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Supplementary Data

Cell interactions and osteogenic differentiation on marine sponge-derived scaffolds: a systematic review

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Analysis	Description				
AlamarBlue®	Quantification of cell viability using a commercial kit				
ALP	Measurement of alkaline phosphatase enzyme activity				
BrdU	Marker for living cells				
Cell Tracker Green	Fluorescent cell staining				
cLSM	Confocal laser scanning microscopy				
FM	Fluorescence microscopy				
HE	Hematoxylin-eosin histological staining				
Hoechst 33342	Fluorescent staining for cellular DNA				
LDH	Measurement of lactate dehydrogenase enzyme activity				
LIVE/DEAD®	Quantification of live and dead cells by fluorescence using a commercial kit				
MTS	Colorimetric assay for evaluating cell proliferation, viability, and cytotoxicity				
MTT	Colorimetric assay for measuring cellular metabolic activity				
PicoGreen®	Quantification of DNA using a commercial kit				
RT-qPCR	Reverse transcription followed by polymerase chain reaction				
SEM	Scanning electron microscopy				
Von Kossa	Histopathological staining				

 Table S1. Summary of analyses conducted in each study.



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Author	Cells	Adhesion assays, period and <i>n</i>	Proliferation assays, period and <i>n</i>	Differentiation assays, period and <i>n</i>	Viability assays, Period and <i>n</i>	Degree of confidence
Clarke et al. [28]	hFOB and gpBMSC ^e	SEM and PicoGreen [®] , 7d, <i>n</i> =4 or 5	LDH and PicoGreen [®] 4, 7 and 14d, <i>n</i> =4 or 5	ALP and RT-qPCR, 7 and 14d, <i>n</i> =4	N/A	<i>p</i> <0.05 and <i>p</i> <0.01
Green <i>et al.</i> [29]	C2C12 i and hOC 1	FM with Cell Tracker Green, 16 h	SEM with Cell Tracker Green, 7 and 21d	ALP and PicoGreen [®] , 7 and 14d	N/A	N/A
Kaya et al. [30]	hOB ^b	FM and SEM, 24, 48 and 72 h	FM and SEM, 24, 48 and 72 h	N/A	Kit WST-1, 24, 48 and 72 h	95%
Lin <i>et al</i> . [31]	C57BL/6J ^h	cLSM, 4, 7, 14 and 21d	cLSM and SEM, 4, 7, 14 and 21d	ALP, RT-qPCR and von Kossa, 7, 14 and 21d, <i>n</i> =3	N/A	<i>p</i> <0.05 and <i>p</i> <0.01
Machałowski <i>et al.</i> [32]	hFOB ª	N/A	Confocal 24 h and 7d	N/A	MTS 1 and 8d	<i>p</i> <0.05 and <i>p</i> <0.1
Mutsenko et al. [33]	hMSC °	SEM and AlamarBlue [®] , 1 and 7d, <i>n</i> =10	BrdU and cLSM, 24 h AlamarBlue [®] , 1 and 7d, n=10	FM, 21d	LIVE/DEAD [®] and cLSM, 1, 7 and 21d	<i>p</i> <0.0001
Mutsenko et al. [34]	hBMSC ^d and hMSC	SEM and cLSM, 7 and 14d	cLSM 1 and 7d	N/A	N/A	<i>p</i> <0.0001
Pallela et al. [35]	MC3T3-E1 ^f and MG-63 ^g	N/A	MTT, 1, 2, 4 and 6d, <i>n</i> =3 and Hoechst 33342, 7d	N/A	MTT 1, 2, 4 and 6d, <i>n</i> =3	N/A
Zheng et al. [36]	hC ^j , rCO ^k	cLSM and SEM, 4 and 7d	ALP, HE and SEM, 14d	N/A	N/A	<i>p</i> <0.01

Table S2. Characteristics of the studies included in the qualitative evaluation (h: hours, d: days, n: number of individuals in sample; N/A: not applicable or data not available).

^a hFOB: human fetal osteoblast; ^b hOB: human osteoblast; ^c hMSCs: human mesenchymal stem cell; ^d hBMSC: human bone marrow stromal cell; ^e gpBMSC: guinea pig bone marrow stromal cell; ^f MC3T3-E1: pre-osteoblastic cell line isolated from rat calvaria; ^g MG-63: isolated human osteosarcoma cell line; ^h C57BL/6J: cell line isolated from rat embryonic stem cells human osteoblast; ⁱ C2C12: cell line isolated from rat myoblasts; ^j hC: Human chondrocytes; ^k rCO: rat calvaria osteoblasts; ¹ hOC: Osteoprogenitor human cells.