Smart Implants as Novel Strategy to Regenerate Well-Founded Cartilage

Laetitia Keller[1,2#], *Pascale Schwinté* [3#], *Enrique Gomez-Barrena*[4], *Manuel Arruebo*[5], *Nadia Benkirane-Jessel*[1,2*]

1 INSERM (French National Institute of Health and Medical Research), "Osteoarticular and Dental Regenerative Nanomedicine" laboratory, http://www.regmed.fr, UMR 1109, Faculté de Médecine, FMTS, F-67085 Strasbourg Cedex.

2 Université de Strasbourg, Faculté de Chirurgie Dentaire, 1 place de l'Hôpital, F-67000 Strasbourg

3 ARTiOS Nanomed, Faculté de Médecine, F-67085 Strasbourg Cedex.

4 Servicio de Cirugía Ortopédica y Traumatología, Hospital Universitario La Paz, IdiPAZ, Universidad Autónoma de Madrid. Pº Castellana 261, Madrid -28046-, Spain.

5 Department of Chemical Engineering and Aragon Nanoscience Institute, University of Zaragoza, C/Mariano Esquillor, s/n, 50018, Zaragoza, Spain.

#, both authors contributed equally; *, corresponding authors

Corresponding authors: Benkirane-Jessel, N. (nadia.jessel@inserm.fr); Schwinté, P. (pschwinte@unistra.fr).

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ABSTRACT

Herein we explore a new generation of smart living implants combining not only active therapeutics but also stem cells, as a novel strategy to regenerate stabilized cartilage and avoid prosthesis, by achieving regeneration of its subchondral bone foundation, requirement which is failing today in the clinic.

Clinical articular cartilage repair: current techniques, opportunities and drawbacks

The growing aging population suffering from articular knee damage boosts the market of cartilage regeneration. The cost of cartilage regeneration techniques will restrain the market in coming years. Emerging technologies such as regenerative nanomedicine is a growing focus in this market. Smart implants, combing active molecules, scaffolds and cells are expected to grow at high rates due to a demonstrated higher efficiency over other cell based therapies. Furthermore, there are about 5.4 million potential patients who will require joint and cartilage regeneration procedures by 2019 in U.S. alone, a significant factor driving the overall market of cartilage regeneration. The rationale to develop a new approach on articular cartilage repair departs from the poor outcome of focal chondral lesions that are left untreated. Those may progress to debilitating joint pain, dysfunction and degenerative arthritis, and the development of an early treatment that controls pain and maintains articular function is a challenging aim. It has been hypothesized that structural repair of articular cartilage may lead to the desired clinical outcome, and this belief has fostered new technologies to obtain the best quality hyaline articular cartilage repair in the focal chondral lesions.

Current clinical treatments include non-transplantation techniques (marrow stimulation techniques like microfractures are considered the current standard), osteochondral autograph transfer (such as mosaicoplasty) and cell therapy (autologous chondrocyte implantation –ACI-), but meta-analysis of randomized controlled trials have not found differences among these techniques to improve function and pain at intermediate-term follow-up [1]. Seeding of autologous chondrocytes in a collagen membrane led to the development of the matrix-applied characterized autologous cultured chondrocytes implantation (MACI) [2], proving that MACI was superior to microfracture treatment for symptomatic cartilage defects of the knee, but this technique showed two associated risks, graft hypertrophy and delamination, and there are insufficient intermediate or

long-term results to ascertain its real value. The combination of structural membranes, pluripotent autologous cells and chondroinductive growth factors can be considered the holy grail of cartilage focal lesions repair. However, it should be stressed that structural repair is not sufficient: pain and function improvement, and a true functional repair of the cartilage are considered the main clinically relevant endpoints, at the end of well-designed clinical trials.

Nanoreservoirs technology for osteochondral regenerative medicine

Nanostructured materials have been widely used in soft tissue engineering as scaffolding materials (to support cell adhesion, proliferation and differentiation), and as nanofillers (within scaffolds) acting as depots, for spatio-temporal controlled release of bioactive molecules. Nanostructured scaffolds are designed to resemble the 3D native extracellular matrix (ECM) of the regenerated tissue, offering adequate porosity for cell infiltration and high surface per volume ratio for protein adsorption, and allowing tunable cell-surface interactions [3]. Nanomedicine allows the emergence of entirely new classes of active devices intended for targeted intracellular delivery for improved efficacies and reduced associated toxicities. In cell therapy, nanoparticles internalized within transplanted cells can also be used as cargos of adjuvant drugs, avoiding the need of systemic drug administration [4]. As building material, the nanoparticle itself can act as a tissue-regeneration inductor as in the case of nano-hydroxyapatite, a bone-like material, which promotes bone regeneration, and has been widely used as nanofiller within scaffolds in the treatment of osteochondral lesions [5]. We reported the use of nanostructured capsules incorporating growth factors for bone regeneration by using stem cells *in vivo* [6]. Nanoencapsulation of differentiation-inducing molecules within nanoparticles has also been proven as an efficient and persistent chondrogenic strategy, preventing an uncontrolled release [7]. In our group, a unique nanotechnology strategy is used to entrap, protect, and stabilize therapeutic agents into polymer coatings: nanoreservoirs, covering nanofibres of implantable nanofibrous membranes for bone and cartilage regeneration [8] (Figure 1). Upon contact with cells, therapeutic agents become available through enzymatic degradation of the nanoreservoirs. As cells grow, divide, and infiltrate deeper into the porous membrane, they trigger slow and progressive release of therapeutic agents that, in turn, stimulate further cell proliferation. The nanoreservoirs technology enables to reduce the quantities of required therapeutic agent (compared to soaked membranes for instance) thereby reducing costs.

Smart implant combining triple 3D technology and double compartment for articular cartilage repair

There are some evidences that intra-articular administration of growth factors and biologics can act on pathologic metabolic changes, but intra-articular injection of cells does not effectively repopulate the damaged areas. Current cartilage repair techniques lead to the formation of low quality fibrocartilage instead of hyaline cartilage and often result in subchondral bone abnormalities. Recently, researches have been focusing their efforts on the development of bilayered implants, to mimic the natural layers present in the osteochondral unit [9]. Mesenchymal stem cells (MSCs) from adult bone marrow provide an exciting and promising stem cell population for the repair of bone in skeletal diseases. We reported a new generation of nanofibrous implant functionalized with BMP (Bone morphogenetic proteins) growth factors nanoreservoirs and equipped with human MSC microtissues (MTs: Cells spheroids) for regenerative nanomedicine [10]. We reported as well an innovative approach improving cartilage repair, by regenerating a robust subchondral bone, supporting articular cartilage [11, 12]. This novel strategy consists in a double compartmented and hybrid implant filled with well-organized 3D stem cells as spheroids. It combines three-dimensional structures: stem cells microtissue, nanofibrous membrane and hydrogel (Figure 2). This triple-3D implant is able to mimic the physiological environment of the osteochondral unit. Thus, compared to the treatments on the market today, it offers a double therapeutic action: Instead of targeting only the cartilage layer, our advanced technology is also engineered to restore a more stable subchondral bone, and proposes a cell-controlled release of the contained BMP, which is expected to enhance the treatment efficiency . All these features will improve the prognostic for the patients by restoring a full articular function.

Future outlook

Osteochondral regenerative nanomedicine provides a new perspective to face clinical challenges thanks to the direct interaction at the cellular and molecular level of the disease and the complete understanding of the interface between biomolecules and biomaterials. For robust and durable articular cartilage regeneration, it is necessary to repair this tissue on a solid subchondral bone basis. We report here an innovative therapeutic medical device based on two designed compartments mimicking the natural

cues of the osteochondral unit which improves subchondral bone regeneration and cartilage stabilisation. We see our technology as an adaptable and easy-to-apply technological platform for various regenerative medicine applications (skin, vasculature…). By functionalizing the structural scaffold with nanoreservoirs of adequate therapeutic agents (angiogenic growth factors, anti-cancer drugs, anti-inflammatory molecules, genes…), seeding it with the appropriate cells and shaping it to the right composite structure, it can be engineered for the treatment of several pathologies. Our technology can also include other vectors such as nanoparticles, cyclodextrins used as tools to protect and solubilise drugs as a second level of action in the nanoreservoirs. Our nanoreservoirs technology can be used by the industry in biopharmaceutical research to design new combination devices with their own drugs and/or cell lines.

Although some efforts have to be dedicated to solve the challenges of these nanotechnologies including industrial scale-up, methods standardization, long-term validation of the medical outcome, safety and efficacy evaluation compared to the current clinical practice, we foresee many other future application avenues for our platform technology in regenerative medicine and eventually in personalized medicine.

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FIGURES

Figure 1. **Nanoreservoirs technology for osteochondral regenerative nanomedicine.** For efficient articular cartilage repair, both the subchondral bone lesion and the cartilage should be targeted in the treatment. Our strategy depicted here consists in: (i) using a nanofibrous membrane as structural scaffold mimicking the extracellular matrix to cover the subchondral bone lesion (small defects), (ii) membrane fibres previously coated with nanoreservoirs containing an osteoinductive agent (BMP) to induce mineralisation, (iii) and stem cells that can differentiate into both bone (osteoblasts) and cartilage (chondrocytes) cells.

Figure 2. **Smart implant combining triple 3D technology and double compartment for articular cartilage repair.**

Our hybrid living implant mimics the biological and structural cues of the native osteochondral unit, leading to both subchondral bone and cartilage repair. The triple 3D environment design is featured by: (i) hMSCs (from bone marrow) as well-organized microtissues (MTs), (ii) nanofibrous membrane (collagen or polycaprolactone) functionalised with nanoreservoirs of BMPs and (iii) alginate/hyaluronic acid hydrogel. The innovative strategy is based on the combination of this triple 3D environment organized in a multi-compartmented well-defined structure:

1st compartment: mineralization capacity of hMSC MTs seeded on nanofibrous membrane; *2nd compartment*: chondrogenic capacity of hMSC MTs in alginate/HA hydrogel; *Compartments combination*: hMSCs in a triple3D environment for the osteoarticular unit regeneration. It generates the natural gradient of mineralization in the physiological osteochondral unit, leading to create the natural ‘glue’ between articular cartilage and subchondral bone.