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506.BONE MARROW MICROENVIRONMENT

Potentiality of Bone Marrow-Derived Mesenchymal Stromal Cells (BM-MSCs) to Differentiate into the Osteogenic Lineage in *in Vitro* Model of Tissue Regeneration

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Introduction: The mesenchymal stromal cells (MSCs) have gained considerable popularity owing to the vast possibilities and lack of ethical constraints. MSCs serve as multipotent progenitors for several niche components in the bone marrow (BM) and play an essential role in the quiescence, proliferation, and differentiation of hematopoietic stem cells. Recently, MSCs from BM (BM-MSCs) represent an attractive cell source for tissue engineering, thanks to their immunomodulatory property, self-renewing and high proliferative capability. BM-MSCs have multi-lineages (adipogenesis, chondrogenesis, osteogenesis) potential and in combination with biomaterials, well support cell-attachments and stimulate extracellular matrix synthesis. For these reasons, BM-MSCs have a great potential for regenerative medicine applications. The present research aims to evaluate the ability of BM-MSCs to proliferate and differentiate toward the osteogenic lineage into innovative three-dimensional bioresorbable scaffold based on gelatin-chitosan hydrogel with a poly(lactic acid) lattice structure (PLA-CH) for regenerative medicine applications in the presence of fetal bovine serum (FBS) or human platelet lysate (HPL) with or without the osteogenic medium (OM).
Methods: BM-MSCs were expanded to the passages 3 or 4 and seeded into the PLA-CH, in dry state, at a cellular density of 7x10 ^{5} cells/scaffolds in growth medium (GM). For inducing osteogenic differentiation, cells/scaffolds constructs were cultured in 24-well plates for 4 weeks with OM consisting of a high-glucose DMEM supplemented with 10% FBS or 5% HPL, 10 $^{-7}$M Dex, 25 mg/ml l-ascorbic acid, and 3mM NaH_2PO_4. For histomorphological analysis at optical microscope, scaffolds were embedded in paraffin using an automatic processor Donatello series 2 (Diapath S.p.A., Bergamo, Italy). Serial paraffin sections (5 μm thick) of each sample were cut, deparaffinized, and rehydrated, according to standard procedures and stained with hematoxylin-eosin stain for general morphology and Von Kossa (Bio-Optica, Milan, Italy) for calcium deposition. Immunohistochemistry for the osteogenic marker Osteocalcin (OSC) (Santa Cruz Biotechnologies, USA) was also performed. For Scanning Electron Microscopy (SEM) (EVO LS-10 manufactured by ZEISS) observation, samples have been progressively dehydrated through immersion in alcohol solutions. The local changes in elemental composition were investigated using the Energy Dispersive X-ray (EDX) analyzer.
Results: The results showed that BM-MSCs have higher affinity to attachment, migrate and differentiate toward the osteogenic lineage inside the novel scaffold PLA-CH both with HPL and FBS as supplement in the culture media. The use of HPL for cell expansion offers the possibility to obtain a safer cellular products without xenogenic contaminants. Cells develop a large spreading area with elongated fibroblast-like morphology preferentially inside the hydrogel pores, showing

great affinity with the scaffold. This is confirmed also by histomorphological analyses at optical microscope, showing homogeneous colonization of cells inside the hydrogel pores (figure 1a). The results showed the presence of calcium deposits in the mash of the scaffold with Von Kossa stain (figure 1b), moreover cells appeared well integrated with a positivity for OSC showing a clear differentiation state as osteoblasts (figure 1c). Calcium and phosphorous were detected with SEM-EDX on the samples incubated in osteogenic medium (figure 1 d,e).

Conclusion: MSCs, derived from bone marrow, associated with scaffold PLA-CH proved to be biocompatible and promising for personalized medicine. In fact, BM-MSCs were able to growth, colonize and osteo-differentiate throughout the hydrogel, demonstrating their potential application for bone regenerative medicine.

Figure 1. *Histomorphological analysis at the optical microscope of PLA-CH with BM-hMSCs in the GM at 100 m m (* ***a****), Von Kossa stain showing calcium deposit distribution on PLA-CH with BM-hMSCs in the OM at 30 m m (* ***b****), immunohistochemistry for OCN with BM-hMSCs differentiated to osteoblast in the OM at 10 m m (* ***c****). Evaluation of calcium (Ca) and phosphorous (P) with SEM-EDX of PLA-CH with BM-hMSCs in OM day 28 (* ***d,e****).*

Disclosures Polverelli: *BMS:* Honoraria; *GSK:* Honoraria; *Abbvie:* Honoraria; *Novartis:* Honoraria. **Malagola:** *Biotest, MSD:* Consultancy, Honoraria. **Russo:** *Medac, Abbvie, MSD, Jazz Pharma, Gilead, Novartis:* Membership on an entity's Board of Directors or advisory committees; *MSD, Novartis, Gilead, BMS, Medac:* Honoraria.

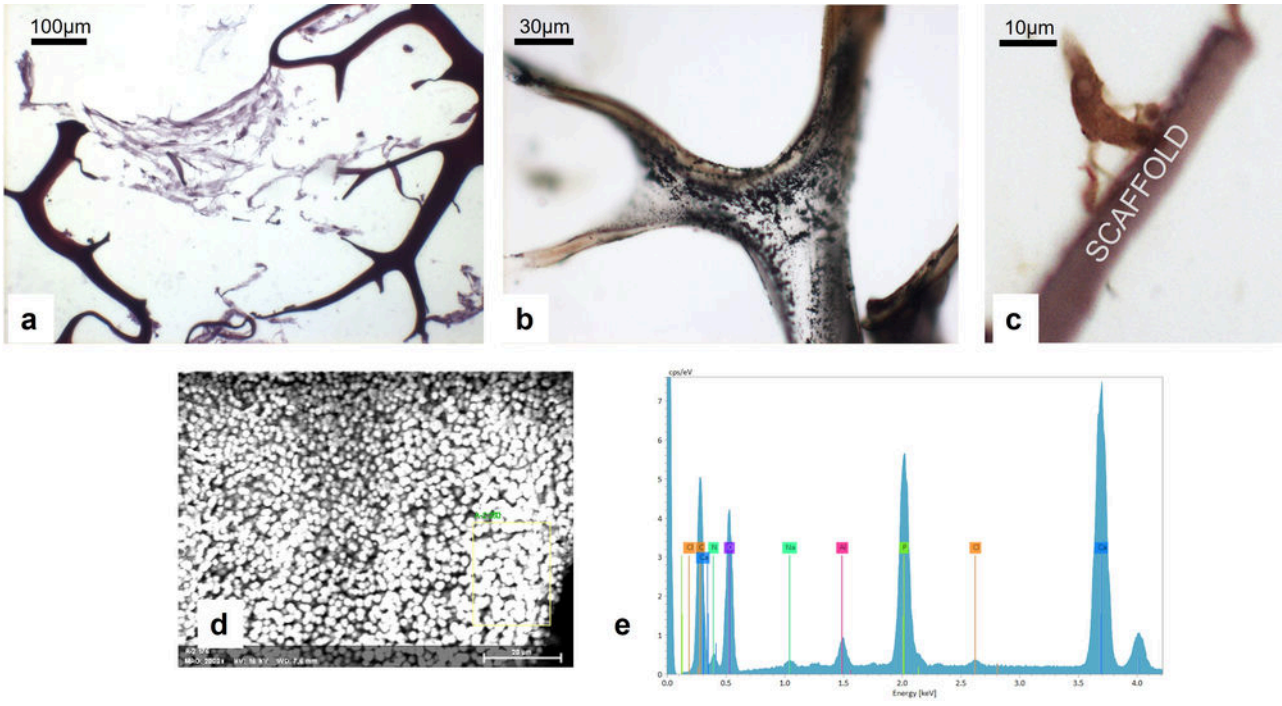

Figure 1

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