ABSTRACT

The human skeleton represents the supporting structure of the organism and accounts for about 20 percent of the total body mass. Despite its intrinsic capacity to regenerate and self-repair, this ability is limited and repair therapies are needed in a large number of clinical cases. Bone engineering holds the potential to alleviate the increasing burden of bone deficiencies by constructing viable substitutes for replacement therapies. However, before bone engineering can realize its full potential, it is critical to assess the suitability of stem cells derived from different sources for the large-scale construction of bone-engineered substitutes.

The aim of the present thesis was to evaluate the potential of stem cells of embryonic origin for bone engineering applications. In particular, we investigated the potential of two cell lines, denoted matrix free-growth human embryonic stem cells (MFG-hESCs) and embryonic stem cell-derived mesodermal progenitors (hES-MPs). Cells were cultured *in vitro* under static and dynamic conditions, with and without ceramic and metal scaffolds as well as implanted *in vivo* as cell/scaffold constructs.

The results demonstrate that, under similar *in vitro* conditions, both MFG-hESCs and hES-MPs undergo osteogenic differentiation and display higher mineralization properties compared to human mesenchymal stem cells (hMSCs). Differentiation was associated with alteration in the expression of genes involved in ossification, and resulted in the synthesis of a matrix with high content of calcium phosphate deposits. Interestingly, following osteogenic differentiation, MFG-hESCs displayed decreased expression of genes involved in pluripotency and self-renewal, which are also responsible for teratoma formation. In particular, hES-MPs displayed morphological and molecular characteristics typical of hMSCs, but exhibited longer telomeric sequences and significantly higher proliferation ability both in monolayer and three-dimensional cultures. In addition, after flow perfusion stimulation, hES-MPs displayed increased tissue formation, denser collagen network and higher calcium content compared to hMSCs. Not least, hES-MPs displayed an immune profile similar to hMSCs, but did not express HLA-DR molecules. Noteworthy, the expression of HLA-DR was not stimulated following expansion, osteogenic differentiation and treatment with INF-γ. In line with this, hES-MPs did not elicit an immune response after subcutaneous implantation in immunocompetent mice. Finally, it was demonstrated that titanium scaffolds supported the attachment and growth of hES-MPs *in vitro*, and did not seem to affect the expression of genes involved in osteogenesis.

In conclusion, this thesis demonstrates that cells of embryonic origin, under experimental *in vitro* conditions, display some comparative advantages over stem cells derived from adult tissues, which are essential prerequisites for the large-scale production of bone substitutes for replacement therapies. Not least, MFG-hESCs and hES-MPs represent optimal cell technology platforms for the generation of experimental models to study bone histogenesis and explore tissue functionality in different conditions.

Keywords: Human skeleton, bone, bone defects, bone regeneration, regenerative medicine, tissue engineering, biomaterials, scaffold, biocoral, titanium, osseointegration, bioreactor, human stem cells, embryonic stem cells, mesenchymal stem cells, embryonic-derived progenitors, osteogenic differentiation, mineralization, immune response, *in vitro*, *in vivo*

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HUMAN EMBRYONIC STEM CELLS FOR BONE ENGINEERING APPLICATIONS

Akademisk avhandling

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This thesis is based on the following original articles and manuscripts:

I. **Superior Osteogenic Capacity of Human Embryonic Stem Cells Adapted to Matrix-free Growth Compared to Human Mesenchymal Stem Cells.**

Narmin Bidgeli, **Giuseppe Maria de Peppo**, Maria Lennerås, Peter Sjövall, Anders Lindahl, Johan Hyllner, Camilla Karlsson. *Tissue Eng Part A. 2010 Nov;16(11) 3427-40.*

II. **Human Embryonic Mesodermal Progenitors Highly Resemble Human Mesenchymal Stem Cells and Display High Potential for Tissue Engineering Applications.**

Giuseppe Maria de Peppo, Sara Svensson, Maria Lennerås, Jane Synnergren, Johan Stenberg, Raimund Strehl, Johan Hyllner, Peter Thomsen, Camilla Karlsson. *Tissue Eng Part A. 2010 Jul;16(7):2161-82.*

III. **Osteogenic Potential of Human Mesenchymal Stem Cells and Human Embryonic Stem Cell-derived Mesodermal Progenitors: a Tissue Engineering Perspective.**

Giuseppe Maria de Peppo, Peter Sjovall, Maria Lennerås, Raimund Strehl, Johan Hyllner, Peter Thomsen, Camilla Karlsson. *Tissue Eng Part A. 2010 Nov;16(11):3413-26.*

IV. **Human Embryonic Stem Cell-derived Mesodermal Progenitors Display Substantially Increased Bone-like Tissue Formation Compared to Human Mesenchymal Stem Cells after Flow Perfusion.**

Giuseppe Maria de Peppo, Martina Sladkova, Peter Sjovall, Anders Palmquist, Peter Thomsen, Herve Petite, Camilla Karlsson. *Submitted.*

V. **Human Embryonic Stem Cell-derived Mesodermal Progenitors do not Elicit Immune Response *in vivo*.**

Giuseppe Maria de Peppo, Christophe Vidal, Camilla Karlsson, Morad Bensidhoum, Johan Hyllner, Peter Thomsen, Herve Petite, Delphine Logeart-Avramoglou. *In Manuscript.*

VI. **Free-form Fabricated Commercially-pure Ti and Ti6Al4V Porous Scaffolds Support the Growth of Human Embryonic Stem Cell-derived Mesodermal Progenitors.**

Giuseppe Maria de Peppo, Anders Palmquist, Peter Borchardt, Maria Lennerås, Johan Hyllner, Anders Snis, Jukkaa Lausmaa, Peter Thomsen, Camilla Karlsson. *Submitted.*