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Keywords

Tissue engineering, regenerative medicine, musculoskeletal diseases, stem cells, MSC, bone morphogenetic proteins, biomimetic peptides, biomaterials, collagen, titanium, bioglass, hydroxyapatite

Research Lines

- ✓ Tissue engineering for bone tissue regeneration
- ✓ Biomaterials
- ✓ Modified bioactive molecules
- ✓ Mesenchymal stem cells

Scientific Activity

The group's current research activity is focused on the search for new therapeutic solutions based on the use of mesenchymal stem cells, active biomolecules and natural and synthetic scaffolds oriented to the obtention of constructs to be applied to bone and cartilage defects.

Research lines:

✓ Tissue engineering for bone tissue regeneration

Bone tissue loses its natural self-healing capacity with age, and becomes prone to fractures that no longer heal. Bone diseases are a major concern for Healthcare Systems because they are common in every period of life. Skeletal reconstructive surgery is needed in infants to correct skeletal malformations, and in adults to treat severe back pain (spinal fusion), nonunion fractures, traumas due to accidents, bone loss as a consequence of infections or cancer, etc. In orthopaedic surgery, autologous bone grafts are considered the gold standard for the repair of defects, but the small amount of available tissue and the risk of morbidity limit their use. On the other hand, allogeneic grafts, sourced from bone banks, can cause rejection and transmission of certain diseases. Tissue engineering (TE) has emerged as a promising alternative to bone grafts by developing in vitro constructs made up of cells, biomolecules and biocompatible scaffolds.

✓ Biomaterials

Bone TE requires the use of biomaterials with a high level of porosity, a broad surface area and specific three-dimensional morphology to facilitate tissue neoformation, including angiogenesis. Collagen can satisfy all these needs, but bone needs as well an appropriate mechanical strength which collagen lacks. In this field the group is dedicated to the development and production of nanostructured ceramics, bioglasses, collagenic and silk biomaterials in order to develop constructs for skeletal tissue engineering, susceptible of being functionalized with biomolecules and mesenchymal stem cells (MSC). We also work on improving the osseointegration of permanent metal prostheses to prevent their current long-term failure. In this particular field, we are developing porous, 3D, custom-made prosthetic implants thanks to EBM (Electron Beam Melting) metal sintering. This is an additive technology that allows reproducing almost any shape and geometry into a metal structure.

✓ Modified bioactive molecules

The most promising molecules for bone repair are the bone morphogenetic proteins (BMPs), which have been shown to act on osteoprogenitor cells and to induce bone formation. BMPs are FDA-approved in Europe and in the United States for the treatment of spinal disc diseases and open tibial fractures. For being effective, BMPs must be combined with an adequate matrix, which is used to retain the proteins at the site of application and to support the migration of osteoprogenitor cells to the implant. In spite of having low affinity to collagen, recombinant human BMPs are currently delivered on absorbable collagen sponges (ACS). As a consequence, most of the BMP is washed away after its application. This not only has economic consequences because high doses (milligrams) of rhBMP-2 or rhBMP-7 (OP-1) must be used to achieve bone repair, but might also be potentially dangerous because of the undesired side effects such amounts of BMP may cause. On the other hand, biomimetic peptides derived from extracellular matrix proteins are widely used to promote the adhesion, survival and differentiation of osteoblastic cells. In the area of active biomolecules the group has developed different molecular tools directed to selectively induce bone formation. Our expertise is oriented to designing chimeric growth factors or biomimetic peptides with an additional domain to increase the affinity of these molecules to a specific biomaterial. The binding of these improved osteogenic factors to the scaffolds has been demonstrated to be very stable during a prolonged period of time. The application of such factors provides controlled delivery systems with high osteoinductive capacity. So far, our group has produced and patented a collagen-targeted rhBMP-2 (rhBMP2-CBD) and a collagen-targeted RGD biomimetic peptide (CBD-RGD), and is currently working on other novel combinations.

✓ Mesenchymal stem cells

The sole application of matrices containing molecular osteoinductive agents might not solve all the problems of orthopedic surgery. Since BMPs are chemical effectors that act on the cells to induce them to repair bone, the use of these molecular triggers without or with insufficient or inefficient effector cells may be a loss of valuable resources in some surgical interventions. In these cases, endogenously provided new cells might be needed to repair the damage. The bone forming cells are the osteoblasts, which differentiate from mesenchymal stem cells (MSCs). These can be obtained from different tissues, being bone marrow and fat the most common clinical sources. However, there is not yet a consensus about the best protocol for the clinical use of MSC.



Should they be used naïve or should they be primed for osseous differentiation prior to implantation? Should they be administered close to the moment of bone trauma or prosthesis implantation, or should they be administered after a bony callus has been initiated? Is the same procedure always advisable or would it depend on the clinical case to be treated? Such are the questions our group is tackling.

✓ Preclinical Research on Animal models

Any type of biomaterial, coating or cell therapy treatment needs to be tested *in vivo*, in an adequate animal model, before following its path to the clinic. Choosing the right animal model is paramount for obtaining significant results, thus accelerating research and sparing laboratory animal lives. Our group has developed animal models of ectopic bone formation, cranial, maxillary and intrasegmental defects in rats, bone and chondro-orthotopic implants in rabbits and spinal fusion in rat and sheep. We are also setting up non-invasive procedures for following-up the outcome of the tested therapies, so as to obtain more accurate results.

Collaborations

- ❖ **Istituto Ortopedico Rizzoli** (Bologna, Italy). Dr. Enrico Lucarelli. Skeletal tissue engineering (biomaterials and MSCs).
- ❖ **Università degli Studi di Bari**, Ospedale Didattico Veterinario DETO (Italy). Dr. Antonio Crovace. Experimental big animal surgery.
- ❖ **University College London, Institute of Child Health** (UK). Dr. Patrizia Ferretti. Bone regeneration for child skeletal malformations.
- ❖ **University California, School of Medicine, Davis** (USA). Dr. Hari Reddi. Bone morphogenetic proteins (BMPs).
- ❖ **Institute of Advanced Chemistry of Catalonia, CSIC** (Spain). Dr. Jerónimo Blanco. MSCs, biomaterials and tissue engineering.
- ❖ **Instituto Tecnológico de Canarias, ITC** (Spain). Donato Monopoli. Bioengineering and personalized prosthesis design and manufacture by EBM metal sintering.
- ❖ **Instituto de Biomecánica de Valencia, IBV** (Spain). Dr. José Luis Peris. Biomechanical tests and histomorfology.
- ❖ **Universidad Complutense de Madrid** (Spain). Dr. María Vallet-Regí. Ceramic biomaterials
- ❖ **Instituto de Cerámica y Vidrio, CSIC** (Spain). Dr. Felipe Orgaz. Bioglass biomaterials

Research Projects in the last 5 years

- Tailored photo-active Keratin nanofibres as advanced wound Dressings, PROPOSAL N° 37, EURONANOMED II. EUROPEAN INNOVATIVE RESEARCH & TECHNOLOGICAL DEVELOPMENT PROJECTS IN NANOMEDICINE (under evaluation). IP1: José Becerra; IP2: Leonor Santos-Ruiz
- Customized bioengineered implants of porous titanium for maxillofacial reconstructive surgery. Proofs of concept and preclinical assay, BIO2015-66266-R. Ministerio de Economía y Competitividad (2016-2018). PIs: José Becerra and Leonor Santos-Ruiz
- Tissue engineering for bone augmentation previous to surgery for dental implant fixation. DENTIMPLANT. TECHNOLOGY TRANSFER PROGRAM PROJECT. CIBER-BBN/ ZVIT médica (Company) (2016-2017). José L. Gómez y José Becerra
- Titanio poroso con recubrimiento nano y microestructurado y su funcionalización con moléculas osteoinductoras y células para aplicaciones ortopédicas, P11-CVI-7245. Consejería de Economía, Innovación Ciencia y Empresa. Junta de Andalucía (2013-2016). PI: José Becerra
- Development of a construct for the treatment of bone defects, based on titanium-collagen implants with biological functionalization, BIO2012-34960. Ministerio de Economía y Competitividad (2013-2015). PI: José Becerra
- Reparación y Regeneración de Defectos Óseos Segmentarios Mandibulares. Modelo Preclínico Basado en Ingeniería de Tejidos, PI-0555-2013, Consejería de Igualdad, Salud y Políticas Sociales (2014-2015). PI: Leonor Santos Ruiz
- Desarrollo de Recubrimientos y Andamios Bioactivos de Material Cerámico Nano estructurado para la Regeneración Ósea (BIOCEREG) P10-CTS-06681 Consejería de Economía, Innovación, Ciencia y Empresa (2011-2014). PI: Aránzazu Díaz Cuenca
- Redes Temáticas de Investigación Cooperativa en Salud, ACCIÓN ESTRATÉGICA EN SALUD (AES-2012). RED DE TERAPIA CELULAR (TERCEL), RD12/0019/0032. Instituto de Salud Carlos III (2013-2016). PI: José Becerra
- New strategies for bone tissue engineering combining advanced biomaterials, bone morphogenetic proteins and progenitor cells. Subproject 2: Biological characterization of a collagen-targeted rhBMP-2 and a mesoporous material (HA-SBA-15), and their combination with cells into implantable composites for bone repair, BIO2009-13903-C02-01/01. Ministerio de Ciencia e Innovación (2010-2012). PI: José Becerra



Publications in the last 5 years

- Orgaz F, Dzika A, Szycha O, Amat D, Barba F, Becerra J, and Santos-Ruiz L. Surface nitridation improves bone cell response to melt-derived bioactive borosilicate glass scaffolds. *Acta Biomaterialia*. 29: 424-434. 2016
- Ramiro-Gutiérrez ML, Santos-Ruiz L, Borrego-González S, Becerra J, Díaz-Cuenca A. In vitro stimulation of MC3T3-E1 cells and sustained drug delivery by a hierarchical nanostructured SiO₂-CaO-P₂O₅ scaffold. *Microporous and Mesoporous Materials*. 229:31-43. 2016
- Vida Y, Collado D, Najera F, Claros S, Becerra J, Andrades JA and Perez-Inestrosa E. Dendrimer surface orientation of the RGD peptide affects mesenchymal stem cell adhesion. *RSC Adv.*, 6: 49839-49844. 2016
- Visser R, Bodnarova K, Arrabal PM, Cifuentes M, Becerra J. Combining bone morphogenetic protein-2 and -6 has additive effects on osteoblastic differentiation in vitro and accelerates bone formation in vivo. *J. Biomedical Materials Research Part A*. 104(1):178-85. 2016
- Orgaz F, Amat D, Szycha O, Dzika A, Barba F, Becerra J, Santos-Ruiz L. Synthesis of novel ICIE16/BSG and ICIE16/BSG-NITRI bioglasses and description of ionic release kinetics upon immersion in SBF fluid: Effect of nitridation. *Data in Brief* 6: 153–157. 2016
- Villatoro AJ, Fernandez V, Claros S, Rico-Llanos GA, Becerra J and Andrades JA. Use of Adipose-Derived Mesenchymal Stem Cells in Keratoconjunctivitis Sicca in a Canine Model. *BioMed Research International* Volume 2015: 1-10. ID 527926. 2015
- Duran I, Csukasi F, Taylor SP, Krakow D, Becerra J, Bombarely A, Marí-Beffa M. Collagen duplicate genes of bone and cartilage participate during regeneration of zebrafish fin skeleton. *Gene Expr Patterns*. 19(1-2):60-9. doi: 10.1016/j.gep.2015.07.004. Epub 2015 Aug 7. 2015
- Visser R, Arrabal PM, Santos-Ruiz L, Fernandez-Barranco R, Becerra J, Cifuentes M. A collagen-targeted biomimetic RGD peptide to promote osteogenesis. *Tissue Engineering Part A*. 20(1-2), pp.34-44. 2014
- Cuenca-López MD, Andrades JA, Zamora-Navas P, Gómez S, Guerado E, Rubio N, Blanco J, Becerra J. Evaluation of Posterolateral Lumbar Fusion in Sheep Using Mineral Scaffolds Seeded with Cultured Bone Marrow Cells. *International Journal of Molecular Sciences*, 15(12): 23359-23376. 2014
- Claros S, Rico-Llanos G, Becerra J, Andrades JA. A Novel Human TGF- β 1 Fusion Protein in Combination with rhBMP-2 Increases Chondro-osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells. *International Journal of Molecular Sciences* 15(7):11255-74. 2014
- Andrades JA, Becerra J, Muñoz-Chápuli R, Martínez S, Raya A, García-Sancho J, Moraleda JM. Stem cells therapy for regenerative medicine: Principles of present and future practice. *J. Biomedical Science and Engineering*, 7, 49-57. 2014
- Bagó JR., Aguilar E, Alieva M, Soler-Botija C, Fernandez O, Claros S, Andrades JA, Becerra J, Rubio N, Blanco J. In vivo imaging of cell differentiation in demineralized bone: a platform for biomaterial development. *Tissue Engineering Part A*. 19(5-6). pp. 593-603. 2013
- Arrabal PM, Visser R, Santos-Ruiz L, Becerra J, Cifuentes M. Osteogenic molecules for clinical applications: improving the BMP-collagen system. *Biol Res*. 46(4):421-9. 2013
- Claros S, Rodríguez-Losada N, Cruz E, Guerado E, Becerra J, Andrades JA. Characterization of adult stem/progenitor cell populations from bone marrow in a three-dimensional collagen gel culture system. *Cell Transplantation* 21(9). pp. 2021-32. 2012
- Andrades JA, Motaung SC, Jimenez-Palomo P, Claros S, Lopez-Puerta JM, Becerra J, Schmid TM, Reddi AH. Induction of superficial zone protein (SZP)/lubricin/PRG 4 in muscle-derived mesenchymal stem/progenitor cells by transforming growth factor- β 1 and bone morphogenetic protein-7. *Arthritis Res Therapy*. 14(2).pp. R72. . 2012
- Visser R, Arrabal PM, Santos-Ruiz L, Becerra J, Cifuentes M. Basic fibroblast growth factor enhances the osteogenic differentiation induced by bone morphogenetic protein-6 in vitro and in vivo. *Cytokine*, 58(1):27-33. 2012
- Becerra J and Santos-Ruiz L, Editors. *Hot Topics in Cell Biology*. 278 pages. Chartridge Books Oxford, Oxford, UK ISBN paperback: 978-1-909287-00-6; ISBN digital (pdf): 978-1-909287-01-3; ISBN digital ebook (epub): 978-1-909287-02-0; ISBN digital ebook (mobi): 978-1-909287-03-7. 2012
- Visser R and Becerra J. An overview of cell biology in the 21st century. *Hot Topics in Cell Biology*. Edited by José Becerra y Leonor Santos-Ruiz. Chapter: Introduction, Pages: 1-6. Publisher: Chartridge Books Oxford, Oxford, UK. ISBN paperback: 978-1-909287-00-6; ISBN digital (pdf): 978-1-909287-01-3; ISBN digital ebook (epub): 978-1-909287-02-0; ISBN digital ebook (mobi): 978-1-909287-03-7. 2012
- Santos-Ruiz L. Cell biology and beyond: Applications of cell biology. Book: *Hot Topics in Cell Biology*. Edited by José Becerra y Leonor Santos-Ruiz. Chapter: 2. Applied Cell Biology, Pages: 106-111. Publisher: Chartridge Books Oxford, Oxford, UK. ISBN paperback: 978-1-909287-00-6; ISBN digital (pdf): 978-1-909287-01-3; ISBN digital ebook (epub): 978-1-909287-02-0; ISBN digital ebook (mobi): 978-1-909287-03-7. 2012



Publications in the last 5 years

- Becerra J. The Stem Cell And Its Neighborhood: The Niche Of Stem Cells In Animal And Plant Organisms. Hot Topics in Cell Biology. Edited by José Becerra y Leonor Santos-Ruiz. Chapter: 2.2. Cell Therapy and Tissue Engineering. Pages: 146-150, Publisher: Chartridge Books Oxford, Oxford, UK. ISBN paperback: 978-1-909287-00-6; ISBN digital (pdf): 978-1-909287-01-3. 2012
- Amat D, Becerra J, Medina MA, Quesada AR and Mari-Beffa, M. Stem cell therapies. Chapter 8, pp: 173-188. EMBRYOLOGY. Updates and highlights on classic topics. Edited by L.A. Violin Pereira. Intech Open Science. ISBN 978-953-51-0465-0. 2012
- Guerado E, Andrist T, Andrades JA, Santos L, Cerván A, Guerado G, Becerra J. Artrodesis del raquis. Ciencia Básica. Rev Esp Cir Ortop Traumatol. 56(3):227-244. 2012
- Andrades JA, Claros S, Becerra J, and Guerado E. Spinal Arthrodesis: Bone Morphogenetic Proteins as More than One Option. Bone Morphogenetic Proteins: New Research, Chapter 4, pp: 77-98. Anja Nohe. Nova Science Publishers Inc. Hauppauge NY (USA). ISBN: 978-1-61942-409-8. https://www.novapublishers.com/catalog/product_info.php?products_id=30774. 2012

Patents

- Basic fibroblastic growth factor with a specific collagen binding domain. ANDRADES J.A.; BECERRA J.; HALL F.L.; NIMNI M.E. P200002811; PCT/ES01/00449. Entity Holder: University of Malaga.
- Cartilage and bone repair composition. BECERRA J, ANDRADES JA, CIFUENTES M, GUERADO, E. P200401227 (PCT/ES2005/000287). WO 2005/112542. Entity Holder: University of Malaga. Licensed to Indas Biotech, Inc.
- Constructo útil para terapia de regeneración de tejidos, procedimiento de obtención y aplicaciones. DÍAZ-CUENCA A, SANTOS-RUIZ L, BECERRA J. P200702694, N° ES2334298. Entity Holder: CSIC (55%) and University of Malaga (45%)
- Bone morphogenetic protein-2 (BMP-2) with a domain for specific binding to collagen type I, procedure for its obtaining and applications.
- ARRABAL PM, VISSER R, CIFUENTES M, BECERRA J. P200900050/1. Entity Holder: University of Malaga and CIBER-BBN (55%) and FPS (45%).
- Mesenchymal cells and multilayer membrane for the treatment of osteochondral lesions. ANDRADES J.A.; LÓPEZ-PUERTA J.M.; CUENCA-LÓPEZ M.D.; JIMÉNEZ-PALOMO P.; BECERRA J. P201031016; PCT/EP2011/061051. Entity Holder: University of Malaga, CIBER-BBN and FPS
- Histological Method optimized for the preservation of antigenic epitopes and the cellular architecture of tissues from vertebrates. DURÁN I, SANTOS-RUIZ L, SANTAMARÍA JA, BECERRA J, MARÍ-BECCA M. P201000096; PCT/ES2011/000016. ENTIDAD TITULAR: Entity Holder: University of Malaga and CIBER-BBN
- RGD-biomimetic peptide with affinity domain for type I collagen and the uses thereof as osteogenic factor. VISSER R, ARRABAL PM, CIFUENTES M, BECERRA J. P201230017; PCT/ES2013/000007. Entity Holder: University of Malaga and CIBER-BBN
- Bioactive glasses, bioactive glass scaffolds, and cell-seeded bioactive glass scaffolds; preparation methods; and uses thereof. ORGAZ F, SANTOS-RUIZ, L, BARBA F, BECERRA J, AMAT D. N° P201400570, 571 Y 572; PCT/ES2015/070535. Entity Holder: University of Malaga, CSIC and CIBER-BBN