA SHORT STEM ON PERIPROSTHETIC BONE REMODELLING IN TOTAL HIP ARTHROPLASTIES: RESULTS AT 3 YEARS AND LITERATURE REVIEW

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## AIMS

Total hip arthroplasty is one of the most performed procedures in orthopaedic surgery. Implantation of a prosthesis determines changes in the distribution of loads on the host bone, and this phenomenon, known as stress-shielding is related to the biomechanical characteristics of the implant. Usually stress-shielding involves the proximal portion of the femur by reducing the mechanical strength and ability to withstand the transmitted loads. The aim of our study is to demonstrate how the use of a short hip stem reduces the stress-shielding phenomenon to the proximal femur.

## METHODS

The study analyzed 20 patients undergoing hip prosthesis surgery with the implant a short stem (B-Braun Metha) at the 1st Orthopaedic Division of Pisa University between Dec 2008 and Jan 2010. Each patient was subjected to analysis of periprosthetic bone mineral density by a Bone Densitometry (DEXA: Dual emission X-ray absorptiometry with the metal removal software) at 0, 6, 12, 18, 24 and 36 months, following a protocol based on the evaluation of the changes of bone density in the 7 Gruen zones.

## RESULTS

We recorded minimal changes in bone mineral density (BMD) at the level of the greater trochanter (-1,44%) and at the level of the calcar (-3,7%). BMD increased significantly after two years at the level of the lateral distal regions (R2 +9,6% - R3 + 12,4%) and at the level of the distal medial regions (R5 + 8,2% - R6 + 13,1%). We compared the results obtained with the literature data at 12 and 24 months with the same stem (B-Braun Metha). At 12 months of follow up we do not recorded significative difference between our data and the data published in literature while after 24 months of follow-up we recorded significative differences in the curves of periprosthetic bone reabsorption at the level of the greater trochanter (Zone 1) and at level of the calcar (Zone 7).

## CONCLUSIONS

The data obtained from our study are in agreement with other studies in the literature which demonstrate how the use of short stems allow to preserve during the time the metaphyseal bone stock at the level of the proximal femur, reducing the stress-shielding phenomenon. From our data, obtained at 24 months and confirmed at 36, stress shielding seems to minimally occur at the level of the calcar. At the level of the great trochanter we saw a good load distribution with maintained of the baseline BMD; these data are in opposition to the literature data that showed a high increased of BMD at the level of the calcar (+12,9%) and a decrease at the level of the great trochanter. From the analysis of the radiographic images of our cases and of the cases published with the same stem, these differences in load transfer encountered between the great trochanter and the calcar seems to be related to the level of the femoral neck osteotomy and the consequent stem position (varus/valgus). We conclude that the amount of periprosthetic bone reabsorption around the Metha stem seems to be strictly related to the surgical technique and the final implant position.

# **TOTAL HIP ARTHROPLASTY BY USING A CEMENTLESS ULTRASHORT STEM: A SUBJECT-SPECIFIC FINITE ELEMENT ANALYSIS FOR A YOUNG PATIENT CLINICAL CASE**

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## **AIMS**

In this research paper, a subject-specific finite element (FE) model of a hip prosthesis and pathological femur by using Computed Tomography (CT) dataset was developed.

## **METHODS**

The FE analysis refers to a clinical case of a surgically misaligned hip prosthesis with an ultra-short stem. It was set out to study the strain energy density pattern, comparing the results obtained with computed tomography images. The authors developed two other numerical models: the first one analyzes the stress and strain distributions in the healthy femur (without prosthesis) and the second one analyzes the same bone-implant biomechanical system of the clinical case but assuming the prosthesis in the proper position.

## **RESULTS**

The misaligned prosthesis produced an overload at the proximal posterior plane of the femur, as confirmed by computed tomography images, which detect the formation of new bone. The numerical model of the correctly positioned prosthesis demonstrated that the bone is not overloaded and that the position of neutral axis does not significantly shift from the physiological condition.

## **CONCLUSIONS**

From the analysis of the numerical results and the CT images, it can be concluded that numerical models can be an effective tool for the evaluation of complex clinical cases.

## FAILURE ANALYSIS OF A MODULAR KNEE PROSTHESIS

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### AIMS

Osteosarcoma is the most common primary malignant tumour of bone. The commonly involved sites are the distal femur, proximal tibia and humerus. Endoprosthetic reconstruction is a highly successful and durable method for restoring skeletal integrity and joint function after resection surgery [1]. Modular prostheses are assembled on the basis of the intraoperative measurement of the residual bone defect after tumour resection. Aseptic loosening is the most frequent complication of reconstructive prosthesis at the knee [2], but other causes of mechanical failure, hinge fracture or polyethylene wear can produce unsatisfactory clinical results.

The aim of this study is the failure analysis of an ex vivo modular endoprosthetic replacement system (MUTARS®) for bone tumors of the distal femur. For over the period of the prosthesis life, our patient was periodically followed-up.

### METHODS

The Modular Universal Tumor and Revision System (MUTARS® system, Implantcast, Buxtehude, Germany) has been considered in this study. With this modular system, it is possible to bridge any bone defect from the proximal femur to the proximal tibia. The fixation is achieved with an intramedullary stem, which was inserted cementless. The stem has a hexagonal cross section and is made of cast alloy (CoCrMo). Different intermediate pieces are connected to this stem to reconstruct the individual length and joint function of the affected extremity. At the knee, a hinged system is used with an axis fixed to the tibial base plate that fits into a polyethylene locking device (Figure 1).

Hardness tests, non-destructive tests and extensive microscopy were carried out to establish the dominant mechanism of fracture and presence of macroscopic internal defects.

### RESULTS

The cause of the failure was determined from the detailed fracture surface analysis and from the background information provided on the implant.

### CONCLUSIONS

The results demonstrated that the experimental techniques applied in this investigation can be a reliable tool for ex vivo failure analysis of biomechanical implants.

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## **TWO STAGE PROTOCOL IN SEPTIC KNEE ARTHROPLASTY REVISION: ARTICULATING VS STATIC ANTIBIOTIC-LOADED SPACERS**

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### **AIMS**

To compare the results achieved using static or articulating antibiotic-loaded spacers in two-stage revision for infected total knee arthroplasty.

### **METHODS**

In the period 1996-2011 we treated 70 knee arthroplasties infections using 15 fixed, 40 standard articulating and 15 augmented articulating spacers. In 7 cases (10%) a second spacer was necessary to eradicate the infection. All patients were re-implanted. In all patients HSS score and range of movement were evaluated.

### **RESULTS**

Mean HSS score improved from 31 preoperatively to 53 at time of second procedure (all patients) and to 81 at 1 year follow-up. Mean flexion improved from 60° to 77° at time of second procedure and to 98° at 1 year follow-up, flexion contracture decreased from 8° to 1° at time of second procedure and to 2°, except for a patient that underwent extensor mechanism transplantation (active ROM: 60°-90°).

### **CONCLUSIONS**

Two-stage reimplantation has been considered state-of-the-art in treatment of chronic knee arthroplasty infections achieving the best results for eradication of infection (88-96%) and maintenance of function.

The antibiotic-loaded preformed cement spacers preserve bone stock, keep collateral ligaments from becoming contracted, avoid quadriceps fibrosis and reduce formation of scar tissue that obliterates the joint space. Moreover, these spacers deliver high-dose local antibiotics in concentrations greater than those achieved with intravenous administration. Articulating spacers allow both weight-bearing and motion during the period of antibiotic therapy (avoiding stiffness and osteopenia and favouring exposure and soft tissue balance at reimplantation) and improve patient satisfaction.

Nevertheless, in some instances (i.e. infection following implantation of hinged or augmented/stemmed prostheses) at time of first procedure bone loss, according AORI classification\*, can be very wide as well as instability; moreover tibial and femoral canal are violated and infection spreads deeply. In those cases preformed commercially available spacers are unable to fill the loss (T2 and F2 type), to achieve enough stability (T3 and F3 type) and to deliver antibiotics deeply into the diaphyseal canals. We present a self-made antibiotic-impregnated cement modular stem, intended to be cemented both to femoral and tibial preformed commercially available spacers. With this device we can intraoperatively obtain a custom-augmented completely antibiotic-loaded spacer that allows to manage wide bone loss (till to T3 and F3 type) and to deliver antibiotic into the diaphyseal canals as deep as needed.

The use of antibiotic-impregnated cement spacers and intravenous antibiotics with delayed exchange arthroplasty demonstrates its reliability in treating infection and in recovering function. In the latter field the custom-augmented spacer allows to satisfactory manage even cases that show the wider bone loss.

\* Engh GA. A classification of bone defects. In: Revision knee arthroplasty. Baltimore: Williams & Wilkins; 1997. p. 63-120.

## STANDARD AND CUSTOM-AUGMENTED ANTIBIOTIC-LOADED SPACERS IN SEPTIC KNEE

### ARTHROPLASTY REVISION

Ivan De Martino<sup>1</sup>, Rocco D'Apolito<sup>1</sup>, Aaron Burrofato<sup>1</sup>, Vincenzo De Santis<sup>1</sup>, Giorgio Gasparini<sup>2</sup>, Carlo Fabbriciani<sup>1</sup>

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Two-stage reimplantation has been considered state-of-the-art in treatment of chronic knee arthroplasty infections achieving the best results for eradication of infection (88-96%) and maintenance of function.

During first procedure debridement and synovectomy are performed: necrotic and infected tissues are excised, implant and cement are removed, antibiotic-loaded acrylic preformed spacer is implanted with antibiotic-loaded cement. After specific systemic antibiotic therapy, for at least 12 weeks, during second stage procedure, spacer is removed, bone and soft tissues are further debrided and a revision implant is inserted with antibiotic-loaded cement.

The antibiotic-loaded preformed cement spacers preserve bone stock, keep collateral ligaments from becoming contracted, avoid quadriceps fibrosis and reduce formation of scar tissue that obliterates the joint space. Moreover, these spacers deliver high-dose local antibiotics in concentrations greater than those achieved with intravenous administration. Articulating

spacers allow both weight-bearing and motion during the period of antibiotic therapy (avoiding stiffness and osteopenia and favouring exposure and soft tissue balance at reimplantation) and improve patient satisfaction.

Nevertheless, in some instances (i.e. infection following implantation of hinged or augmented/stemmed prostheses) at time of first procedure bone loss, according AORI classification (Engh, 1997), can be very wide as well as instability; moreover tibial and femoral canal are violated and infection spreads deeply. In those cases preformed commercially available spacers are unable to fill the loss (T2 and F2 type), to achieve enough stability (T3 and F3 type) and to deliver antibiotics deeply into the diaphyseal canals. We present a self-made antibiotic-impregnated cement modular stem, intended to be cemented both to femoral and tibial preformed commercially available spacers. With this device we can intraoperatively obtain a custom-augmented completely antibiotic-loaded spacer that allows to manage wide bone loss (till to T3 and F3 type) and to deliver antibiotic into the diaphyseal canals as deep as needed.

In the period 1996-2010 we treated 59 knee arthroplasties infections using 21 fixed, 29 standard articulating and 9 augmented articulating spacers. In 6 cases (15%) a second spacer was necessary to eradicate the infection. At reimplantation a CCK or a RHK modular prosthesis was used. A failure was observed in 10 patients (6%) at 3-17 years follow-up due to a late deep infection. Mean HSS score improved from 31 preoperatively to 53 at time of second procedure (all patients) and to 81 at 1 year follow-up (except of failed cases). Mean flexion improved from 60° to 77° at time of second procedure and to 98° at 1 year follow-up, flexion contracture decreased from 8° to 1° at time of second procedure and to 2°, except for a patient that underwent extensor mechanism transplantation (active ROM: 60°-90°).

The use of antibiotic-impregnated cement spacers and intravenous antibiotics with delayed exchange arthroplasty demonstrates its reliability in treating infection and in recovering function. In the latter field the custom-augmented spacer allows to satisfactorily manage even cases that show the wider bone loss.

## **POSTERIOR STABILIZED VERSUS DEEP-DISH POLYETHYLENE IN TOTAL KNEE ARTHROPLASTY. A CLINICAL RANDOMIZED PROSPECTIVE STUDY**

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The excellent results of the posterior stabilized (PS) implants in total knee replacement (TKR) are largely recognized. Nevertheless, some disadvantages are described: removal of large bone stock, paradoxical kinematics, wear or disruption of the post, post-cam dislocation (short horizontal and vertical jumping distances). On the other hand, the polyethylene wear continues to represent the major cause of failure in primary TKR. The deep-dish polyethylene component has a deeper concavity with an elevated anterior lip that is conforming in the sagittal plane with the femoral component imparting anteroposterior stability without needing a central cam and post. This insert may decrease polyethylene debris formation so reducing

osteolysis and loosening, allows to save the intercondylar femoral bone decreasing the risk of fracture and preserving bone stock for possible revisions and permits a more physiological kinematics and distribution of the loads. Furthermore a deepdished component has increased jumping distances that reduces the risk of dislocation. We performed a clinical randomised prospective study on 100 patients with primary osteoarthritis who received the same computer-assisted TKR using a CT-free navigation system. In all patients the posterior cruciate ligament was resected: in 50 PS tibial and femoral components were implanted whereas in 50 a deep-dish tibial polyethylene was coupled to a cruciate retaining femoral component. There were 74

females and 26 males with an average age of 71 years, with a maximum follow-up of three years. Flexion increased from 65° to 113°, whereas range of motion improved from 62° to 112°. HSS score increased from 31 to 93 at the last follow-up. There were no statistical difference between two groups in the mean range of all examined parameters. The deep-dish sagittal conforming component may reduce the polyethylene wear so increasing the implants survivorship; preserving the femoral intercondylar bone, the deep-dish implant may improve the results of possible revisions. Further longer term investigations are necessary on larger series.

## THE APPLICATION OF EXTERNAL CIRCULAR FIXATOR IN BONE TRANSPORT OF TIBIAL INFECTED NONUNIONS: CLINICAL AND BIOMECHANICAL ASPECTS

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### AIMS

The infected nonunion is one of the most complex problems in orthopaedics, Ilizarov method gives the opportunity to fill large bone defects, to solve *infection* and to consolidate *non-union*, restoring the physiological length of the limb. The real benefit of Ilizarov's apparatus is to reduce the "parasites" movements (angles and rotations), without removing the "elasticity" which, concerning to biomechanical studies, promotes the bone healing through axial micromotions.

### METHODS

First of all, it is necessary to restore load's axis, so that the applied forces to the bone result in a compression-distraction component, with the complete elimination of the "cutting forces", which are absolutely damaging for the formation of the reparative callus. After an accurate debridement and the removal of the non-union focus, it proceeds to the assembly of the apparatus followed by the execution of an osteotomy in the biological vital place (metaphysis), limited to the cortical bone (compactotomy).

Undifferentiated mesenchymal cells develop into osteoblasts and start the formation of bone tissue. The application of a continuous and progressive distraction force "ad longitudinem" on the two stumps, guides the osteoid toward the force's lines. The newly formed bone lamellae are arranged in parallel to form the "regenerate".

### RESULTS

At the end of the lengthening, the bone regenerative activity resumes morphological maturation and functional differentiation, to the integration of the newly formed segment. At the same time, at the site of non-union, forces, involved in progressive compression, operate guaranteed by the stability of the stumps, so you get a rapid transformation of the inter-fragmentary tissue (fibro-cartilage) in bone tissue, with the recovery of non-union.

### CONCLUSIONS

This method allows the surgeon to be radical, removing the necrotic and infected tissues which could extend the dangerous infection to the healthy adjacent tissues.

Thanks to bone and soft tissues regeneration, it is possible to fill important bone and cutaneous losses, so that the shape and functionality of the limb can be restored with just one operation, followed by a period of time in "Ilizarov apparatus", which is correlate with the length of bone resection.

## DEGENERATIVE FACET JOINT CHANGES AFTER LUMBAR PERCUTANEOUS PEDICLE SCREW FIXATION

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### AIM

Aim of the study was to evaluate degenerative lumbar facet-joints changes after percutaneous pedicle screw fixation (PPSF) for treatment of lumbar amyelic fractures.

### METHODS

Thirty patients underwent short PPSF without fusion. CT scan was performed in the pre and post-operative time at 4, 8 and 12 months. The 6 zygoapophyseal joints adjacent the fracture's level were evaluated .

### RESULTS

Eleven patients presented an A3.1, 4 an A3.2, 15 an A.3 fracture. Patients were divided into 3 groups considering CT scan execution time: group A 4 months, group B 8 months, group C 12 months. At 4 months patients showed no joint radiographic differences between pre and post-operative time. At 8 and 12 months CT scan demonstrated a progressive degeneration only in the middle joints respectively in 21,42% and in 76,92% of the cases. Considering screws placement there was an impingement with the articular surface in 6 cases, responsible of an early degeneration of the related facet joint.

### CONCLUSION

Lumbar percutaneous fixation without fusion induces little degenerations only in the middle joints close to fracture level at 8 and 12 months. In the proximal and distal joints adjacent the screws degenerative changes can be seen only associated to pedicle-screw encroachment.

# **A NEW STRUCTURAL METHOD PERMITS THE EVALUATION OF BONE QUALITY AND FRACTURE RISK IN A CASE OF RAPID AND PROGRESSIVE OSTEOPOROSIS OF THE HAND (CRPS TYPE II)**

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## **AIMS**

Even if densitometry is still currently considered to be the gold standard in bone quality assessment, however, the medical community has long been aware that two are the factors that increase bone weakness: bone mineralization loss and micro-architectural deterioration. In effect, without prejudice to the importance of the contribution to bone resistance provided by mineralization, it is well known that the ability of bone to resist the applied loads also depends on the structural architecture of trabeculae, an aspect that the techniques currently available are not able to take into account (Liu X.S. et al., 2009). Estimating bone density alone is not, therefore, a parameter sufficient for a complete evaluation of the load bearing capability of this tissue, which, instead, requires also the consideration of the spatial arrangement of its trabecular component.

Micro-numerical models have already been developed to compute the elastic properties of 3D reconstructions of trabecular structures and to perform strength predictions. Nevertheless, a widespread clinical application of 3D methods seems unlikely in the next future, given the examination costs and computational requirements.

## **METHODS**

The approach followed in this work focuses on the characterization of the bone structure by numerical simulation, obtained from conventional radiographic images of suitable anatomical districts. In a few seconds, the test can quantify the possible pathological alterations of bone micro-architecture by means of a structural index (SI), which is calculated from the elastic response of the reconstructed structure and the normalized sum of gray tones, indicative of the mineralization in the region under examination (Cosmi, 2008).

## **RESULTS**

Here we present the results obtained by applying the test to a particular case of rapidly progressing osteoporosis, occurred in a Complex Regional Pain Syndrome (CRPS) of the hand. CRPS is a chronic progressive disease, formerly known as "reflex sympathetic dystrophy" and "causalgia" (now classified as CRPS type I and CRPS type II, respectively), whose pathophysiology is still currently poorly understood (de Mos et al., 2008).

## **CONCLUSIONS**

The results obtained show how the developed tool may be able to open important prospective applications for the study of bone pathologies (namely osteoporotic ones), in regards to the assessment of the illness course and the efficacy of potential therapies. Moreover, interesting possibilities of application exist also in the medical forensic field, where this new method can help the physician in the quantification of the biological damage.

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## **PERCUTANEOUS TECHNIQUE FOR INCISIONAL BIOPSY OF SOFT TISSUE TUMORS: PRELIMINARY EVALUATION OF AN ACCURATE ALTERNATIVE TO OPEN BIOPSY**

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**AIMS.** Biopsy is a crucial step in diagnosis of soft tissue tumors(STT). Current methods are open incisional or excisional biopsy, core-needle biopsy(CNB) and fine needle aspiration(FNA). Open biopsy is the gold standard: even if it requires an incision, an operative suite and general anesthesia, diagnostic accuracy is more than 90%. Complications as seroma, hematoma, infection, wound dehiscence occur more frequently after open biopsies (4%-19%), versus 1% of complication rate of percutaneous techniques. Recent literature suggests satisfactory results for CNB and FNA in terms of minimal morbidity, cost and time-consumption; however percutaneous techniques are associated to an acceptable but definitely lower rate of accuracy compared with open biopsy. The main problem is the lack of needle large enough to cut adequate tissue fragments and able to maintain the core inside during removal. The aim of the study is to describe an innovative procedure of percutaneous incisional biopsy for STT.

**METHODS.** Patients who had a palpable soft tissue mass were included in this prospective study conducted between January and May 2013. Any patient with a bony lesion without palpable mass, or who had undergone a recent biopsy, or had an inaccessible mass were excluded. Ultrasonography and MRI investigations were performed before the procedure to appropriately approach the mass and obtain the most vital tissue sample. The skin prepared in a sterile technique and local anesthetic injected subcutaneously, the mass was located by palpation and a stab wound was made with a n°11 surgical blade. The stab incision was placed such that it could be incorporated in subsequent definitive surgery. A sharp tip instrument inserted, through the incision to slightly open wide the access, then a closed rongeur was inserted, opened, the mass was bitten and the fragment pull out. Three passes were made through the mass in different directions depending on the quality of specimen obtained through the same incision. The specimens were immediately. An experienced pathologist was provided with all clinical and radiological details along with the biopsy specimen fixed in formalin. Diagnostic accuracy and complications were evaluated.

**RESULTS.** A total of 18 patients were biopsied during the study period with a mean age of 46 years (range 14-78). The most frequent location was the thigh (8=44,5%). Benign tumors were diagnosed in 11 cases, with lipoma being the most common histotype (4=22,2%), while 5 were malignant STTs (28%). Only 1/18 biopsied specimens were reported as inadequate; overall adequacy rate was 94%. Among 17 cases available, 16(94%) had true-positive result, 1(5,8%) case of false negative. The sensitivity and specificity of this technique in our series was 88,9% and 100%. No serious post-procedural complications occurred; difficulty was encountered only in biting with rongeur, without tearing off, some fragments from fibrous masses.

**CONCLUSIONS.** Diagnostic accuracy versus complications is the main concern in any STT biopsy. Percutaneous incisional biopsy with rongeur is a safe effective compromise solution between core needle and open biopsy. Although no complications are present in our series, further studies with larger series are necessary to evaluate potential bleeding and consequent major risk of contamination.

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## **CARBON FIBER-REINFORCED PEEK INTRAMEDULLARY NAILING FOR TREATMENT OF PATHOLOGIC FRACTURES DUE TO METASTASES: SHORT-TERM RESULTS**

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**AIMS.** Pathologic fractures of long bones due to metastases seriously affect the quality of life of cancer patients resulting in severe pain and disability. Locked intramedullary nailing is currently used in the treatment of pathologic fractures of femur, humerus and tibia when proximal or distal metaphyses or the shaft are mainly involved in patients with multiple metastases. The advantageous properties of carbon fiber reinforced polyetheretherketone (CF-PEEK) composites (CarboFix™) for use as orthopedic implants, such as nails or plates, include similar modulus to bone and ability to withstand prolonged fatigue strain. Moreover, the material makes nails and other devices radiolucent, which is beneficial during surgery and follow-up. This study aims to assess the use of a new CarboFix locked intramedullary nail to treat pathologic fractures of long bones. Although the results of the commercially available nail have been described in some case reports, short-terms results of a case series have not previously been presented.

**METHODS.** Five consecutive patients affected by long bone metastases were treated with CarboFix locked intramedullary nails (Piccolo Composite™, Unimedical): 3 for humeral and 2 for femoral pathologic fractures. All patients were female, presenting multiple visceral or bone metastatic lesions due to carcinomas (4) and myeloma (1). Three fractures were complete and 2 impending; 4 nails were inserted anterograde and 1 femoral nail was retrograde due to a previous prosthesis implantation. No cement augmentation was applied and adjuvant therapy was used in 3 patients. Surgical complications, clinical and functional outcomes were evaluated. Follow up was performed with radiograph, CT or MRI images. Average follow up was about 3 months (range 1-6).

**RESULTS.** No failure fixation nor hardware breakage occurred. Radiopaque markers around the drill holes and along the nails' length axis facilitated positioning and drilling. Nails were available in different humeral and femoral diameters, respectively from 8 and 10 mm which is suitable for female sex, and in different lengths. One patient suffered from non-fatal deep venous thrombosis post-surgery. Wound infection occurred in 1 patient treated for humeral fracture. All patients attained satisfactory pain relief and functional improvement during the first 2-4 postoperative months. Two patients died within 2 postoperative months due to cancer progression. At last follow up there were no local progression of metastasis requiring surgical revision. Avoiding artifacts at CT or MRI imaging, the system revealed to improve the accuracy of follow up and the definition of target volume and dose distribution of adjuvant radiotherapy.

**CONCLUSIONS.** It is very well known that nailing fixation provides pain relief, early return of function, and easier nursing care in advanced cancer patients with long bones metastases. As more patients with skeletal metastases live longer, further efforts are required to counteract the increasing risk for implant failure. Although the smallness of this series, CarboFix locked nail shows satisfactory preliminary mechanical and clinical results, provided the appropriateness of surgical indications in pathologic fractures. CarboFix offers a valuable advantage in identification of target volume in patients who need radiotherapy. Improvement of the system in terms of recon nailing and entire bone length stabilizations is necessary.

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## **PATIENT-SPECIFIC TEMPLATES FOR PEDICLE SPINE SCREWS PLACEMENT: LITERATURE REVIEW AND PROPOSAL OF A NEW DESIGN**

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### **AIMS**

Pedicle screws fixation to stabilize lumbar spinal fusion has become the gold standard for posterior stabilization. However their positioning remain difficult due to variation in anatomical shape, dimensions and orientation, which can determine the inefficacy of treatment or severe damages to close neurologic structures. Image guided navigation allows to drastically decrease errors in screw placement but it is used only by few surgeons due to its cost and troubles related to its using, like the need of a localizer in the surgical scenario and the need of a registration procedure. An alternative image guided approach, less expensive and less complex, is the using of patient specific templates similar to the ones used for dental implants or knee prosthesis.

### **METHODS**

Like proposed by other authors we decided to design the templates using CT scans. (slice thickness of 2.0 mm). Template developing is done, for each vertebra, using a modified version of ITK-SNAP 1.5 segmentation software, which allow to insert cylinders (full or empty) in the segmented images. At first we segment the spine bone and then the surgeon chose screw axes using the same software. We design each template with two hollow cylinders aligned with the axes, to guide the insertion in the pedicle, adding contact points that fit on the vertebra, to obtain a template right positioning. Finally we realize the templates in ABS using rapid prototyping. After same in-vitro tests, using a synthetic spine (by Sawbones), we studied a solution to guarantee template stability with simple positioning and minimizing intervention invasiveness. Preliminary ex-vivo animal testing on 2 porcine specimens has been conducted to evaluate template performance in presence of soft-tissue in place, simulating dissection and vertebra exposure. The spine has been scanned with CT, we realized the a total of 14 templates and we performed the insertion of 28 Kirschner wires. For verification, the surgeon examined post-operative CT-scans to evaluate Kirschner wires positioning.

### **RESULTS**

During the ex-vivo animal test sessions, template alignment resulted easy thanks to the spinous process contact point. Their insertion required no additional tissue removal respect to the traditional approach. The positioning of contact points on vertebra's lamina and articular processes required just to shift the soft tissue under the cylinders bases. The surgeon in some cases evaluated false stable template positions since not each of the 4 contact points were actually in contact with the bone surface and tried the right position. CT evaluation demonstrate that 1 Kirschner wire (3.5%) has been wrongly positioned (grade II pedicle cortex violation).

### **CONCLUSIONS**

Our approach allows to obtain patient specific templates that does not require the complete removal of soft tissue around vertebra. Guide positioning is facilitated thanks to the using of the spinous processes contact point, while false stable positions can be avoided using four redundant contact points. The templates can be used to guide the drill or the insertion of Kirschner wires and screws. Results obtained during our preliminary ex-vivo animal tests encourage further studies comprising cadaver or in-vivo human evaluation.

## **PLEXUR M™ IN RECONSTRUCTION OF SUBCHONDRAL BONE: A NEW PERSPECTIVE IN ONCOLOGICAL ORTHOPEDICS**

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### **AIMS**

Several bones substituted are commercially available in literature, yet none of these are suitably moldable to repair or be placed in the bone defect to reconstruct the subchondral bone after curettage of benign tumors located in the epiphysis. Our aim is to verify the application of this new engineered biomaterial bone to adapt and support articular cartilage.

### **METHODS**

We enrolled the first ten consecutive cases referred to our department, affected by a benign epiphyseal tumor destroying the subchondral bone till articular cartilage. Every patient underwent curettage of the disease, apposition of a new-engineered biomaterial bone and filling with homoplastic morselized bone.

### **RESULTS**

In seven out of eight cases of lesions located in the lower limbs the quality of reconstruction was considered good, restoring an adequate support to the articular cartilage. The quality of the remaining case was considered poor probably due to the extent of the spread of the disease which destroyed the entire proximal tibial epiphysis. In the two cases located in the upper limbs, the PM application restored support to the articular cartilage sufficiently well. However, unfortunately in a case of giant cell tumor of the distal radial epiphysis there was a slight reabsorption of the morselized homoplastic bone.

### **CONCLUSIONS**

PM is a valid option for the orthopedic surgeon in restoring adequate mechanical support to the articular cartilage; nevertheless further studies are necessary to verify the integration process and the effects on the survival of cartilage damaged by the disease.

## **LA PROTESI MUTARS X-PAND NEL TRATTAMENTO DELLE NEOPLASIE PRIMITIVE MALIGNI: TECNICA CHIRURGICA E CONSIDERAZIONI SUL MECCANISMO DI ALLUNGAMENTO**

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### Scopo

Le moderne tecniche di "limb salvage" in pazienti affetti da neoplasie maligne primitive dell'osso hanno posto nuove sfide riguardo le tecniche di ricostruzione. Gli autori presentano una breve storia dello sviluppo delle protesi di accrescimento, discutendone le complicanze chirurgiche, fino alle più moderne tecniche di impianto.

### Materiali e Metodi

Viene presentato un caso clinico di Osteosarcoma del femore distale in un paziente di 11 anni, sottoposto a resezione intra-articolare, dopo ciclo di chemioterapia neoadiuvante, ed un caso clinico di Condrosarcoma di II° del femore distale in una donna di 50 anni andata incontro ad un ritorno di malattia con lo stesso istotipo dopo 20 anni; in entrambi è stata effettuata una ricostruzione con Protesi Mutars X-pand. Si discutono i principi chirurgici di scelta di resezione e le caratteristiche ingegneristiche dell'impianto protesico.

### Risultati

Entrambi i pazienti, ad un follow-up di 1 anno, presentano un recupero della funzione dell'arto, riuscendo a deambulare anche con un solo bastone e presentando al termine una eterometria residua < ai 3 cm. Si premette che nel soggetto adulto l'impiego di tale tipo di protesi è giustificato solo dalla presenza di dismetrie > ai 5 cm.

L'impiego di protesi espandibili con meccanismo magnetico di trasmissione sottocutanea rappresenta la migliore soluzione protesica attualmente disponibile nella ricostruzione dopo resezione di tumori ossei maligni primitivi nei soggetti in accrescimento e, a conoscenza degli Autori, l'impiego di tale protesi non è riportato in letteratura per pazienti in età adulta. L'elevato costo di questa tecnologia riduce il numero dei pazienti che possono beneficiarne; tale chirurgia presenta una curva di apprendimento particolarmente lunga e necessità di chirurghi ortopedici oncologi di provata esperienza.

## **INTRA-ARTICULAR INJECTIONS FOR DEGENERATIVE CARTILAGE LESIONS OF THE KNEE: PLATELET RICH PLASMA VS HYALURONIC ACID**

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### **AIMS**

In recent years the conservative management of degenerative cartilage lesions has received particular attention. Hyaluronic Acid is a widely used product, but newer solutions have been introduced in this field. Platelet Rich Plasma represents a promising method to manage degenerative cartilage lesion, despite its mechanism of action is still troublesome, its positive effects is encouraging. This study aims to compare clinical outcomes in patients undergoing injections of Hyaluronic Acid and Platelet Rich Plasma to the knee for treatment of degenerative chondral lesion.

### **METHODS**

One hundred and fifty (73 men and 77 women, mean age 55.6) patients with clinical and radiographic evidence of degenerative changes to the knee were enrolled. The lesions were classified according to the Kellgren-Lawrence radiographic system. Patients were randomized into 3 groups: 50 patients (Group A) received 3 intra-articular injections of 5.5 mL PRP "Regen", 50 patients (Group B) received 3 injections of 5.5 mL "Home made" PRP, and 50 patients (Group C) underwent 4 injections of Hyaluronic Acid. IKDC, KOOS and VAS score were administered to all patients before starting the treatment, at 1, 6 and 12 months from the end of the management.

### **RESULTS**

At 1 month and 6 months, PRP Regen Lab provided the best outcomes. Patients underwent home made PRP administration reported significant higher score than those undergoing hyaluronan injections ( $<0.005$ ), but lesser than PRP Regen Lab since the 6th post-injection month.

### **CONCLUSION**

The overall cohort improved significantly from the pre- to the post-treatment time, but PRP is markedly more effective than hyaluronic acid. In particular, Regen Lab PRP injections provided significantly better outcomes than home made PRP.

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# **COMPARISON OF CLINICAL RESULTS BETWEEN MICROFRACTURE TECHNIQUE WITH THE USE OF PRF INTRAOPERATIVE INJECTION, PRP POSTOPERATIVE INJECTION AND MICROFRACTURE ONLY FOR OSTEOCHONDRAL LESION OF THE KNEE – A PRELIMINARY STUDY**

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## **AIMS**

Cartilage lesions are the most common cause of chronic knee pain. The current treatment options consist in conservative strategies, such as viscosupplementation and platelet-rich plasma injections, and surgical management including debridement of the chondral lesion, bone marrow stimulation with microfractures and osteochondral transplant. In this study we combined arthroscopic microfracturing with platelet concentrates, Platelet-rich Plasma (PRP) and "Vivostat Platelet-Rich Fibrin" (PRF).

## **METHODS**

We conducted a prospective trial on 51 patients divided into 3 groups with clinical and radiological evidence of cartilage lesions. All patients underwent a knee arthroscopy with microfracture. The platelet concentrate was administered intra-operatively to the "PRF Group" and postoperatively to the "PRP Group", as a cycle of 3 intra-articular injection. Finally, the "Microfractures Group" underwent only microfractures (control group). Clinical Scores (IKDC, KOOS, VAS) have been administered at 6 months postoperative.

## **RESULTS**

All patients achieved a statistically significant clinical improvement from preoperative to postoperative time. In particular, patients who were treated with platelet concentrates achieved better clinical results compared to patients treated with microfracture only ( $P < 0.0001$ ). Comparing "PRF Group" and "PRP Group" patients, the first group showed a significant increase compared to the second of the IKDC scores ( $P 0.0349$ ), KOOS ( $P 0.0003$ ), VAS ( $P 0.0023$ ) at last follow-up. From a radiological point of view the "PRF Group" obtained better results earlier than the other two groups.

## **CONCLUSIONS**

The application of intra-operative platelet concentrate associated with microfractures has led to significant improvement 6 months postoperatively both clinically and radiologically. The rationale is that a greater concentration of platelets and growth factors leads to a greater stimulation of the bone marrow and a earlier formation of repair tissue. Comparing "Vivostat PRF" application with PRP injections a more rapid and less painful recovery is evident. However, the repair tissue does not have the same characteristics of the articular cartilage. Studies of histology and microscopic anatomy are required to assess the quality of this tissue to define its characteristics.

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Mechanism of action and in vivo role of platelet-derived growth factor. Heldin, C. H. and B. Westermark. 4, 1999, Physiol Rev, Vol. 79, p. 1283-1316.

## THE CLINICAL AND SCIENTIFIC "FRIENDSHIP" BETWEEN ORTHOPAEDICS AND PLASTICS

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Orthopaedic and plastic surgery are similar disciplines. Both specialties have the entire body as a field of interest. They are craft-based and technique-dependent.

Orthopaedic surgery has a longer history than plastic surgery, whose major modern development is in coincidence with the World Wars.

These two surgical specialties interact with each other in many areas of medical and surgical care and in several parts of the body.

In addition, the superficial soft tissues, main area of plastic surgical competence, represent the biological soil for the bone, therefore bone and soft tissues need to be treated together when lesions involve both.

Hand surgery is the most obvious multidisciplinary orthoplastic area, due to the unique, tight and inseparable relationship between musculoskeletal frame and integument. Open fractures are another typical example of lesions requiring multidisciplinary orthoplastic treatment, as well as hand deformities, hand trauma and oncology, peripheral nerve lesions, diabetic foot, ischaemic lesions of the bone.

Several scientific evidences proved the importance of the union between bone and soft tissues in the healing processes from various types of lesions.

Plastic and orthopaedic surgery can achieve important results from a coordinated collaboration in both clinical and scientific sectors.