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REVIEW ARTICLE

Nanosized biomaterials for regenerative medicine

Raffaele Conte [1,]; Anna Calarco [2]; Gianfranco Peluso [2]*

[1]*Institute of Biosciences and Bio Resources, National Research Council of Italy*
[2]*Institute of Agro-Environmental and Forest Biology, National Research Council, Naples, Italy*

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Abstract

This review discusses recent developments in the field of nanosized biomaterials and their use in tissue regeneration approaches. The aim is to provide an overview of the research focused on nanoparticle-based strategies to stimulate regeneration. In particular, nanoparticles improve the regenerative capabilities of biomaterials offering ways to control surface and mechanical properties. Moreover, incorporation of nanoparticles within biomaterials increases cellular adhesion, differentiation and integration of stem cells into the surrounding environment. Finally, the drug delivery capabilities of nanoparticles offer additional possibilities to increase the biological performance of biomaterials. As the development of nanoparticles continues, incorporation of this technology in the field of regenerative medicine will ultimately lead to new tools that can diagnose, track and stimulate the growth of new tissues and organs.

Keywords: *Biomaterials; Nanotechnology; Nanocomposites; Nanomedicine; Regeneration.*

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INTRODUCTION

Regenerative medicine and the regeneration process

Regenerative medicine is an emerging field of biomedicine that deals with tissue engineering and molecular biology. It is defined as the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects [1]. Such procedure differs from tissue reparation on the fact that the repair involves the healing of dead tissue by fibrous patching, while regeneration is the re-growth of parenchymal and stromal cells. Consequently, reparation induces tissue replacement with scar lacking of the functional capacity, while regeneration restores damaged tissues both structurally and functionally (*restitutio ad integrum*) [2]. Hence, regenerative medicine is focused on human cells (somatic, adult stem cells and embryo-derived stem cells) and their mobilization, recruitment and integration into functional tissues. Therefore, the key issue in regeneration relates with the implementation of an appropriate environment ('niche') for cells recruitment and complete functional integration [3]. Two alternative regenerative routes are available with the use of these tailored biomaterials. In "Tissue engineering" progenitor cells are seeded onto modified scaffolds. The cells grow outside the body, become differentiated and mime naturally occurring tissues. These tissue-engineered constructs are then implanted into the patients to replace diseased or damaged tissues. Clinical applications of these biomaterials include repair of articular cartilage, skin and vascular system. Differently, the "In situ tissue regeneration" approach involves the use of biomaterials in the form of powders, solutions, or doped microparticles to stimulate local tissue repair. Bioactive materials release chemicals by diffusion or network breakdown and activate the cells in contact with these stimuli. Clinical applications of this technique include surgery, surgical implants and hospital procedures [1, 4]. Fig. 1 schematizes the regeneration process.

The critical prerequisite for successful tissue regeneration is an appropriate extracellular matrix

* Corresponding Author Email: *raffaele.conte@ibbr.cnr.it*

(ECM) remodeling, as ECM provides physical scaffolding for the cellular constituents and initiates crucial biochemical and biomechanical cues required for tissues morphogenesis, differentiation and homeostasis. Moreover, the ability of ECM to produce a "bridge" for normal tissue edges to regenerate counteracts the tissue's natural response of fibroblast deposition and scar formation that leads to reparation instead of regeneration [2]. Biomaterials scaffolds mime the actions of ECM. In fact, these constructs provide an environment that supports stem cells differentiation in the presence of other co-factors, such as serum-containing cell culture medium or biochemical supplements. Moreover, the synergic action of material inherent properties and released factors determines cells shift in phenotype [4].

Development of regenerative biomaterials

The development of novel regenerative biomaterials is an iterative process that involves the creation of increasingly safer, more reliable and more inexpensive replacements for damaged or diseased human tissues. In the last sixty years, four generations of products were developed with increased advantages. The first group, the "Bio-inert material", is engineered to provide appropriate mechanical properties for surgical applications, corrosion resistance, and absence of injurious effects such as carcinogenicity, toxicity, allergy and inflammation. Examples are the hydroxyapatites, bioactive ceramics, metals and alloys [5].

The second class of biomaterials is the "Resorbable polymers". These composites have natural or synthetic origin and the most used are polylactides, polyglycolides, polycaprolactones and trimethylcarbonates. The most prominent use of resorbable polymers regards the synthesis of drug-eluting stents that are used to maintain patency of the coronary arteries. Such devices contain cytostatic, cytotoxic, antithrombotic and/or anti-inflammatory agents [5]. The third class of biomaterials is "Biocompatible nanocomposites" created to promote or inhibit specific cell activities. The nanoscale dimension enables nutrient transport and supports cell proliferation. Consequently, this morphology mimes the natural extracellular environment [6]. Finally, the fourth generation of biomaterials is the "Biomimetic composites", characterized by the ability to release bioactive molecules and to interact with stem cells. The stem cells/materials interfaces are complex and dynamic microenvironment in which cells and materials cooperate, leading to the remodeling of cells surroundings. In particular, the inherent properties of materials (e.g. adhesivity, stiffness, nanotopography, molecular flexibility or degradability) induce lineage-specific stem cells differentiation [7].

Enhancing regenerative approaches with nanoparticles

Since the 1970s, when Nobel Prize winner Christian de Duve described the structure and properties of lysosomes in biological tissues, drug administration protocols have significantly evolved due to the introduction of nanosized drug delivery systems [8]. These latter can be defined as ultra-dispersed solid organic or inorganic

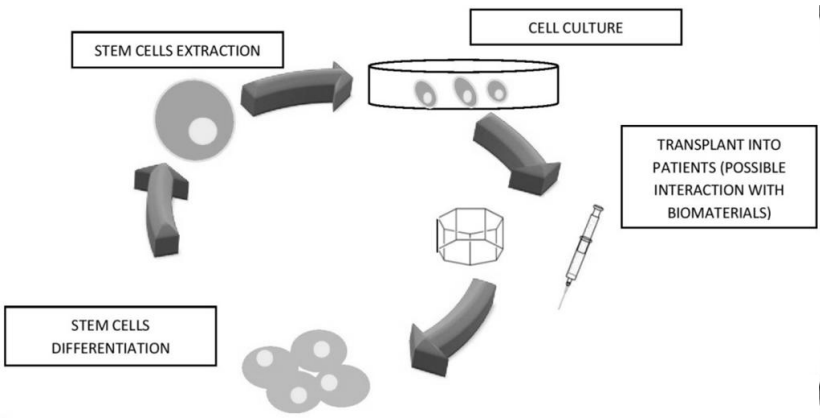

Fig. 1: Regeneration process.

structures displaying a sub-micrometer size, typically comprised between 10 and 100 nm. The upper limit is dictated by the vector's ability to pass cellular interstices, while the lower limit is fixed by the threshold for first-pass elimination by kidneys. Moreover, such dimensions permit a good biodistribution of long-circulating nanocarriers [9]. Then, the use of nanotechnology to improve current approaches in tissue and organ regeneration has received increased attention over the years thanks to the great versatility that they offer in terms of size and surface chemistry, allowing the utilization as carriers for the delivery of drugs, genetic material or growth factors (GFs). Indeed, a variety of nanoparticles has been developed for therapy; among them dendrimers, liposomes, polymer-based nanoparticles, micelles, carbon nanotubes and many more (Fig. 2). [10] In regeneration, nanoparticles improve the regenerative capabilities of biomaterials offering ways to control surface and mechanical properties. Moreover, incorporation of nanoparticles within biomaterials increases cellular adhesion, differentiation and integration of stem cells into the surrounding environment. Finally, the drug delivery capabilities of nanoparticles offer additional possibilities to increase the biological performance of biomaterials. [11]

Nanocomposite materials

The first way to improve the tissue regenerating capabilities of biomaterials is to combine them with nanomaterials to create nanocomposites. This new class of materials showed improved mechanical and/or biological performance, compared to analogous composites without nanoparticles, due to the changes in the classic laws of physics consequent to the manipulation at scales of around 100 nm. [12] In particular, the nanostructural topographical properties (nanotopography) of the materials is able to mime natural tissue, that can be defined as a nanostructured material consisting of collagen fibrils and proteins with dimensions in the 100 nm size or less, or bone tissue, which is a nanostructured composite composed of a polymer matrix (mainly collagen) reinforced with nanomater-sized ceramic particles (mainly carbonated HA) in order to stimulates the cells to grow. [13] Nanocomposites are produced using a wide range of nanostructured materials (e.g. ceramics, polymers and hydrogels). For example, hydroxyapatite (HA) the native mineral structure of bone, in its microscale dimension is a poor material for bone reconstruction due to its brittleness and slow degradation rate. However, incorporation of HA nanoparticles

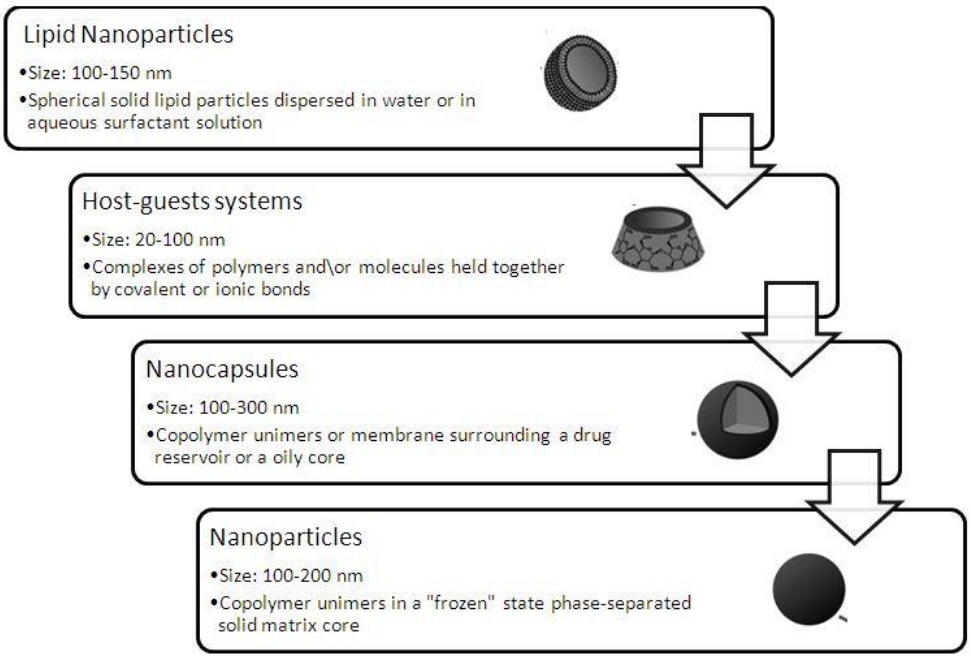

Fig. 2: Examples of nanoparticles enclosed into biomaterials.

into polymeric materials has created promising scaffolds for bone tissue engineering. In fact, HA nanoparticles coated on polymeric poly lactide-co-glycolide (PLGA), scaffolds facilitated bone formation in a concentration-dependent manner [14]. Similarly, polylactide (PLA) scaffolds coated with HA nanoparticles stimulated the expression of osteogenic proteins (e.g. BMP-2, osteopontin) on scaffold-attached bone marrow-derived mesenchymal stem cells and facilitated bone regeneration [15]. Incorporation of metallic nanoparticles prepared from iron oxide or titanium into polymeric scaffolds, increased collagen and calcium deposition by osteoblasts, leading to enhanced tensile strength compared to non-metallic incorporated materials [16-17]. A study by Khang *et al.* showed that bone cells respond differently on submicrometer and nanometer scale titanium surfaces, and that small changes in nanomater surface features can have larger consequences towards bone regeneration [18]. Nanoparticles of biphasic calcium phosphate have been shown to increase the tensile strength of a biomaterial composed of polyvinyl alcohol/gelatin nanomater [19]. Table 1 recaps the synthesized nanocomposite materials.

Controlled release of biomolecules from biomaterials

Another strategy to build bioactive nanomaterials for tissue regeneration is by incorporating biomolecules directly into the materials, in order to promote stem cell attachment in situ. Thus, such materials not only act as a scaffold but also as a delivery vehicle for controlled release of bioactive molecules. The use of nanoparticles gives advantages because of their drug delivery capabilities, inherent mechanical and biological properties and ease of functionalization. For example, plasmids coated PLGA nanoparticles within a fibrin hydrogel complex were found to be capable of enhance bone regeneration [20]. Similarly, PLGA nanospheres encapsulating plasmid and enclosed within a nanofibrous PLA scaffold showed controlled release of BMP-7 (plasmid) followed by ectopic bone formation [21]. In another approach, block copolymer nanolithography was used to tune the size of gold nanoparticles on a polymeric surface. The selective immobilization of the plasmid BMP-2 on the gold nanoparticles offered the possibility of exactly controlling the release [22]. Also proteins can be incorporated into nanosized biomaterials in order to stimulate bone tissue regeneration. For example, the extracellular matrix molecule osteopontin, a protein that plays an important role in bone remodeling, was incorporated in HA nanoparticles enclosed into a degradable matrix. The release was analyzed for its osteoinductive potential in a dog bone defect model [23]. Biomaterials with chitosan nanocomplexes delivering angiogenesis and osteogenesis, stimulating factors demonstrated great bone tissue regeneration potential [24].

Biomaterials with PLGA nanoparticles and alginate microcapsules encapsulating, respectively, the factors BMP-2 and VEGF showed a positive effect on the formation of vascularized bone [25]. Last developments of research regarded the development of 'smart' biomaterials with the ability to spatio-temporally control the dose, sequence and profile of release of several regeneration factors. Types of carriers are nanogels, cross-linked gelatin–polymer composites or gelatin-based coatings [26]. In these systems, the biomaterials were produced by incorporating different layers that serve as matrices enabling internal architecture with controlled release properties. Temporal controlled release of nanoparticles from biomaterials can be achieved via responsive linkers. For example, in a study by Tokatlian *et al.* nanoparticles were immobilized to a biomaterial through the use of matrix metalloproteinases (MMPs) sensitive linkers.

In this way, cell-secreted MMPs are able to release the nanoparticles from the biomaterial in a temporally controlled manner [27]. Another method to remotely control biomolecule release is with external stimuli such as light. For example, Shas S. *et al.* produced Photo-triggerable hydrogel-nanoparticle hybrid scaffolds for remotely controlled drug delivery [28]. Table 2 recaps the examples of controlled release of biomolecules from biomaterials.

Concluding Remarks

In the past few decades, nanoparticles have revolutionized the field of drug delivery due to their unique physical characteristics. Then, nanostructures were designed to fit multiple purposes. Specifically, their application in the field of regenerative medicine regarded the incorporation within biomaterials to increase

Table 1: Nanocomposite materials.

Material	Reference
Hydroxyapatite (HA) nanoparticles coated on polymeric poly lactide-co-glycolide (PLGA) scaffolds	[14]
Polylactide (PLA) scaffolds coated with HA nanoparticles	[15]
Nanometre scale titanium surfaces in scaffolds	[18]
Nanoparticles of biphasic calcium phosphate on a polyvinyl alcohol/gelatin nanomat scaffold	[19]

Table 2: Controlled release of biomolecules from biomaterials.

Material	Reference
Plasmid coated PLGA nanoparticles within a fibrin hydrogel complex	[20]
PLGA nanospheres encapsulating plasmid and enclosed within a nanofibrous PLA scaffold	[21]
Gold nanoparticles incorporating plasmid BMP-2 on a polymeric surface	[22]
HA nanoparticles with osteopontin enclosed into a degradable matrix	[23]
Biomaterials with chitosan nanocomplexes delivering angiogenesis and osteogenesis, stimulating factors	[24]
Biomaterials with PLGA nanoparticles and alginate microcapsules encapsulating, respectively, the factors BMP-2 and VEGF	[25]
Nanoparticles immobilized to a biomaterial through the use of matrix metalloproteinases (MMPs)	[27]
Photo-triggerable hydrogel-nanoparticle hybrid scaffolds for remotely controlled drug delivery	[28]

cellular adhesion, differentiation and integration of stem cells into the surrounding environment. Such research is still in its beginning phase but is already making important contributions.

CONFLICT OF INTEREST

All authors declare no conflicts of interest in this paper

REFERENCES

[1] Mason C., Dunnill P., (2008), A brief definition of regenerative medicine. *Regen. Med.* 3: 1-5.

[2] Atala A., Irvine D. J., Moses M., (2010), Wound healing versus regeneration: Role of the tissue environment in regenerative medicine. *M. R. S. Bull. Mater. Res. Soc.* 35: 597-606.

[3] Mironov V., Visconti R. P., Markwald R. R., (2004), What is regenerative medicine? Emergence of applied stem cell and developmental biology. *Expert Opinion on Biol. Therap.* 4: 773-781.

[4] Hench L. L., Polak J. M., (2002), Third-generation biomedical materials. *Science.* 295: 1014-1017.

[5] Narayan R. J., (2010), The next generation of biomaterial development. *Philosop. Transac. Royal Soc. London A: Mathemat. Phys. Eng. Sci.* 368: 1831-1837.

[6] Kretlow J. D., Mikos A. G., (2008), From material to tissue: Biomaterial development, scaffold fabrication, and tissue engineering. *AIChE J. l.* 54: 3048-3067.

[7] Murphy W. L., McDevitt T. C., Engler A. J., (2014), Materials as stem cell regulators. *Nat. Mater.* 13: 547-557.

[8] Couvreur P., (2013), Nanoparticles in drug delivery: Past, present and future. *Adv. Drug. Deliv. Rev.* 65: 21-23.

[9] Nair H. B., Sung B., Yadav V. R., (2010), Delivery of antiinflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. *Biochem. Pharmacol.* 80: 1833-1843.

[10] Conte R., Marturano V., Peluso G., (2017), Recent advances in nanoparticle-mediated delivery of anti-inflammatory phytocompounds. *Int. J. Mol. Sci.* 18: 709-714.

[11] Van R. S., Habibovic P., (2017), Enhancing regenerative approaches with nanoparticles. *J. R. Soc. Interf.* 14. 20170093.

[12] Streicher R. M., Schmidt M., Fiorito S., (2007), Nanosurfaces and nanostructures for artificial orthopedic implants. *Nanomedicine (Lond).* 2: 861-874.

[13] Khang D., Carpenter J., Chun Y. W., (2010), Nanotechnology for regenerative medicine. *Biomed. Microdev.* 12: 575-587.

[14] Kim S. S., Ahn K. M., Park M. S., (2007), A poly(lactide-co-glycolide)/hydroxyapatite composite scaffold with

enhanced osteoconductivity. *J. Biomed. Mater. Res. A.* 80: 206-215.

[15] Guo J., Meng Z., Chen G., (2012), Restoration of critical-size defects in the rabbit mandible using porous nanohydroxyapatite-polyamide scaffolds. *Tissue Eng. Part A.* 18: 1239-1252.

[16] Tran N., Webster T. J., (2011), Increased osteoblast functions in the presence of hydroxyapatite-coated iron oxide nanoparticles. *Acta Biomater.* 7: 1298-1306.

[17] Goto K., Tamura J., Shinzato S., (2005), Bioactive bone cements containing nano-sized titania particles for use as bone substitutes. *Biomaterials.* 26: 6496-6505.

[18] Khang D., Lu J., Yao C., (2008), The role of nanometer and sub-micron surface features on vascular and bone cell adhesion on titanium. *Biomaterials.* 29: 970-983.

[19] Ba Linh N. T., Lee K-H., Lee B-T., (2013), Functional nanofiber mat of polyvinyl alcohol/gelatin containing nanoparticles of biphasic calcium phosphate for bone regeneration in rat calvaria defects. *J. Biomed. Mater. Res. Part A.* 101A: 2412-2423.

[20] Qiao C., Zhang K., Jin H., (2013), Using poly(lactic-co-glycolic acid) microspheres to encapsulate plasmid of bone morphogenetic protein 2/polyethylenimine nanoparticles to promote bone formation in vitro and in vivo. *Int. J. Nanomed.* 8: 2985-2995.

[21] Wei G., Jin Q., Giannobile W. V., (2007), The enhancement of osteogenesis by nano-fibrous scaffolds incorporating rhBMP-7 nanospheres. *Biomaterials.* 28: 2087-2096.

[22] Schwab E. H., Pohl T. L., Haraszti T., (2015), Nanoscale control of surface immobilized BMP-2: Toward a quantitative assessment of BMP-mediated signaling events. *Nano Lett.* 15: 1526-1534.

[23] Jensen T., Baas J., Dolathshahi-Pirouz A., (2011), Osteopontin functionalization of hydroxyapatite nanoparticles in a PDLLA matrix promotes bone formation. *J. Biomed. Mater. Res. A.* 99: 94-101.

[24] Liu Y., Deng L. Z., Sun H. P., (2016), Sustained dual release of placental growth factor-2 and bone morphogenic protein-2 from heparin-based nanocomplexes for direct osteogenesis. *Int. J. Nanomed.* 11: 1147-1158.

[25] Subbiah R., Hwang M. P., Van S. Y., (2015), Osteogenic/angiogenic dual growth factor delivery microcapsules for regeneration of vascularized bone tissue. *Adv. Health. Mater.* 4: 1982-1992.

[26] Santo V. E., Gomes M. E., Mano J. F., (2013), Controlled release strategies for bone, cartilage, and osteochondral engineering—part II: Challenges on the evolution from single to multiple bioactive factor delivery. *Tissue Eng. Part B. Rev.* 19: 327-352.

[27] Tokatlian T., Shrum C. T., Kadoya W. M., (2010), Protease degradable tethers for controlled and cell-mediated release of nanoparticles in 2- and 3-dimensions. *Biomater.* 31: 8072-8080.

[28] Shah S., Sasmal P. K., Lee K-B., (2014), Photo-triggerable hydrogel-nanoparticle hybrid scaffolds for remotely controlled drug delivery. *J. Mater. Chem. B.* 2: 7685-7693.