Bone Regeneration Medicine

Subjects: Cell & Tissue Engineering

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Bone regenerative medicine is a clinical approach combining live osteoblast progenitors, such as mesenchymal stromal cells (MSCs), with a biocompatible scaffold that can integrate into host bone tissue and restore its structural integrity.

clinical trials | mesenchymal stromal cells | scaffolds | hydrogels

1. Overview of Published Clinical Trials with MSCs and Scaffolds (2018–2023)

A total of nine clinical trials were included in the analysis: six were present in the literature and three were reported on Clinicaltrials.gov (**Table 1** and **Table 2**, **Figure 1**). Background information from each trial was summarized including clinical phase, condition, controls used, and follow-up. Studies were conducted in Spain [1], Mexico [2], Norway [3], the Czech Republic [4], Italy [5][6][7], Western Australia (NCT01742260), and Spain and Portugal [8]. One trial was reported in three publications [5][6][7]. Two clinical trials were at Phase I [1] (NCT01742260), two were at Phase I/II [3][5], one was at Phase IIa [4], and phase was not reported in one clinical trial [2][8]. The studies were prospective, open, non-randomized [1][3][5][6][7], randomized [2][8], interventional (NCT01742260), and in particular, one trial was multicentric [5][6][7]. The most common indications concerned the treatment of lumbar intervertebral degenerative disc disease (DDD) [1], the alveolar ridge [3][8], large skeletal defects [4], and fracture of long bones [1][2][3][4][5][6][9][10][11][12][13][14]. Clinical evaluation showed that the patients achieved lumbar fusion in up to five years using TCP and autologous MSCs [1]. An increase in bone mineralization in association with a decrease in inflammation were obtained thanks to the combination of MSCs from dental pulp and a collagen sponge scaffold in periodontal disease at the 6-month follow-up [2]. In particular, Hernandez et al. evaluated 10 controls for which only collagen scaffold without DPMSCs had been placed, observing a less impressive clinical outcome with respect to the cell-added scaffold group [2]. Successful ridge augmentation without adverse events in maxillofacial bone defects was pursued using BCP and autologous MSCs [3]. No significant differences were obtained using cancellous allografts compared to the combination of TCP and MSCs in promoting the healing of bone defects, whereas significant differences were documented following the implantation of TCP only and cancellous allografts in femoral bone defects [4]. Clinical and radiological evaluation confirmed complete bone consolidation in long bone non-unions at 12 months using biphasic calcium phosphate bioceramic granules and autologous MSCs [5][6][7]. In addition, some clinical trials reported no findings, results, or publications in ClinicalTrials.gov although the study's expected completion dates were 2017 for NCT01742260, 2018 for NCT03682315, and 2022 for NCT03797963. Hydrogels are the new generation of scaffolds for bone reconstruction and DEXGEL Bone, a hydrogel used for alveolar ridge preservation, was shown to stimulate natural bone regeneration without side effects [8]. DEXGEL

Bone is derived from the association of Bonelike by Biosckin® (BL®), a glass-reinforced hydroxyapatite synthetic bone substitute, with a dextrin-based hydrogel named DEXGEL. In particular, Machado et al. compared the synthetic bone substitute BL® (control) to its hydrogel-reinforced version, DEXGEL Bone (test), in the preservation of alveolar ridge dimensions following tooth extraction, demonstrating bone quantity and quality and primary stability of the implant [8]. Finally, considering all studies, the follow-up periods ranged between 1 and 60 months.

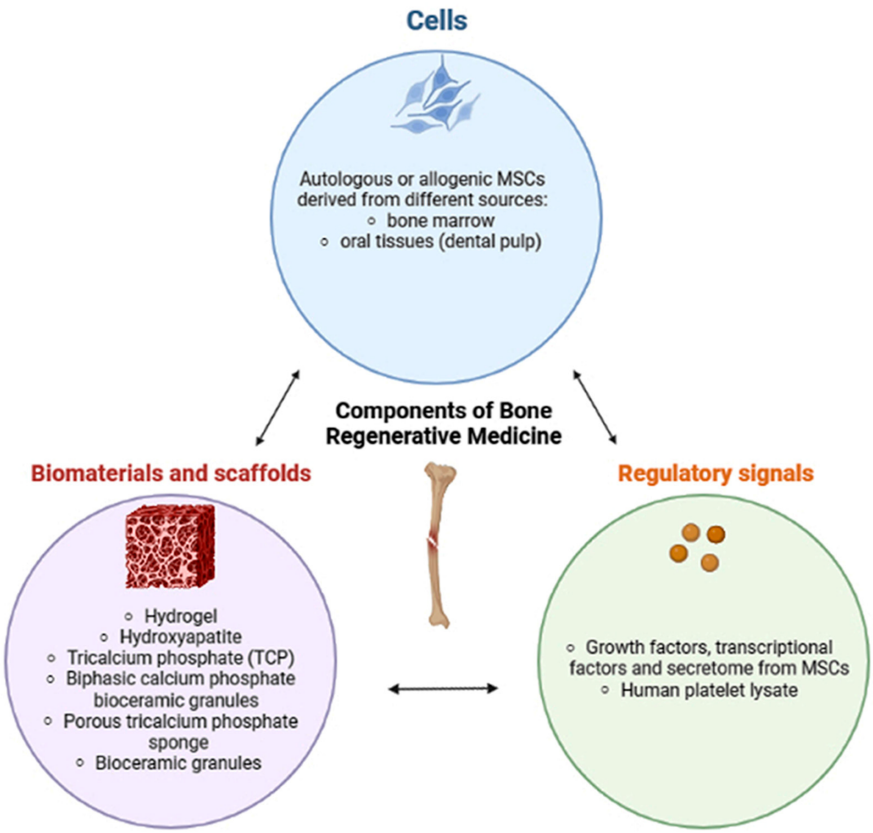

Figure 1. Bioengineering strategy beyond bone regeneration in clinical practice. The nature and structure of the scaffolds have great importance to support cell growth. The MSCs represent a source of growth factors, cytokines, and extracellular vesicles to their surrounding cells, which may favor bone regeneration and osteogenesis [15].

Table 1. Summary of clinical trials using MSCs and scaffolds for bone regeneration from 2018 to 2023.

Cells	Scaffolds	Condition	Number of Patients (Age)	Number of Cells Seeded (Scaffolds Dimensions)	Follow Up	Control	Evaluation Methods and Outcomes	References
Autologous bone marrow-derived MSCs	ß-tricalcium phosphate (TCP)	Lumbar degenerative disc disease (DDD) at L4-L5 or L5-S1	11 (18–65)	1.5×10^{-6} cells/kg from the patient (20 mL of TCP)	1, 3, 6, 12, 60 months	_	Radiography and clinical evaluation revealed that 80% of patients achieved	Blanco et al., 2019 [1]

Cells	Scaffolds	Condition	Number of Patients (Age)	Number of Cells Seeded (Scaffolds Dimensions)	Follow Up	Control	Evaluation Methods and Outcomes	References
							lumbar fusion in up to five years. Both the visual analog scale (VAS) and the Oswestry disability index (ODI) improved after surgery. The Short-Form Health Survey (SF-36) evaluated the physical and mental status that showed a significant improvement in the first year after surgery. There were no adverse effects related to cell implantation.	
MSCs obtained from the dental pulp of two male patients ages 7 and 8 and a 10-year-old patient (hDPSCs)	Scaffold of lyophilized collagen-polyvinylpyrrolidone sponge (Fibroquel; Aspid, Mexico City, Mexico)	Deep infra bony defect ≥ 4 mm deep caused by periodontal disease	22 (55–64)	5×10^6 hDPSCs (0.5 cm^2)	6 months	11 scaffolds without hDPSCs	Increase in the bone mineral density of the alveolar bone; increased salivary superoxide-dismutase and decreased levels of salivary IL1β	Beatriz Hernández-Monjaraz et al., 2020 [2]
Autologous bone marrow-derived MSCs	Biphasic calcium phosphate granules (BCP)	Maxillofacial bone defects	11 (52–79)	20×10^6 cells (1 cm^3)	1, 2, 4,12 months	–	All patients had successful ridge augmentation and an	Gjerde et al., 2018 [3]

Cells	Scaffolds	Condition	Number of Patients (Age)	Number of Cells Seeded (Scaffolds Dimensions)	Follow Up	Control	Evaluation Methods and Outcomes	References
							adequate amount of bone for dental implant installation without adverse events. The alveolar ridge increased both in width and volume.	
Autologous bone marrow-derived MSCs	ß -tricalcium phosphate (TCP)	Femoral bone defect	37 (44–75)	15 ± 4.5 × 10^6 cells (dimensions not reported)	6 weeks, 3, 6, 12 months	Group A: 19 patients with ß -TCP and autologous MSC, group B: 19 patients with ß -TCP alone, group C: 19 patients with cancellous allografts only	The combination between TCP and MSCs appears safe and promotes the healing of bone defects. No significant differences were observed between groups A and B. Significant differences were observed between group B and C. Adverse events emerged from the demanding and extensive character of revision hip replacement without a causal relationship to the suspension of autologous MSCs.	Pavel Sponer et al., 2018 [4]

Cells	Scaffolds	Condition	Number of Patients (Age)	Number of Cells Seeded (Scaffolds Dimensions)	Follow Up	Control	Evaluation Methods and Outcomes	References
Autologous bone marrow-derived MSCs	Biphasic calcium phosphate bioceramic granules (BCP)	Long bone non-unions (fractures of the femur, tibia, and humerus)	28 (3 months), 27 (6 months), 25 (12 months) (18–65). E. Gómez-Barrena et al., 2020 [16] 26 (18–65). E. Gómez-Barrena et al., 2020 [17] 28 (18–65). E. Gómez-Barrena et al., 2020 [18]	20×10^6 cells (5–10 cc of bioceramic granules)	3, 6, 12 months For E. Gómez-Barrena et al., 2020 [18] subgroup analysis of gender, tobacco use, time since the original fracture	_	The ATMP combined with the bioceramic was surgically delivered to the non-unions, and 26/28 treated patients were found radiologically healed at one year (3 out of 4 cortices with bone bridging). E. Gómez-Barrena et al., 2020 [16] The REBORNE bone healing score, defined to perform an evaluation of long bone non-union consolidation in radiograph and computed tomography (CT), proved valid to assess consolidation	E. Gómez-Barrena et al., 2020 [5] E. Gómez-Barrena et al., 2020 [6] E. Gómez-Barrena et al., 2020 [7]

23.

Cells	Scaffolds	Condition	Number of Patients (Age)	Follow Up	Control	Evaluation Methods and Outcomes	References	Registration ID and Country
No cells	Anorganic bovine bone (BioOss Xenograft)	Bilateral Maxillary Sinus Floor Augmentation	8 (>18 years)	6 months	Active Comparator (contralateral: biphasic phycogenic biomaterial and autogenous cortical bone)	CBCT scans before the sinus floor elevation and 6 months later before implant placement to calculate vertical bone height change from the crestal bone to the floor of the maxillary sinus. Histomorphometric quantification of new mineralized tissue, non-mineralized tissue and remaing graft	No publication, no results posted. Actual study completion date 2018	NCT03682315 Responsable: Pablo Galindo-Moreno, Universidad de Granada, Spain Phase not applicable

Cells	Scaffolds	Condition	Number of Patients (Age)	Follow Up	Control	Evaluation Methods and Outcomes	References	Registration ID and Country
						particles in a bone biopsy collected 6 months after the grafting procedure. NO outcomes.		
No cells	Anorganic bovine bone (BioOss Xenograft) + autogenous cortical bone	Bilateral Maxillary Sinus Floor Augmentation	10 (>18 years)	6–12–18 months	Active Comparator (contralateral: Porcine bone mineral (Symbios Xenograft) + autogenous cortical bone)	CBCT scans after the sinus floor elevation and 6–12–18 months later before implant placement to calculate vertical bone height change. Histomorphometric quantification of new mineralized tissue, non-mineralized tissue and remaing graft particles in a bone biopsy collected 6–12–18 months after the grafting procedure. NO outcomes.	No publication, no results posted. Actual study completion date 2022	NCT03797963 Responsable: Pablo Galindo-Moreno, Universidad de Granada, Spain Phas not applicable
No cells but BL® was mixed with autologous blood previously extracted from the alveolar defect and applied with a spatula.	DEXGEL Bone: Bonelike by Biosckin® (BL®), a glass-reinforced hydroxyapatite synthetic bone substitute, in association to dextrin-based hydrogel, DEXGEL	Alveolar ridge preservation	12 (above 18 years)	6 months	BL® granules (250–500 µm) were administered to 6 randomized participants whereas the other 6 received DEXGEL Bone.	Both treatments showed good osseointegration. DEXGEL Bone exhibited increased granule resorption accompanied by a tendency for more new bone ingrowth compared to the BL® group. DEXGEL was rapidly resorbed and accelerated BL® resorption as well, freeing up space that favored	Machado et al., 2023 [8]	EUDAMED: CIV-PT-18–01–02,705. RNEC: 30122. Portugal Phase not reported

on

Bone marrow cells represented the cells most commonly used, particularly autologous cells [1][3][4][5][6][7]. Cells from donors were also used in [2] and NCT01742260. The term ‘stem’ was much more commonly used than ‘stromal’. MSCs from bone marrow were expanded in vitro using GMP, according to common standard operating procedures (SOP), in a specific medium enriched with PL without animal products at different concentrations: 5% PL [1], and 8% PL [5][6][7]. Cells were used at two or three passages [1][4][5][6][7] and one passage [3]. Mononuclear cell isolation after density-gradient centrifugation was performed by Blanco et al. [1], while details of viability analysis by flow cytometry for the positivity of CD90, CD73, and CD105 markers and the negativity of CD14 and CD45 markers were reported by Gjerde et al. [3] and in the ORTHO-1 study [5][6][7]. Other tested markers were reported: MHCI, MHCII, CD16, CD45, CD34, CD19, CD3, CD14, and CD80 [4]. Additional analyses, such as bacteriological tests

Cells	Scaffolds	Condition	Number of Patients (Age)	Follow Up	Control	Evaluation Methods and Outcomes	References	Registration ID and Country
[5][6][7]	[5][6][7]	[5][6][7]	[5][6][7]	[5][6][7]	[5][6][7]	[5][6][7]	[5][6][7]	[1]
[8]						new bone ingrowth, without compromising mechanical support. The healing of defects was free of any local or systemic complications.	2	

al. used sterility, g to the included NCA) of 0 mL [3], rate in a to other units [5][6][7]. hDPSCs from young donors were only used in the research of Hernández-Monjaraz et al. [2]. The nature of the growth factors used in cell culture for cell expansion was not detailed, but it was declared that the experiments were conducted under the strict criteria of GMP, using animal-origin-free reagents [2].

3. Scaffolds for Bone Regeneration

The majority of scaffolds were composed of calcium phosphate ceramic, such as β-tricalcium phosphate (TCP) [1][4], biphasic calcium phosphate bioceramic granules [3][5][6][7], anorganic bovine bone (NCT03682315, NCT03797963), and hydrogel in association with hydroxyapatite [8]. A total of 1.5×10^{-6} cells/kg from the patient were mixed with 20 mL of TCP support [1], 20×10^6 cells/cm^3 were cultivated in BCP [3], $15 \pm 4.5 \times 10^6$ cells were applied onto an absorbable porous β-tricalcium phosphate sponge [4], and 20×10^6 cells per mL were suspended in 10 mL solution with bioceramic granules to obtain the ORTHO-1 MSC tissue-engineered product [5][6][7]. Processing of bone biopsies, after scaffolds were seeded with implanted cells, was performed for histological staining using hematoxylin/eosin and Masson trichrome [5]. In addition, immunohistology was performed to identify macrophages with human CD68 primary antibody by Gómez-Barrena et al. [5][6][7]. Only in the research of Hernández-Monjaraz et al., 5×10^6 DPMSCs dripped suspended in PBS were seeded onto a scaffold of lyophilized polyvinylpyrrolidone sponge® (clg-PVP) in 0.5 cm^2 fragments, while the control group only received PBS without DPMSCs [2]. Finally, in both groups, collagen membranes (Biomed extend®, ZimVie, CA, USA) were placed on the flap. Moreover, in the clinical trial of Herrmann (NCT01742260), the researchers created a skull-like scaffold composed of medical-grade bioceramic granules of beta-tricalcium phosphate by ChronOS (Synthes GmbH, Oberdorf) and cells (concentration not reported) were placed between the specially molded plastic scaffolds (PLA such as 70:30 poly(L-lactide-co-D,L-lactide) [2].

Not all of the studies used scaffolds in association with cells. In particular, the study by Machado et al. demonstrated the ability of the hydrogel to stimulate newly formed bone and biological compatibility with the host tissues [8]. The authors used DEXGEL, an in situ gelling hydrogel with oxidized dextrin as the base, as a moldable carrier of BL® granules in the management of alveolar bone regeneration. BL® is a synthetic bone graft designed to mimic the inorganic composition of bone [8]. Even if no cells were used in association with DEXGEL Bone, BL® (control) was mixed with autologous blood previously extracted from the alveolar defect and applied with a spatula [8]. Moreover, two other studies tested scaffolds without cells for sinus floor augmentation but no results were reported. The first study used xenograft bovine hydroxyapatite (BioOss) (NCT03682315) with contralateral active

control of the biphasic phycogenic biomaterial and autogenous cortical bone. The second study added BioOss to the autogenous cortical bone (NCT03797963) with contralateral active control of the porcine bone mineral (Symbios Xenograft) mixed with autogenous cortical bone.

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