

# **3<sup>rd</sup> 3B's Symposium on *Biomaterials and Stem Cells in Regenerative Medicine***

3B's Research Group Auditorium– AvePark, Caldas das  
Taipas, Guimarães, Portugal  
Date: 22 May, 2013

## Program

### 3rd 3B's symposium on *biomaterials and stem cells in regenerative medicine*

Date: 22 May, 2013

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**Chairmen: João F. Mano and Rui L. Reis** – 3B's Research Group, University of Minho, Portugal

#### Program

|   |  |                                   |
|---|--|-----------------------------------|
| 9:00-09:10  | Welcome  | <i>João F. Mano / Rui L. Reis</i> |
| <b>Stem cells and biological aspects in regenerative medicine (Chair: Rui L. Reis)</b>              |  |                                   |
| 9:10-9:35   | Adult stem cells for TERM: sources and manipulation                    | <i>Ana Rita Costa-Pinto</i>       |
| 9:35-10:00  | Induced pluripotent stem cells   | <i>Ana M. Martins</i>             |
| 10:00-10:25   | Vascularization strategies in Regenerative Medicine                    | <i>Rogério P. Pirraco</i>         |
| 10:25-11:00   | Keynote Lecture: Extracellular matrix-driven tissue regeneration       | <i>Alexandra P. Marques</i>       |
| BREAK   |  |                                   |
| <b>Biomaterials: from <i>nano</i> to <i>macro</i> (Chair: Ricardo A. Pires)</b>                     |  |                                   |
| 11:20-11:45   | Polymeric biomaterials from marine-origin                              | <i>Anabela Alves</i>              |
| 11:45-12:10   | Shaping biomaterials into porous 3D constructs                         | <i>Ana R. Duarte</i>              |
| 12:10-12:35   | Nanobiomaterials in tissue engineering                                 | <i>Albino Martins</i>             |
| LUNCH BREAK   |  |                                   |
| 14:00-14:25   | What is common between pintarolas and cells?                           | <i>Iva Pashkuleva</i>             |
| 14:25-14:50   | Shaping biomaterials into spherical objects                            | <i>Clara R. Correia</i>           |
| 14:50-15:15   | Combinatorial analysis of biomaterials for Tissue Engineering          | <i>Mariana B. Oliveira</i>        |
| BREAK   |  |                                   |
| <b>Using natural-based biomaterials in case studies of Tissue Engineering (Chair: João F. Mano)</b> |  |                                   |
| 15:40-16:00   | Regeneration strategies in the Central Nervous System                  | <i>Susana R. Cerqueira</i>        |
| 16:00-16:20   | Skin tissue engineering  | <i>Mariana Cerqueira</i>          |
| 16:20-16:40   | Cartilage tissue engineering   | <i>Marta L. Silva</i>             |
| 16:40-17:00   | Regeneration of the intervertebral disk                                | <i>Joana S. Correia</i>           |
| 17:00-17:20   | Strategies for the regeneration of the tendon                          | <i>Márcia Rodrigues</i>           |
| 17:20-17:40   | Sports & regenerative medicine   | <i>Hélder Pereira</i>             |
| <b>Creative thinking (Chair: Nuno M. Neves)</b>   |  |                                   |
| 17:40-18:15   | Keynote Lecture: 3C's - Cultura, Ciência e Criatividade em gastronomia | <i>Renato Cunha</i>               |

## ADULT STEM CELLS FOR TERM: SOURCES AND MANIPULATION

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The body comprises different types of progenitor cells (stem cells) capable of giving rise to cells with more restricted developmental potential. Stem cells (SCs) occur as unspecialized cells, lacking tissue specific characteristics, but maintaining undifferentiated phenotype until they are exposed to appropriate signals. SCs have capacity for extensive self-renewal, and apparently maintain themselves throughout the entire life of an organism. Under the influence of specific signals, SCs can differentiate into specialized cells of different lineages. SCs include embryonic stem cells (ESCs), isolated from the inner cell mass of the blastocyst [1], and mesenchymal stromal cells (MSCs), isolated from fetal and adult tissues.

Adult MSCs are multipotent cells isolated from different tissues for example, adipose tissue [2] and bone marrow, among others. These cells can also be isolated from extra-embryonic tissues, including placenta, amniotic fluid, and umbilical cord [3].

The International Society for Cellular Therapy (ISCT) stated that MSCs are characterized by: plastic adherence to tissue culture flasks; 95% of the MSC population must express the surface markers CD105, CD73 and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II; Finally, the cells must differentiate in vitro into osteoblasts, adipocytes and chondrocytes under standard differentiating conditions [4]. Another important characteristic is the colony forming unity fibroblasts (CFU-Fs) assays to assess the clonogenicity of MSCs i.e., the ability of a cell to grow in a density-insensitive fashion [5].

Autologous approaches to use MSCs, namely from bone marrow, have difficulties regarding the limited availability of cells from the patient. Cell expansion protocols are based on the use of media supplemented with fetal bovine serum (FBS) as a source of

nutrientes and growth factors. The animal serum is not completely safe, once there is a possibility of contamination by animal viruses, prions or other contaminants and it is described that FBS used systematically in MSCs subcultivation induces more humoral immune response [6]. Platelet lysate (PL) has enormous possibilities in cell therapy, namely because of the high concentration of growth factors that promotes higher cell expansion, such as tissue regeneration [7].

### References:

1. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *Journal of Cellular Physiology* 1999; 181(1):67-73.
2. Gimble JM, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy* 2003; 5(5):362-369.
3. Secco M ZE, Vieira NM, Fogaça LL, Cerqueira A, Carvalho MD, Jazedje T, Okamoto OK, Muotri AR, Zatz M. Mesenchymal stem cells from umbilical cord: do not discard the cord! *Neuromuscul Disord* 2008; 18(1):17-18.
4. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8(4):315-317.
5. Castro-Malaspina H, Gay R, Resnick G, Kapoor N, Meyers P, Chiarieri D, et al. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood* 1980; 56(2):289-301.
6. Bernardo ME, Avanzini MA, Ciccocioppo R, Perotti C, Cometa AM, Moretta A, et al. Phenotypical/functional characterization of in vitro-expanded mesenchymal stromal cells from patients with Crohn's disease. *Cytotherapy* 2009; 11(7):825-836.
7. Anitua E, Sanchez M, Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. *Advanced Drug Delivery Reviews* 2010; 62(7):741-752.

## INDUCED PLURIPOTENT STEM (IPS) CELLS IN CARDIAC RESEARCH: WHERE WE STARTED, WHERE WE ARE NOW AND WHERE WE ARE GOING?

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The first study reported by Yamanaka et al. (1) in 2006, showed that fully differentiated somatic cells (e.g. fibroblasts) could be reprogrammed to generate cells similar to embryonic stem (ES) cells with the introduction of four genes (Oct 3/4, Sox2, Klf4, and c-Myc) expressing transcription factors through retroviral transduction. These cells induced to embryonic-stem-cell-like cells are called induced pluripotent stem (iPS) cells. The main advantages of iPS cells are the obvious edge of not having to be derived from human embryos, and may also enable scientists to sidestep other controversial methods, notably somatic cell nuclear transfer (cloning). Also, iPS cells derived from the patient, and it allows the creation of cell lines that are genetically customized to the same individual. There are no immunological compatibility problem, circumventing the important issue of tissue rejection associated with transplantation and allowing a patient-specific therapy (2). Several studies using iPS cells have been shown to differentiate into cells of the cardiovascular lineages (3, 4), and no significant differences in the characteristics of cardiomyocytes generated from ES cells or iPS cells were found (5). Since iPS cells are obtained from patient's own tissue represents a unique and individual in vitro testing, opening a new era of "personalized medicine". Advances made in the generation of iPS cell-based therapies can serve as platform to perform drug screenings with the aim of developing cell-based therapies against cardiovascular diseases.

## References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug 25;126(4):663-76.
2. Egashira T, Yuasa S, Fukuda K. Induced pluripotent stem cells in cardiovascular medicine. *Stem Cells Int*. 2011;2011:348960.
3. Mauritz C, Schwanke K, Reppel M, Neef S, Katsirntaki K, Maier LS, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation*. 2008 Jul 29;118(5):507-17.
4. Zhang JH, Wilson GF, Soerens AG, Koonce CH, Yu JY, Palecek SP, et al. Functional Cardiomyocytes Derived From Human Induced Pluripotent Stem Cells. *Circ Res*. 2009 Feb 27;104(4):E30-E41.
5. van Laake LW, Qian L, Cheng P, Huang Y, Hsiao EC, Conklin BR, et al. Reporter-Based Isolation of Induced Pluripotent Stem Cell- and Embryonic Stem Cell-Derived Cardiac Progenitors Reveals Limited Gene Expression Variance. *Circ Res*. 2010 Aug 6;107(3):340-7.

## VASCULARIZATION STRATEGIES IN REGENERATIVE MEDICINE

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A comprehensive network of blood vessels is responsible for the critical role of transporting gases, nutrients and other molecules to and from most organs and tissues in the human body. Without such a system, tissue thickness would be restricted to 100 - 200  $\mu\text{m}$ , the diffusion limit of oxygen[1]. Blood vessel formation can occur by two processes, de novo (vasculogenesis) and from preexisting vessels (angiogenesis). During embryonic development, the first blood vessels form from progenitor cells through vasculogenesis while angiogenesis is the main process responsible for post-natal vessel formation[2]. Any defect or problem affecting the blood supply to a given organ (ischaemia) can result in cellular death and, ultimately, loss of function. It is therefore of the utmost importance to develop adequate Regenerative Medicine strategies to address such issues, both in the context of tissue regeneration in general and in the context of tissue engineering. Recent approaches for the regeneration of ischemic tissues are based on boosting endogenous angiogenesis by growth factor or cell delivery. However, the lack of an adequate cell source [3] and the complexity of growth factor response [4] pose great challenges necessary to be overcome. In the case of tissue engineering applications, inadequate vascularization of the engineered constructs lead to cell death at the bulk of the scaffolds, implant failure and, ultimately, rejection[5]. The strategies researchers have used to deal with these issues often involve the use of angiogenic growth factors and scaffold design to promote vessel ingrowth[6], endothelial cells to prevascularize the constructs[7] and combinations of both [8]. The pitfalls of such strategies are deeply related with inadequate cell type used or limited control of growth factor delivery strategies[9]. Therefore, novel strategies encompassing an adequate cell source for neo-vascularization as well as

deeper knowledge regarding the controlled release of angiogenic factors will be required for successful Regenerative Medicine and Tissue Engineering Strategies.

## References

1. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249-57.
2. Patan S. Vasculogenesis and Angiogenesis as Mechanisms of Vascular Network Formation, Growth and Remodeling. *J Neurooncol* 2000;50:1-15.
3. Deb A, Patterson C. Hard Luck Stories: The Reality of Endothelial Progenitor Cells Continues to Fall Short of the Promise. *Circulation* 2010;121:850-2.
4. Cooper LT, Hiatt WR, Creager MA, Regensteiner JG, Casscells W, Isner JM, et al. Proteinuria in a placebo-controlled study of basic fibroblast growth factor for intermittent claudication. *Vascular Medicine* 2001;6:235-9.
5. Pirraco RP, Marques AP, Reis RL. Cell interactions in bone tissue engineering. *Journal of Cellular and Molecular Medicine* 2010;14:93-102.
6. Muller D, Chim H, Bader A, Whiteman M, Schantz J-T. Vascular Guidance: Microstructural Scaffold Patterning for Inductive Neovascularization. *Stem cells international* 2011;2011.
7. Unger RE, Ghanaati S, Orth C, Sartoris A, Barbeck M, Halstenberg S, et al. The rapid anastomosis between prevascularized networks on silk fibroin scaffolds generated in vitro with cocultures of human microvascular endothelial and osteoblast cells and the host vasculature. *Biomaterials* 2010;31:6959-67.
8. Dawood AF, Lotfi P, Dash SN, Kona SK, Nguyen KT, Romero-Ortega M. VEGF Release in Multiluminal Hydrogels Directs Angiogenesis from Adult Vasculature In Vitro. *Cardiovasc Eng Tech* 2011;2:173-85.
9. Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. *Advanced Drug Delivery Reviews* 2011;63:300-11.

## POLYMERIC BIOMATERIALS FROM MARINE-ORIGIN

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Biomaterials can assume a pivotal role in diverse strategies aimed at restoring and sustaining normal tissue function. In this context, they are usually incorporated and conjugated with diverse factors into the design of medical structures. The choice of the appropriate material to be used becomes critical, and can contribute to the holistic failure of the intended structure in its final application. The library of available (raw) biomaterials is vast and this choice is dictated by numerous requirements that need to be addressed for the right control over the natural niche tissue environment.

In this regard, marine biological materials are being prized for their uniqueness. In fact, marine-origin organisms (including vertebrates, invertebrates, plants, algae or bacteria) can be regarded as a wealthy and considerably untapped reserve of valuable molecules with special importance, which inspire their exploitation in a biomedical context.

Diverse biopolymers and inorganic materials originating from various marine organisms are establishing their value within this field [1][2]. Chitin and its derivative chitosan [3][4], alginate [5], carrageenan [6] or ulvan [7] are remarkable examples. Marine origin collagen [8] or glycosaminoglycans (sulphated or not) [9] are also the focus of intense research. These can demonstrate the distinctiveness of the biological materials originating from the sea, but represent only a fraction of the available materials.

The richness and availability of marine biological materials allied with sustainable exploitation constitutes a highly attractive and strategic platform for the development of novel biomaterials, both with socio-economic and environmental benefits.

Within the highly translational field of regenerative medicine, marine-origin biopolymers are receiving considerable interest and we hypothesize and may foresee the significant impact that these materials will have in this field and related applications.

## References

1. Silva TH, Alves A, Ferreira BM, Oliveira JM, Reys LL, Ferreira RJF, et al. Materials of marine origin: a review on polymers and ceramics of biomedical interest. *International Materials Reviews* 2012;57:276–306.
2. Silva TH, Alves A, Popa EG, Reys LL, Gomes ME, Sousa R a, et al. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. *Biomatter* 2012;2:278–89.
3. Custódio CA, Frias AM, Del Campo A, Reis RL, Mano JF. Selective cell recruitment and spatially controlled cell attachment on instructive chitosan surfaces functionalized with antibodies. *Biointerphases* 2012;7:65.
4. Costa-Pinto AR, Correlo VM, Sol PC, Bhattacharya M, Srouji S, Livne E, et al. Chitosan–poly (butylene succinate) scaffolds and human bone marrow stromal cells induce bone repair in a mouse calvaria model. *Journal of Tissue Engineering and Regenerative Medicine* 2012:21–8.
5. Correia CR, Reis RL, Mano JF. Multilayered hierarchical capsules providing cell adhesion sites. *Biomacromolecules* 2013;14:1250.
6. Popa EG, Gomes ME, Reis RL. Cell delivery systems using alginate-carrageenan hydrogel beads and fibers for regenerative medicine applications. *Biomacromolecules* 2011;12:3952–61.
7. Alves A, Sousa RA, Reis RL. A practical perspective on ulvan extracted from green algae. *Journal of Applied Phycology* 2012;25:407–24.
8. Tillet E, Franc JM, Franc S, Garrone R. The evolution of fibrillar collagens: a sea-pen collagen shares common features with vertebrate type V collagen. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology* 1996;113:239–46.
9. Luppi E, Cesaretti M, Volpi N. Purification and characterization of heparin from the Italian clam *Callista chione*. *Biomacromolecules* 2005;6:1672–8.

## WHAT IS COMMON BETWEEN PINTAROLAS AND CELLS

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Similar to the Pintarolas the surface of virtually every living cell is decorated with a layer of sugars called glycocalyx. Like the candy shell of Pintarolas, which preserves the chocolate fillings from melting, the cellular glycocalyx keeps the cell entire - it has a structural role. However, it also has other biological roles that are crucial for the development, growth, function or survival of an organism: blood transfusion, virus infections or cancer development are all related with the structure and quantity of the sugars on the cell surface. Evidences that remodelling of glycocalyx is an integral part of diseases progression have led to speculation that sugars can be used as key diagnostic and prognostic indicators as well as therapeutic targets of interest. The lecture will give brief overview on the recent developments and utility of different biomedical tools based on the carbohydrates. Vaccines, arrays, biosensors, imaging agents are some of the examples that are going to be presented and discussed during the talk.

### References

1. Varki A et al, Essentials of Glycobiology, Cold Spring Harbor Laboratory Press (USA), 2nd edition.
2. Astronomo RD and Burton DR, Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov (2010) 9: 308-324.
3. Fuster MM and Esko JD, The sweet and sour of cancer: Glycans as novel therapeutic targets, Nat Rev Cancer (2005) 2:526-542

4. Hsu KL and Mahal LK, Sweet tasting chips: microarray-based analysis of glycans, *Curr Opin Chem Biology* (2009) 13:427-432.
5. Pashkuleva I and Reis RL, Sugars: burden or biomaterials of the future? *J Mat Chem* (2010) 20: 8803-8818.

## NANOBIOMATERIALS IN TISSUE ENGINEERING

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Nanobiomaterials are defined as materials with building block size scales (e.g. grains, particles, fibers, tubes, etc.) less than 100 nm. Nanomaterials possess numerous unique properties such as large specific surface area, enhanced mechanical properties, and exceptional magnetic, optical and electrical properties [1]. The herein presented contributions comprise new nanobiomaterial designs to develop highly functional scaffolds for TERM applications.

The fibrous nature of the natural extracellular matrix ECM has led researchers to focus on the development of fiber-based scaffolds for Tissue Engineering and Regenerative Medicine strategies. Electrospinning has emerged as a very promising technology enabling to produce synthetic polymeric ultrafine fibers [2]. Despite the claimed similarity to the morphology of natural ECM, the surface chemical properties of electrospun nanofibers must be optimized for the intended application. It was shown that defined plasma treatments enable improving the proliferation of different cell types (fibroblastic, chondrogenic and osteogenic) when culture in the optimized surfaces [3].

The chemical and topographical properties of the scaffolds should provide an appropriated environment for tissue development, allowing also for the incorporation of biological signals to enhance tissue formation. Therefore, electrospun fibrous structures were also proposed as drug release systems of an established osteogenic differentiation agent (i.e. dexamethasone) of human bone marrow mesenchymal stem cells (hBMSCs) [4]. The phenotypic and genotypic expression of osteoblastic markers confirmed the osteogenic inducing potential of the loaded growth/differentiation factor.

The complex ordered organization of the natural ECM is not usually well replicated in the typical random alignment of electrospun structure. Therefore, complex ordered microporous fibrous structure were developed, designated as patterned nanofiber meshes, composed of both random/orthogonal and parallel/uniaxial aligned fibers [5]. Those patterned nanofiber meshes, not only induced hBMSCs guidance at the early culture periods, but also influence the cell ECM deposition along the predefined fiber direction.

### References

1. Yang L, Zhang L, Webster TJ. Nanobiomaterials: State of the Art and Future Trends. *Advanced Engineering Materials* 2011, 13(6):B197-B217.
2. Martins A, Araujo JV, Reis RL, Neves NM. Electrospun nanostructured scaffolds for tissue engineering applications. *Nanomedicine* 2007, 2(6):929-942.
3. Martins A, Pinho ED, Faria S, Pashkuleva I, Marques AP, Reis RL, et al. Surface modification of electrospun polycaprolactone nanofiber meshes by plasma treatment to enhance biological performance. *Small* 2009, 5(10):1195-1206.
4. Martins A, Duarte AR, Faria S, Marques AP, Reis RL, Neves NM. Osteogenic induction of hBMSCs by electrospun scaffolds with dexamethasone release functionality. *Biomaterials* 2010, 31(22):5875-5885.
5. Martins A, da Silva MLA, Faria S, Marques AP, Reis RL, Neves NM. The Influence of Patterned Nanofiber Meshes on Human Mesenchymal Stem Cell Osteogenesis. *Macromolecular Bioscience* 2011, 11(7):978-987.

## SHAPING BIOMATERIALS INTO POROUS 3D CONSTRUCTS

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One of the most important stages of tissue engineering is the design and development of a porous 3D construct. Ideal scaffolds should be biocompatible, biodegradable and promote cellular interactions and tissue development and possess proper mechanical and physical properties. In terms of morphological properties the scaffolds should present high porosity, with interconnected and adequate mean pore size for cell growth and proliferation and vascularisation. A variety of different processing techniques have been developed and include fibre bonding, freeze drying, solvent casting and particle leaching, wet spinning, particle aggregation, electrospinning, 3D potting and supercritical fluid technology among others.[1-3] The choice of the most suitable polymer processing technique depends greatly on the characteristics of the polymer itself, particularly their solubility in aqueous or organic solutions and their thermal properties as these will ultimately determine the feasibility to successfully produce matrices with the desired features.[4] The emerging next generation of engineered structures requires the incorporation of bioactive molecules able to create an environment for cellular function, for example proliferation and differentiation factors or to interact with the contact tissues, such as anti-inflammatory agents or antibiotics.[5] Processing thermosensitive bioactive compounds requires, however, the use of mild processing conditions and the reduction of the amount of organic solvents used. This presents an increase challenge in materials processing for tissue engineering and regenerative medicine. The integration of different technologies could provide interesting developments in shaping biomaterials into 3D porous constructs, opening a wide range of opportunities for the preparation of enhanced materials as structural

supports for tissue development. In this communication the major advantages and limitations of different processing technologies will be presented and the perspectives of future applications will be discussed.

## References

1. Gomes ME, PB M, RL R. Methodologies for processing biodegradable and natural origin hybrid scaffolds for bone and cartilage tissue engineering applications. In: Hollander A, editor. In Biopolymer Methods in Tissue Engineering - Methods in Molecular Biology Series. Totowa: The Humana Press Inc, 2003. p.65.
2. Duarte ARC, Mano JF, Reis RL. Supercritical fluids in biomedical and tissue engineering applications: a review. International Materials Reviews 2009;54:214.
3. Martins A, Reis RL, Neves NM. Electrospinning: processing technique for tissue engineering scaffolding. International Materials Reviews 2008;53:257.
4. Mano JF, Silva GA, Azevedo HS, Malafaya PB, Sousa RA, Silva SS, Boesel LF, Oliveira JM, Santos TC, Marques AP, Neves NM, Reis RL. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. Journal of the Royal Society Interface 2007;4:999.
5. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Advanced Drug Delivery Reviews 2007;59:207.

## SHAPING BIOMATERIALS INTO SPHERICAL OBJECTS

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The aim of cell encapsulation technique is to physically isolate a cell mass from an outside environment within the confines of a membrane. The membrane is engineered to have a selective permeability, by controlling the exchange of nutrients, oxygen, metabolites and waste products, while avoiding the entrance of high molecular weight immune system components, such as immunoglobulins and immune cells [1]. Particularly, polymeric multilayer capsules (PMLCs) produced by the layer-by-layer (LbL) technique have emerged as a promising source of transplantable materials for bioencapsulation strategies [2]. This method is based on the sequential adsorption of layers of oppositely charged macromolecules onto a sacrificial template. Subsequently, the sacrificial core can be dissolved or eliminated originating liquified or hollow capsules, respectively. This powerful assembly process allows tailoring the size, morphology, and membrane thickness of capsules, and also their composition, permeability, and surface functionality. Capsules have been widely used particularly in cell encapsulation systems, but also have found great applicability in drug delivery or screening systems, biosensors, catalysis, reactors, immunoisolation, and medical image due to their versatile wall functions, capability to load active substances, and unique permeability [3-6]. Recently, we reported the production of liquified capsules featuring (i) an external LbL membrane, and encapsulating (ii) surface functionalized poly(L-lactic acid) microparticles [7,8]. We hypothesize that, while the liquified environment enhances the diffusion of essential molecules for cell survival, microparticles dispersed in the liquified core of capsules provide the physical support required for cellular functions of anchorage-dependent cells. Results show that

capsules containing microparticles revealed an enhanced biological outcome in cell metabolic activity and proliferation, suggesting their potential to boost the development of innovative biomaterials designs for bioencapsulation systems and tissue engineering products.

## References

1. Hernández RM, Orive G, Murua A, Pedraz JL. Microcapsules and microcarriers for in situ cell delivery. *Adv. Drug Deliv. Rev.* 2010;62:711-730.
2. van Dongen SFM, Verdurmen WPR, Peters RJRW, Nolte RJM, Brock R, van Hes JCM. Cellular Integration of an Enzyme-Loaded Polymersome Nanoreactor. *Angew. Chem. Int. Ed. Engl.* 49(40):7213-7216.
3. Kim J, Arifin DR, Muja N, Kim T, Gilad AA, Kim H, Arepalley A, Hyeon T, Bulte JWM. Multifunctional Capsule-in-Capsules for Immunoprotection and Trimodal Imaging. *Angew. Chem.* 2011;23:2365-2369.
4. Szarpak A, Cui D, Dubreuil F, de Geest BG, de Cock LJ, Picart C, Auzély-Velty R. Designing Hyaluronic Acid-Based Layer-by-Layer Capsules as a Carrier for Intracellular Drug Delivery. *Biomacromolecules* 2010;11(3):713-720.
5. Amali AJ, Awwad NH, Rana RK, Patra D. Nanoparticle assembled microcapsules for application as pH and ammonia sensor. *Anal. Chim. Acta* 2011;708:75-83.
6. Yuan W, Lu Z, Li CM. Controllably layer-by-layer self-assembled polyelectrolytes/nanoparticle blend hollow capsules and their unique properties. *J. Mater. Chem.* 2011;21:5148-5155.
7. Correia CR, Sher P, Reis RL, Mano JF. Liquified chitosan–alginate multilayer capsules incorporating poly( L-lactic acid) microparticles as cell carriers. *Soft Matt* 2013;9:2115-2130.
8. Correia CR, Reis RL, Mano JF. Multilayered Hierarchical Capsules Providing Cell Adhesion Sites. *Biomacromolecules* 2013;14:743–751.

## COMBINATORIAL ANALYSIS OF BIOMATERIALS FOR TISSUE ENGINEERING

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The achievement of the effective regeneration of injured tissues is the ultimate goal of tissue engineering (TE). Research in this field has been mainly based in trial/error logic and usually a low number of conditions are tested in each study. However, the complete understanding of the therapeutic potential of a given system requires the full study of all possible combinations as each of them lead to unpredictable results. The large number of combinations of biomaterials, cells and other stimuli that can be varied during a TE system development make this area especially resource spending. High-throughput methods play an important role in the streamline of such studies [1, 2].

A cytocompatible chip consisting of superhydrophobic surfaces patterned with wettable regions was showed to be suitable to study different aspects of TE product development. Firstly, the wettability contrast of these platforms was used to pattern different types of nanoparticles and study their bioactivity [3]. Proteins were also patterned in the hydrophilic spots and 2D cells-proteins interactions were studied individually [4]. As 3D milieus were proved to be more similar to native tissues, the platforms were used to perform combinatorial studies of both cells-3D hydrogels [5] and cells-porous scaffolds interactions [6]. Growth factors and other biomolecules play an important role in TE approaches, mainly for determining stem cell fate. Their presence as well as their delivery rate from biomaterials may determine the success of a tissue regeneration approach. A new application for these chips was developed in order to evaluate biomolecules' release profiles from biomaterials using microscopy and image analysis methods [7].

We believe that the proposed uses for the superhydrophobic chip are a promising breakthrough in integrated technologies for the rapid development of TE systems.

## References

1. Titmarsh DM, Chen H, Wolvetang EJ, Cooper-White JJ. Arrayed cellular environments for stem cells and regenerative medicine. *Biotechnology Journal*. 2013;8:167-79.
2. Zonca MR, Yune PS, Heldt CL, Belfort G, Xie Y. High-Throughput Screening of Substrate Chemistry for Embryonic Stem Cell Attachment, Expansion, and Maintaining Pluripotency. *Macromolecular Bioscience*. 2013;13:177-90.
3. Luz GM, Leite AJ, Neto AI, Song WL, Mano JF. Wettable arrays onto superhydrophobic surfaces for bioactivity testing of inorganic nanoparticles. *Materials Letters*. 2011;65:296-9.
4. Neto AI, Custodio CA, Song WL, Mano JF. High-throughput evaluation of interactions between biomaterials, proteins and cells using patterned superhydrophobic substrates. *Soft Matter*. 2011;7:4147-51.
5. Salgado CL, Oliveira MB, Mano JF. Combinatorial cell-3D biomaterials cytocompatibility screening for tissue engineering using bioinspired superhydrophobic substrates. *Integr Biol*. 2012;4:318-27.
6. Oliveira MB, Salgado CL, Song W, Mano JF. Combinatorial On-Chip Study of Miniaturized 3D Porous Scaffolds Using a Patterned Superhydrophobic Platform. *Small*. 2013;9:768-78.
7. Oliveira MB, Mano JF. On-Chip Assessment of the Protein-Release Profile from 3D Hydrogel Arrays. *Analytical Chemistry*. 2013;85:2391-6.

## DRUG-LOADED NANOPARTICLES FOR SPINAL CORD INJURY REGENERATION

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Unlike fish, amphibia and even mammalian peripheral nerves, human central nervous system (CNS) axons have a very limited regeneration capability and do not spontaneously re-grow if lesioned. After damage or disruption, for instance caused by brain/ spinal cord injury (SCI) or stroke, a cascade of cellular and biochemical reactions occurs around the lesion site that creates a harsh environment for axons to regenerate. Immune and glial cells around the injury are also responsible for the production of molecules that restrain the axon re-growth, such as myelin associated inhibitors and chondroitin sulfate proteoglycans. Additionally, astrocytes and fibroblasts contribute to the formation of a scar around the damaged tissue that physically blocks axon repair. Thus, and in view of the latest findings it is imperative to block these inhibitory reactions and induce a more adequate environment for tissue repair and regeneration. In our lab, we are currently developing biomaterial-based strategies to repair the injured CNS, focusing on spinal cord. The absence of effective therapies in SCI repair is in part due to its extreme complexity, but also to the lack of efficiency and targeting of the existing drugs. In order to target the detrimental cellular responses that follow the injury in a more specific and sustained manner, we developed a nanoparticle-based drug delivery system intended to target glial cells and modulate the inflammatory processes in SCI. Poly/(amido)amine (PAMAM) dendrimer nanoparticles grafted with carboxymethylchitosan (CMChT) were loaded with the anti-inflammatory corticosteroid methylprednisolone. The nanoparticles were shown to be internalized by glial cells without affecting its metabolic viability, while releasing the drug in a sustained and prolonged manner. Nanoparticle administration in spinal cord injured rats induced

improved recovery in these animals, suggesting that nanoparticles can limit the damage extent and contribute to nerve repair/ sparing.

We believe that strategies such as this, intending to minimize the secondary events that follow nervous injury can be an opportunity for successful treatments in CNS tissue repair.

## References

1. Kordower J, Tuszynski MH. CNS regeneration: basic science and clinical advances: Academic Press; 2011.
2. Horner PJ, Gage FH. Regenerating the damaged central nervous system. *Nature*. 2000;407:963-70.
3. Oliveira JM, Kotobuki N, Marques AP, Pirraco RP, Benesch J, Hirose M, et al. Surface Engineered Carboxymethylchitosan/Poly(amidoamine) Dendrimer Nanoparticles for Intracellular Targeting. *Advanced Functional Materials*. 2008;18:1840-53.
4. Salgado AJ, Oliveira JM, Pirraco RP, Pereira VH, Fraga JS, Marques AP, et al. Carboxymethylchitosan/Poly(amidoamine) Dendrimer Nanoparticles in Central Nervous Systems-Regenerative Medicine: Effects on Neuron/Glial Cell Viability and Internalization Efficiency. *Macromolecular Bioscience*. 2010;10:1130-40.
5. Cerqueira SR, Oliveira JM, Silva NA, Leite-Almeida H, Ribeiro-Samy S, Almeida A, et al. Microglia Response and In Vivo Therapeutic Potential of Methylprednisolone-Loaded Dendrimer Nanoparticles in Spinal Cord Injury. *Small*. 2013;9:738-49.

## SKIN TISSUE ENGINEERING

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Dysfunctional healing has been associated to lifelong disability, causing pain and the formation of non-functional skin. Hence, the route to skin regeneration is an important concern in the tissue engineering (TE) field, especially for massive skin loss cases where current treatments are yet not capable of leading to a satisfying tissue response and to a permanent outcome (1).

Skin analogues have the longest clinical applicability history, but the idea of perfect skin grafts turned out to be unrealistic (2) and major hurdles such as slow preparation time, high production costs, immunogenicity issues, lack of appendages, variable engraftment rates and consequently delayed vascularisation, are still of foremost concern (3). Despite these limitations, Skin TE strategies remain as the strongest alternative to commonly used skin grafting, offering the possibility of off-the-shelf availability and the option of producing cell-based constructs in sufficient quantities capable of responding to the full biological signaling complexity, as well as providing environmental cues to attain permanent wound closure.

Current state of the art, exploring stem cells potential and the healing microenvironment/matrix, is indicative of skin substitute's defective signals to induce skin regeneration (4). Stem cells act either by direct or indirect signalling through paracrine interactions (5) or transdifferentiation (6-9), privileging an integrated and orchestrated remodelling, while the extracellular matrix (ECM) dynamics during wound healing is also seen as a determinant factor in the presentation of cues for tissue morphogenesis, accurately leading regeneration (10). We have been exploring novel routes to target skin regeneration problematic taking advantage of powerful tools such as 3D hydrogels and cellular key players, such as stem cells and endothelial cells, circumventing the prolonged cell culture period. Improved cell-compatible

polyssacharide-based spongy-like hydrogels promoted a superior neo-tissue vascularization and its assemblage with microvascular endothelial and stem cells, synergized to improve skin wound healing.

Thus, this complex interplay between ECM, cells and growth factors, within a particular 3D environment, where a supported and dynamic healing interaction occurs, leads to a new era of skin regeneration, rather than the traditional replacement.

## References

1. Priya SG, H Jungvid and A Kumar. (2008). Skin tissue engineering for tissue repair and regeneration. *Tissue Eng Part B Rev* 14:105-118.
2. Bottcher-Haberzeth S, T Biedermann and E Reichmann. (2009). Tissue engineering of skin. *Burns* 36:450-460.
3. MacNeil S. (2007). Progress and opportunities for tissue-engineered skin. *Nature* 445:874-880.
4. Yildirimer L, NT Thanh and AM Seifalian. (2012). Skin regeneration scaffolds: a multimodal bottom-up approach. *Trends Biotechnol.*
5. Hanson SE, ML Bentz and P Hematti. (2010). Mesenchymal stem cell therapy for nonhealing cutaneous wounds. *Plast Reconstr Surg* 125:510-516.
6. Kamolz LP, A Kolbus, N Wick, PR Mazal, B Eisenbock, S Burjak and G Meissl. (2006). Cultured human epithelium: human umbilical cord blood stem cells differentiate into keratinocytes under in vitro conditions. *Burns* 32:16-19.
7. Sasaki M, R Abe, Y Fujita, S Ando, D Inokuma and H Shimizu. (2008). Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 180:2581-2587.
8. Altman AM, Y Yan, N Matthias, X Bai, C Rios, AB Mathur, YH Song and EU Alt. (2009). IFATS collection: Human adipose-derived stem cells seeded on a silk fibroin-chitosan scaffold enhance wound repair in a murine soft tissue injury model. *Stem Cells* 27:250-258.
9. Altman AM, N Matthias, Y Yan, YH Song, X Bai, ES Chiu, DP Slakey and EU Alt. (2008). Dermal matrix as a carrier for in vivo delivery of human adipose-derived stem cells. *Biomaterials* 29:1431-1442.
10. Tsang KY, MC Cheung, D Chan and KS Cheah. (2010). The developmental roles of the extracellular matrix: beyond structure to regulation. *Cell Tissue Res* 339:93-110.

## TISSUE ENGINEERING AS A REMARKABLE TOOL FOR CARTILAGE REPAIR

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Articular cartilage is a very specialized tissue with outstanding load-bearing capacity. It consists mainly of a dense extracellular matrix (ECM) with chondrocytes embedded on it. Cartilage has very low capacity of self-repair and regeneration after traumatic, degenerative or inflammatory injury. Current available surgical treatments for cartilage repair present several drawbacks, such as possible implant rejection or infection, or the need for revision after some years of implantation. Autologous chondrocyte implantation (ACI) is an autologous therapy that was proposed as a basis for tissue engineering strategies to repair cartilage (1). Modifications on various aspects of this surgical technique have been developed, comprising the use of natural-based scaffolds as supports for chondrocyte expansion (2).

Many strategies and systems have been developed along the years for cartilage regeneration and repair. Scaffolds play a major role in those strategies, as they provide the support for cell growth and to promote extracellular matrix production. Both natural based (3) or synthetic scaffolds (4) have been successfully used as supports for chondrogenic differentiation or cartilage-like tissue production.

The interest in cells cross-talk and communication has been growing in the past years, revealing that signalling pathways are pivotal elements when understanding the tissue formation and its repair mechanisms (5). Chondrocytes release morphogenetic signals that influence the surrounding cells, for example, stem cells, to differentiate into the chondrogenic lineage (5). In fact, the increased cartilage formation on co-cultures using stem cells and articular chondrocytes has been reported (6). Therefore, the study of co-cultures using chondrocytes and undifferentiated cells is a very promising strategy to develop engineered cartilage.

## References

1. Brittberg M. Articular Cartilage Repair in the Knee Joint with Autologous Chondrocytes and Periosteal Graft. *Orthopedics and Traumatology*. 2001;3:185 - 94.
2. Welsch GH, Trattinig S, Hughes T, Quirbach S, Olk A, Blanke M, et al. T2 and T2\* mapping in patients after matrix-associated autologous chondrocyte transplantation: initial results on clinical use with 3.0-Tesla MRI. *Eur Radiol*. 2010 Jun;20(6):1515-23.
3. Alves da Silva ML, Crawford A, Mundy JM, Correlo VM, Sol P, Bhattacharya M, et al. Chitosan/polyester-based scaffolds for cartilage tissue engineering: assessment of extracellular matrix formation. *Acta Biomater*. 2010 Mar;6(3):1149-57.
4. Alves da Silva ML, Martins A, Costa-Pinto AR, Costa P, Faria S, Gomes M, et al. Cartilage Tissue Engineering Using Electrospun PCL Nanofiber Meshes and MSCs. *Biomacromolecules*. 2010 Dec 13;11(12):3228-36.
5. Hwang NS, Varghese S, Puleo C, Zhang Z, Elisseeff J. Morphogenetic signals from chondrocytes promote chondrogenic and osteogenic differentiation of mesenchymal stem cells. *J Cell Physiol*. 2007 Aug;212(2):281-4.
6. Meretoja VV, Dahlin RL, Kasper FK, Mikos AG. Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. *Biomaterials*. 2012 Sep;33(27):6362-9.

## REGENERATION OF THE INTERVERTEBRAL DISC

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Degeneration of intervertebral disc (IVD) seems to be one of the main causes associated to lower back pain (LBP), one of the most common painful conditions that lead to work absenteeism, medical visits, and hospitalization in actual society [1,2]. This complex fibro-cartilaginous structure is composed by two structures, an outer multilayer fiber structure (annulus fibrosus, AF) and a gel-like inner core (nucleus pulposus, NP), which are sandwiched in part between two cartilage endplates (CEP) [1]. Existing conservative and surgical treatments for LBP are directed to pain relief and do not adequately restore disc structure and mechanical function [2]. In the last years, several studies have been focusing on the development of tissue engineering (TE) approaches aiming to substitute/regenerate the AF or NP, or both by developing an artificial disc that could be implanted in the body thus replacing the damaged disc [3]. TE strategies aiming to regenerate NP tissue often rely on the use of natural hydrogels, due to the number of advantages that these highly hydrated networks can offer. Nevertheless, several of the hydrogel systems developed still present numerous problems, such as variability of production, and inappropriate mechanical and degradation behaviour. Recently, our group has proposed the use of gellan gum (GG) and its derivatives, namely the ionic- and photo-crosslinked methacrylated gellan gum (GG-MA) hydrogels, as potential injectable scaffolds for IVD regeneration [4,5]. Work has been conducted regarding the improvement of GG mechanical properties either by chemically modifying the polymer (allowing to better control in situ gelation and hydrogel stability) [4] or by reinforcing it with biocompatible and biodegradable GG microparticles (enabling the control of degradation rate and cell distribution) [5].

Another strategy currently under investigation relies on the development of a biphasic scaffold that mimics the total disc by using a reverse engineering approach.

## References

1. Richardson SM, Mobasheri A, Freemont AJ, Hoyland JA. Intervertebral disc biology, degeneration and novel tissue engineering and regenerative medicine therapies. *Histol Histopathol* 2007;22:1033-41.
2. Kalson NS, Richardson S, Hoyland JA. Strategies for regeneration of the intervertebral disc. *Regen Med* 2008;3:717-29.
3. Silva-Correia J, Correia SS, Pereira H, Espregueira-Mendes J, Oliveira JM and Reis RL. Tissue engineering strategies applied in the regeneration of the human intervertebral disc. *Biotechnol Adv* (accepted for publication).
4. Silva-Correia J, Oliveira JM, Caridade SG, Oliveira JT, Sousa RA, Mano JF, et al. Gellan gum-based hydrogels for intervertebral disc tissue-engineering applications. *J Tissue Eng Regen Med* 2011;5:e97-e107.
5. Pereira DR, Silva-Correia J, Caridade SG, Oliveira JM, Salgado AJ, Sousa N, et al. Development of Gellan gum-based microparticles/hydrogel matrices for application in the intervertebral disc regeneration. *Tissue Eng Part C* 2011;17:961-72

## BIOENGINEERING STRATEGIES FOR THE REGENERATION OF TENDON TISSUES

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Tendons are connective tissues that usually connect muscle to bone and are capable of withstanding tension, and passively modulate forces during locomotion.

Tendons are highly prone to injury and the intrinsic hypo-cellularity and hypo-vascularity make their natural healing extremely slow and inefficient when severely damaged[1]. Surgical repair with grafts is common but unsuccessful in a long-term basis. The development of tissue engineering strategies proposes to explore for alternative solutions to grafting with potential for tendon regeneration applications[1].

To progress in development of functional approaches, it is critical to understand how tendon resident cells interact with the natural micro-environment under non-pathological conditions[2]. Also, with tendon-specific markers to be identified, tenogenesis and the process of tendon regeneration have been barely explored. Following a cellular-based strategy, we studied resident cells isolated from tendon and ligament tissues during arthroplastic surgeries to the knee. Nevertheless, tendon-resident cells are scarce as tendons are hypocellular tissues. To overcome this limitation we proposed a natural endogenous system for tissue maintenance and neotissue formation based on stem cells[3]. Stem cell potential towards the tenogenic phenotype was assessed and results interpreted with basis on the results obtained for tendon resident cells.

In severe tissue injuries, cells alone may have limited regenerative ability due to the lack of structural support to promote gradual load transfer and extracellular deposition. Envisioning this critical issue, we designed a bioengineered strategy combining cells with tendon customized scaffolds. Focusing on the naturally parallel orientated fibers of

native tendons, fibrous scaffolds were developed with an aligned orientation using different processing methodologies, and cultured with cells. Ongoing studies suggest that the biomimetic scaffolding contributes to modulate cellular behavior aimed at tendon-related strategies.

## References

1. Rodrigues MT, Reis RL, Gomes ME. Engineering tendon and ligament tissues: present developments towards successful clinical products. *J Tissue Eng Regen Med.* 2012;DOI:10.1002/term.
2. Zeugolis DI, Chan JCY, Pandit A. Tendons: Engineering of Functional Tissues. In: Pallua N, Suschek CV, editors. *The Tissue Engineering Book: State of the Art, Visions, and Limitations*: Springer; 2011. p. 537-72.
3. Rodrigues MT, Lee BK, Lee SJ, Gomes ME, Reis RL, Atala A, et al. The effect of differentiation stage of amniotic fluid stem cells on bone regeneration. *Biomaterials.* 2012;33:6069-78.