

1 Smart Implants as Novel Strategy to Regenerate Well-Founded Cartilage
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22 regeneration; regenerative nanomedicine; osteoarticular diseases; stem cells.

1 ABSTRACT

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

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8 **Clinical articular cartilage repair: current techniques, opportunities and**
9 **drawbacks**

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

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

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

7 8 **Nanoreservoirs technology for osteochondral regenerative medicine**

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

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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

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

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

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21 ACKNOWLEDGMENTS

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1 FIGURES

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3 **Figure 1. Nanoreservoirs technology for osteochondral regenerative nanomedicine.**

4 For efficient articular cartilage repair, both the subchondral bone lesion and the cartilage
5 should be targeted in the treatment. Our strategy depicted here consists in: (i) using a
6 nanofibrous membrane as structural scaffold mimicking the extracellular matrix to
7 cover the subchondral bone lesion (small defects), (ii) membrane fibres previously
8 coated with nanoreservoirs containing an osteoinductive agent (BMP) to induce
9 mineralisation, (iii) and stem cells that can differentiate into both bone (osteoblasts) and
10 cartilage (chondrocytes) cells.

11

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

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

21 *1st compartment:* mineralization capacity of hMSC MTs seeded on nanofibrous
22 membrane; *2nd compartment:* chondrogenic capacity of hMSC MTs in alginate/HA
23 hydrogel; *Compartments combination:* hMSCs in a triple3D environment for the
24 osteoarticular unit regeneration. It generates the natural gradient of mineralization in the
25 physiological osteochondral unit, leading to create the natural ‘glue’ between articular
26 cartilage and subchondral bone.

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