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Advanced therapeutic scaffolds of biomimetic periosteum for functional bone regeneration

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Abstract

Treating chronic bone injuries and defects remains a significant challenge in orthopedic medicine, impacting patient mobility, recovery time, and healthcare costs. The periosteum, a specialized, vascularized connective tissue covering the outer bone surface, plays a crucial role in osteogenesis and skeletal repair. While regenerating the periosteum is critical to restoring bone structure and function, current treatments face substantial limitations, including limited donor tissue availability, donor site complications, and the risk of immunological rejection. Recent advances in biomaterial engineering have driven the development of therapeutic platforms specifically designed to promote periosteal regeneration. These biomaterial-based platforms mimic the biochemical and biomechanical properties of the native periosteal microenvironment. By facilitating key cellular processes involved in osteogenesis and angiogenesis, these materials enable controlled spatiotemporal delivery of bioactive molecules, ion release, modulation of reactive oxygen species (ROS), and enhancement of pro-angiogenic factors. In this review, we discuss the recent advancements in engineered biomaterials, focusing on their mechanisms of action and applications in periosteum restoration. We also provide insights into current challenges and future research directions, emphasizing the critical role of these strategies in clinical practice. The perspectives offered here aim to guide the development of targeted, effective therapies for periosteum repair, ultimately advancing functional bone regeneration.

Keywords Periosteum, Bone regeneration, Biomimetic, Biomaterial, Angiogenesis

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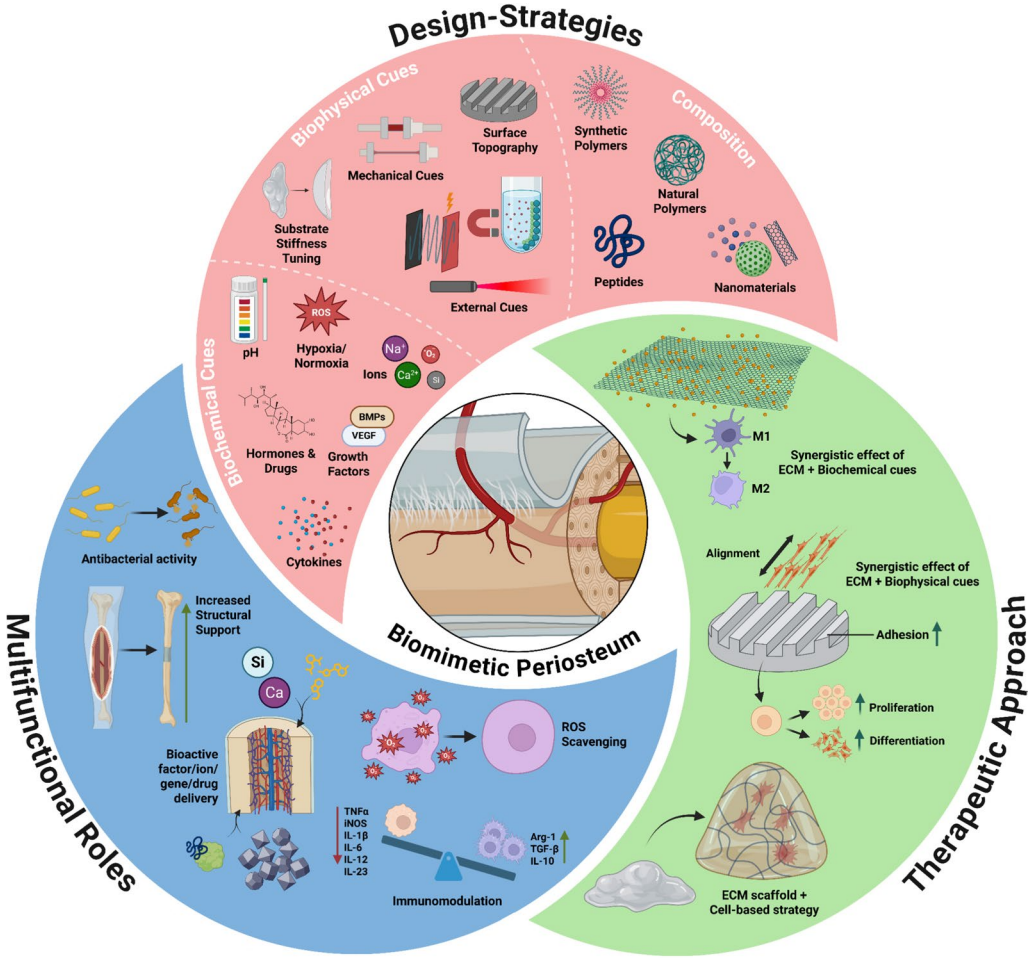
Graphical Abstract



Introduction

The periosteum is a fibrous, multilayered connective tissue that forms the outermost coverage of the bone. This highly osteogenic and vascularized tissue is the niche of progenitor cells and serves as the supply house of the nutrients, minerals, and osteoinductive cues to the bone cortex for tissue remodeling [1]. The absence of periosteum can significantly impair bone regeneration due to reduced vascularization. The remarkable regenerative potential of periosteum has thus intrigued clinicians toward natural tissue transplantation for accelerated bone repair. However, problems such as infections, immune rejection, donor site morbidity, and limited availability have spurred research into the development of artificial periosteum constructs that mimic natural tissues [2–4]. Tissue-engineered scaffolds for periosteum-mediated therapy thus represent a promising approach to supporting functional bone regeneration.

The biomimetic design of the periosteum has engrossed researchers due to the natural microarchitecture of the periosteum. Researchers have developed various approaches to create periosteum-like structures, including replicating periosteal morphologies onto biomaterial surfaces [5], culturing periosteum-derived cells on scaffolds [6], and assembling cell-laden nanofiber mats into multilayered constructs [7]. These engineered periostea have demonstrated improved osteogenic potential compared to traditional scaffolds. Advanced techniques like 3D bioprinting to precisely pattern periosteal-derived cells have also been explored [8]. These approaches aim to mimic the structure and function of periosteum, including its role as a source of osteogenic cells and growth factors. By incorporating periosteum-like components,

tissue-engineered constructs have shown enhanced bone regeneration capabilities, offering promising solutions for treating critical-sized bone defects and advancing the field of bone tissue engineering [9]. The microenvironment rendered by mechanical factors, biochemical cues, and different cells can facilitate cell proliferation and differentiation during regeneration [10]. Recently, a lot of research has been advancing in the areas of developing artificial periosteum using native tissues from intestinal submucosa, induced membranes, cell sheets, and scaffold-cell composites. The cell sheets mimicking the osteogenic cell layer of the periosteum have been successful in neovascularization and bone formation [11]. However, challenges with cell detachment and viability have shifted efforts towards incorporating biochemical factors, such as growth factors and biomolecules, to simulate natural periosteum. Moreover, topographic designs with enhanced biophysical features offer further potential to replicate periosteal functions [12]. Biomaterial designs incorporating multiple biomimetic factors hold great promise for the therapeutic regeneration of functional bone tissue.

Recent advances in periosteum research, illustrated in Fig. 1, underscore the rapid progress in this emerging field. Significant strides have been made in the development of periosteum-mimicking scaffolds, including hydrogel-based structures, nanofibrous membranes, and multilayered scaffolds. A central question in ongoing research is identifying the optimal material characteristics and designs for artificial periosteum. In this review, we summarize the recent biomaterial-based advancements in periosteum regeneration, beginning with an overview of the structural organization of the native periosteum and its crucial role in functional bone regeneration, which provides critical insights for designing artificial periosteum. We further examine recent trends in biomaterial design, emphasizing the design parameters, physicochemical properties, and material characteristics required for effective regeneration. Key strategies are highlighted, including cell-laden heterogeneous structures, physical–chemical hybrids, and topography-specific biomimetic periosteum scaffolds to create multifunctional biomimetic periosteum scaffolds. Lastly, we discuss the clinical implications and challenges faced in periosteum regeneration, offering insights into innovative design strategies for clinical applications of biomimetic periosteum.

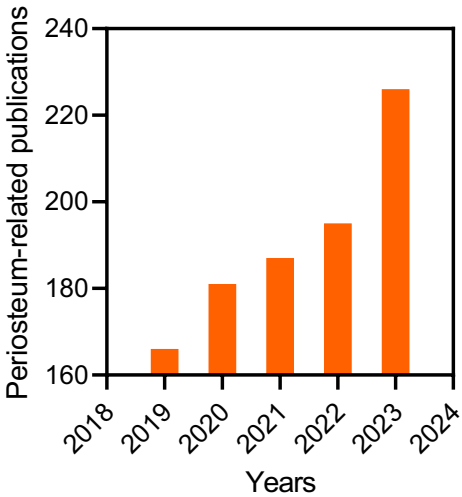


Fig. 1 Number of publications on the periosteum over the last 5 years, identified using the keyword "bone-periosteum" in the Web of Science. This data highlights the significant increase in research focused on the periosteum, emphasizing its critical role in bone biology and regenerative medicine

Periosteum anatomy and function: a brief overview

Periosteum, represented as the vascular outer covering of the bone, comprises osteoprogenitor cells that can potentially differentiate into osteo- and chondroblast [6]. This fibrous and cellular layer, apart from providing structural integrity, is also a critical source of growth factors and can recruit stem cells for the growth, development, and regeneration of bone [13–15]. Initially periosteum was portrayed as a bilayer membrane with osteoblast-like cells in the inner layer [16]; but with the advancements and histological analysis of the periosteum was revealed to consist of different layers: an outer fibrous layer, the inner cambium layer, and an undifferentiated transparent layer in between [15, 17, 18] (Fig. 2). The outer layer is further subdivided into a superficial and a fibroblastic layer. The superficial layer is composed of a majorly collagenous matrix with very few cells, making it inelastic but highly vascularized. However, on the other hand, the fibroblastic layer is highly elastic due to many elastic fibers and contains most of the fibroblasts but less vascularization compared to the superficial layer [13, 14]. The outer fibrous layer, as the name suggests, is highly fibrous due to the presence of collagenous matrix and elastic fibers, whereas the inner cambium layer, also known as the cellular layer, is highly rich in cells. The cambium layer features niches for different cells, like osteoprogenitor cells, mesenchymal stem cells, fibroblasts, and osteoblasts. The majority of cells found in this layer are osteogenic cells under different developmental phases (quiescent, proliferating, differentiating) and osteoblasts [13, 14]. The mature osteoblasts are on the interior side of the periosteum membrane towards the bone lining, while the more differentiating cells (osteoprogenitor cells) are present towards the surface [19]. The cambium layer is also rich in vascularization, and neural networks allow pericytes to stimulate the differentiation of the less

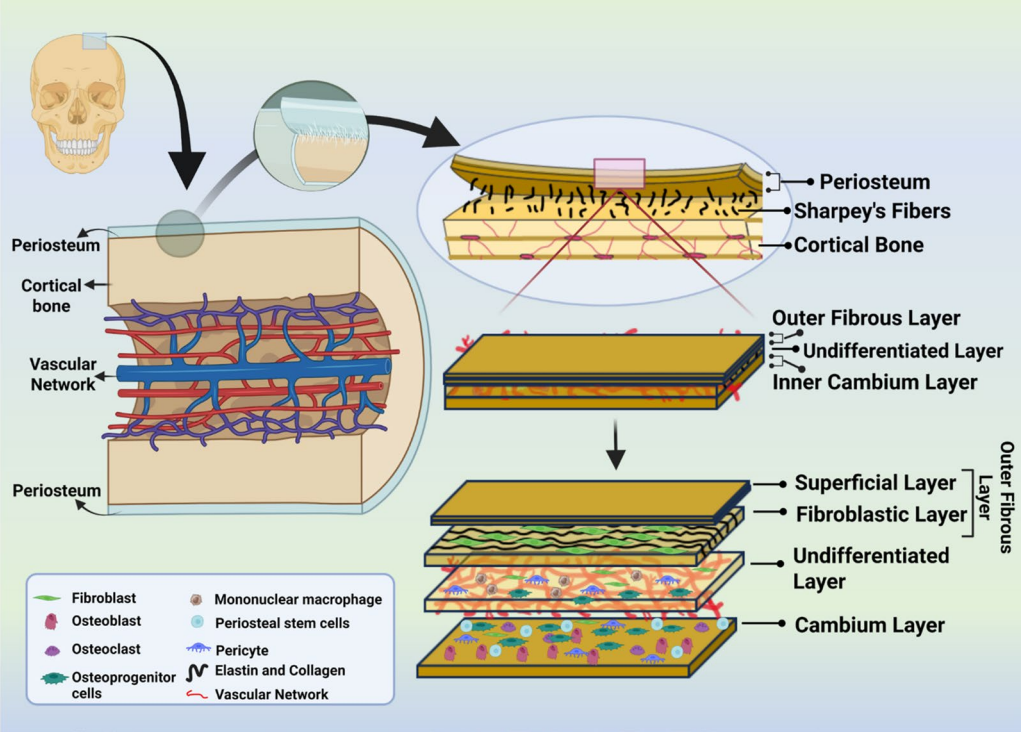


Fig. 2 Schematic illustration depicting the anatomical structure of the natural periosteum

mature cells to more mature osteoblasts for augmented bone formation [20, 21]. The cambium layer has the majority of cells, and the periosteal cells originate from the bottom layer and end in the fibroelastic layer [22]. The undifferentiated layer in between is a house for capillaries, extracellular matrix (ECM), collagen fibers, osteoprogenitor cells, and some fibroblasts. It helps regulate and support bone formation while providing osteoprogenitor cells to both layers [23–25]. It is also considered a protector from different pressures and tensions by transferring the same to initiate tissue remodelling [15].

The healing of a fracture is divided into three imbricating phases: inflammatory, reparative, and remodeling [26–29]. As the injury occurs, there is an immediate occurrence of inflammatory responses followed by a reparative phase comprising new bone formation and finally remodeling, representing a restoration of the injured site with newly formed bone micro- and macroscopically. The periosteum plays a crucial role in fracture healing by providing osteoprogenitor cells and growth factors [15]. It contains a heterogeneous population of progenitor cells, including αSMA-expressing osteochondroprogenitors, which are essential for bone formation during fracture repair [30]. The periosteum's blood supply is vital for fracture healing, as it sustains viability on both sides of the fracture site and revascularizes the distal fragment [31]. However, the importance of the periosteum in rigidly immobilized fractures has been questioned, with some suggesting it may even retard healing under these conditions [32]. In fracture healing, the periosteum can provide a niche for the majority of cells. Other sources, such as the bone marrow, pericytes, and adjacent soft tissues, can contribute to its absence [15]. Nevertheless, the complete absence of periosteum and damage to the intramedullary vascular network can lead to non-union [33].

Periosteum plays a significant role in osteogenesis by providing inductive cues, cells, and physicochemical regulatory factors [34]. Due to an ample blood supply, the periosteum can easily provide nutrients, oxygen, minerals, and other substances required for bone repair. After bone damage, the periosteal blood supply is recruited to the damaged site, and newly formed vessels connect to form neo-vasculature, providing the basis for new bone formation. The pericytes present in the cambium layer of the periosteum can differentiate into osteoblasts during periosteal osteogenesis [35]. The periosteal layer harbors cells that have the potential to differentiate into the osteogenic lineage under different physical, chemical, and biological stimulation. The extracellular matrix of the periosteum provides microarchitecture and inductive cues for cell differentiation required for osteogenesis. The matrix promotes heterotrophic ossification and acellular mineralization of bone [36]. The gaps between

the collagen fibers of the periosteum allow the deposition and stabilization of calcium, phosphate, carbonate, and further growth of apatite. Many growth factors like BMP-2, TGF-β, and IGF-1 are present in the matrix of the periosteum, which plays a substantial role in bone repair and regeneration [15, 37]. Apart from the chemical stimulation, physical cues are also equally involved in the functioning of the periosteum. The periosteum acts as a barrier of the elastic membrane to avoid any cartilage formation resulting from any applied stress to the bone. Because of the close connection of the periosteum and bone, whenever bone bends, the tension from the periosteum (due to contraction) is transferred to the bone surface. During bone remodeling, the stress on the periosteum is transferred onto the bone to regulate the cellular response, where the role of an undifferentiated layer of the periosteum comes into play [15]. Under mechanical stimulation, different signaling pathways and cytokines collectively promote osteogenic differentiation [38]. The structural organization, cell localization, composition, and physicochemical functions of the periosteum can provide great avenues for developing periosteum-mimicking therapeutic options for guided functional bone regeneration.

Engineered biomaterial scaffolds for periosteum regeneration

Researchers have explored various material-based approaches to develop artificial periosteum and promote bone regeneration, including native tissues, cell sheets, and cell-scaffold composites [2]. Despite the advantages of scaffold-free cell sheets, challenges such as stem cell detachment, limited cell survival, and poor graft localization persist. To address these issues and better emulate the functions of the periosteum, it is essential to develop scaffold-cell composites that facilitate proper cell adhesion and growth. For developing an artificial periosteum, the biomaterial that needs to mimic the native periosteum has specific physicochemical properties. Different biomaterials with various compositions have been generated to get a therapeutic reaction for functional bone repair in terms of tissue regeneration, cell proliferation, migration, recruitment, immunomodulation, and others, as shown in Fig. 3. The regenerative potential of periosteum is closely associated with its mechanical and biological properties [39, 40]. Since the periosteum is present between the bone and muscles experiencing dynamic mechanical tension and pressure, the biomaterial with appropriate mechanical properties becomes essential [41]. Also, after implantation, the biomaterial should match the degradation rate with the rate of bone formation. About half of the periosteum can be regenerated naturally within a month post-fracture [39]. Therefore, the biomaterial must remain intact for about 2 weeks to provide enough mechanical support for the cells and biological cues to induce osteogenesis [3]. After that, the material should be degraded to allow ingrowth of the tissues and should be replaced by newly regenerated tissue within a month. Different biomaterials have been developed to mimic the natural periosteum, as presented in Table 1.

Design strategies for artificial periosteum

Scaffolds structure and composition-based strategies

Different natural polymers like collagen, gelatin, fibrin, chitosan, and cellulose have been employed in the polymeric scaffolds for fabricating artificial periosteum. These polymers have high biocompatibility and can enhance cell-material interaction. Engineering scaffolds with polymers can influence biomineralization by the tissues [42]. Collagen is a fibrous protein constituting about 25% of the total protein content in the animal body [43]. It has high viscoelasticity with high tensile strength and low extensibility when reinforced in elastic sheets. Its triple helix structure, arranged in the form of fibers, is mainly responsible for its mechanical and viscoelastic properties. Due to its similar composition to periosteum and low immunogenicity, Collagen has demonstrated high potential in promoting bone formation. Collagen induces bone formation by regulating osteoblast and osteoclast activities through different cell signaling pathways [44]. Bioinspired gradient scaffolds have recently been under advanced research for osteochondral regeneration [45]. However, the scaffold that mimics the natural structure of bone and periosteum offers significant potential for effective bone defect repair; however, developing a gradient scaffold with a cohesive and mechanically stable interface continues to present substantial challenges. Recently, a bioinspired collagen-based bilayer scaffold has been prepared, mimicking the bone-periosteum architecture. A 3D-printed collagen scaffold as the bottom layer for osteogenic differentiation, and a dense collagen electrospun top layer were prepared to mimic the periosteum (Fig. 4A) [46]. The upper layer consists of a collagen-rich scaffold fabricated via electrospinning, designed to emulate the periosteum and inhibit infiltration by reticular fibrous tissue. The lower layer is formed by an in situ mineralized collagen scaffold (IMCS), which supports osteogenic differentiation. Through a sequential fabrication strategy combining 3D printing and electrospinning, a unified scaffold (BP-IMCS) is produced, exhibiting significantly enhanced structural integrity-approximately ten times greater than that of conventionally assembled composite scaffolds. The scaffold improved the bone formation by 32.47% compared to the individual layers. Some other researchers have developed bio-artificial

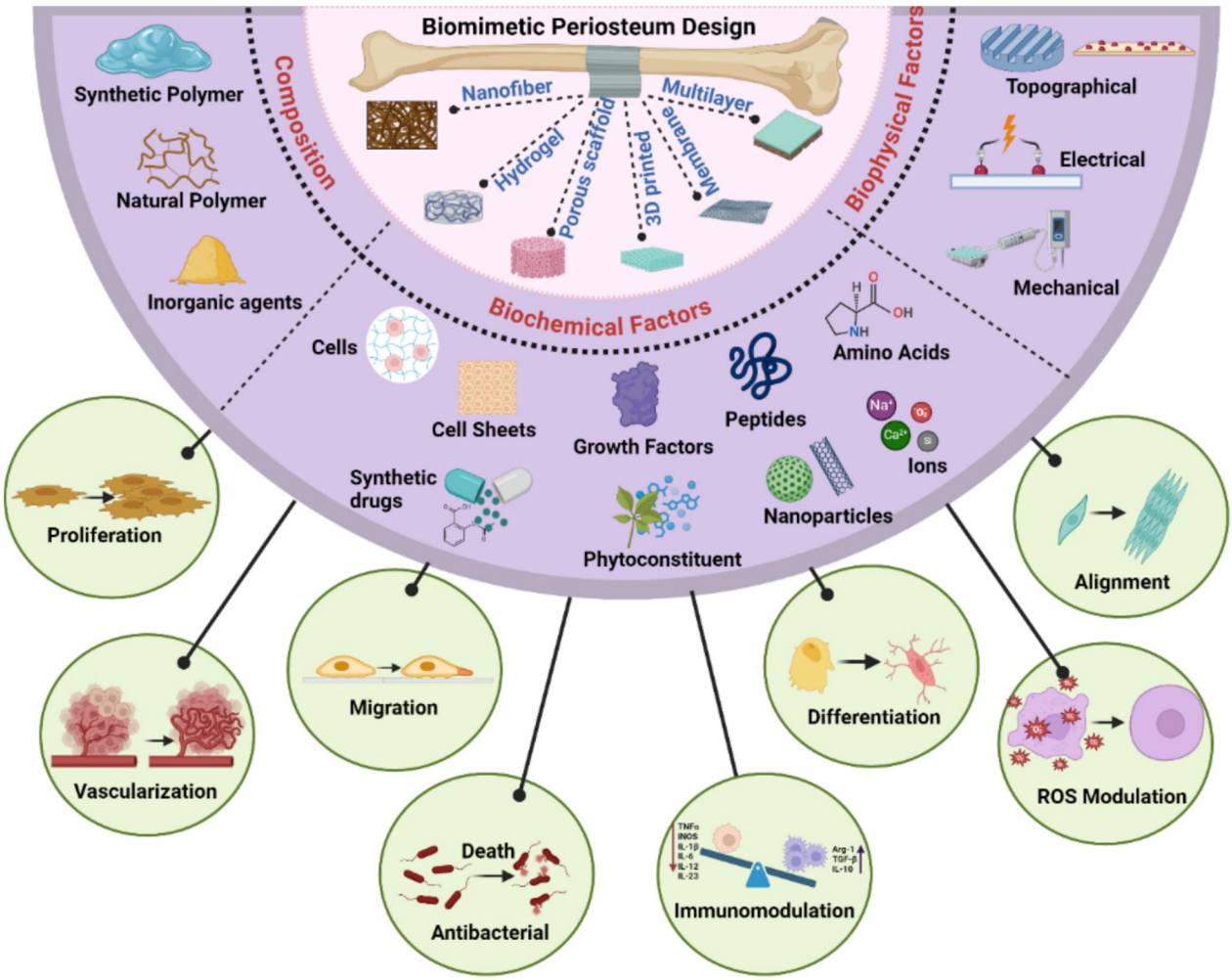


Fig. 3 Biomimetic periosteal design strategies and the therapeutic effects for guided functional bone regeneration

periosteum that is made of collagen sponges and osteogenic cells. The scaffold facilitated the bone formation in the center as well as the edges of the scaffold [47]. In another study, collagen-based scaffolds have allowed bone growth in the mandibular defects in a centripetal manner, wherein bone growth starts from the edges and progresses toward the center [48]. Although collagen has high osteoinductive potential and provides mechanical support to connective tissues, its low in vivo mechanical properties have limited its clinical applications [49, 50]. Moreover, the high degradation rate (within 3–5 weeks) under enzymatic conditions has shifted the research toward other polymer-based scaffolds or integrating collagen scaffolds with nanomaterials to form composites [44, 51].

Gelatin, a commonly used natural polymer, is the precursor of collagen formed by its partial hydrolysis. Due to its ECM-mimicking properties, stemming from unique RGD sequences, gelatin-based biomaterials provide a favorable microenvironment for cellular functions such as adhesion and proliferation [52]. Thanks to its remarkable biodegradability, biocompatibility, and excellent water absorption properties, gelatin has become the most favored natural polymer among researchers for tissue engineering applications. Despite the development of numerous gelatin-based scaffolds for bone tissue engineering, gelatin remains limited by its lower mechanical and thermal stability. However, recently, researchers have reported the synthesis of electrospun scaffolds of gelatin only by modulating the concentration of the solvents and crosslinking agent [53]. A bilayer biomimetic periosteum layer from electrospun gelatin was generated with an aligned and random orientation of fibers with a controlled release of bioactive molecules to regulate inflammation, angiogenesis, and osteogenesis [54]. In another study, gelatin-based electrospun fibers were generated as an artificial periosteum exhibiting appropriate degradation, permeability, biocompatibility, flexibility,

Table 1 Different biomimetic periosteum designs comprising multiple factors targeting periosteal healing for bone regeneration

Composition	Biochemical Cues	Biophysical Cues	Mechanism	In vitro model	Therapeutic application	References
PCL + gelatin nanofiber	Deferoxamine, Aspirin, Silicon nanoparticles	Topographical	Anti-inflammatory, Angiogenic, Osteogenesis, Anti-osteoclastogenesis	MC3T3-E1, HUVECs, NIH3T3, RAW 264.7 cells	Rat skull defect	[54]
	Bioglass, COD liver oil, Magnesium-doped zinc oxide nanoparticles	Topographical	Cell metabolic function	NIH3T3	Biomimicking structure	[69]
	Icariin	–	Cell metabolic function	MC3T3-E1	Biomimicking structure	[55]
	Icariin, Moxifloxacin	–	Antibacterial, Osteogenesis	MC3T3-E1	Rabbit radius defect model under infection	[85, 86]
	Magnesium oxychloride ceramics nanoneedle	–	Cell adhesion, Osteogenesis	MC3T3-E1, EA. Hy926, rBMSCs	Rat calvarial defect regeneration	[87]
PCL + decellularized nerve matrix nanofiber	Black phosphorus	Electrical	Promote axon growth, Neurotransmitter secretion	Schwann cell line (RSC96), BMSCs	C57BL/6 mice neuronal bone regeneration	[88]
PCL nanofiber	MgO, AS-IV	–	Antimicrobial, Cell proliferation, Control inflammatory response, Osteogenesis	MC3T3-E1	SD rat bone regeneration under infection	[89]
	Tantalum, Zinc oxide nanoparticles	–	Antibacterial, Vascularization, Osteogenesis	BMSCs, EPCs	SD rat bone regeneration under infection	[82]
	Whitlockite	Topographical, Mechanical	Osteogenesis, Angiogenesis, Neurogenesis	BMSCs	Rat skull defect	[90]
PCL + TCP nanofiber	E7-BMP-2	Topographical	Cell proliferation, Osteogenesis	rBMSCs	SD rat calvarial defect regeneration	[91]
PLGA nanofiber	Lidocaine, Vancomycin, Ceftazidime	–	Antibacterial, Osteogenesis	–	New Zealand rabbits segmental long bone open fracture	[70]
PLA-TCP nanofiber	Germanium, Selenium	Electrical	Osteogenesis, Nerve fiber ingrowth	BMSCs, SCs, and Saos-2	New Zealand rabbit innervated bone regeneration, treatment for osteosarcoma	[73]
PLA + Periosteal decellularized extracellular matrix nanofiber	–	–	–	hBMSCs	SD rat critical-sized bone defects	[74]
PLA nanofiber	Leptin receptor antibody, BMP2-loaded hollow MnO_2	–	Cell recruitment, Cell metabolic function	BMSCs	C57BL/6 mice sequential bone regeneration	[66]
PLA + Collagen nanofiber	APY29 loaded liposomes	Topographical	Immunomodulation, Angiogenesis, Osteogenesis	RAW264.7, BMSCs, HUVECs	Diabetic rat bone regeneration	[92]
PLA + PVP nanofiber	Graphene oxide, Urolithin A	Mechanical	Antibacterial, Immunomodulation, Osteogenesis	BMSCs, RAW 264.7, HUVECs	Rat bone regeneration under infection	[93]
PVFT nanofiber	Bioactive glass microparticles	Electrical, Topographical	Cell metabolic function	mBMSCs	SD rat critical-sized bone regeneration	[80]
PVB + Bioactive glass nanofiber	Magnesium ion,Zinc ion	–	Immunomodulation, Vascularization, Osteogenesis	RAW264.7, HUVECs, BMMSCs	SD rat calvarial defect regeneration	[94]

Table 1 (continued)

Composition	Biochemical Cues	Biophysical Cues	Mechanism	In vitro model	Therapeutic application	References
Hyaluronan-PLLA nanofiber	VEGF	Topographical	Angiogenesis, Cell metabolic function	BMSCs, HUVECs	SD rat cranial bone regeneration	[95]
GelMA nanofiber	Phage 11	Topographical	Immunomodulation, Cell recruitment, Osteogenesis	BMSCs, RAW264.7, HUVECs	Balb/c mice femoral defect regeneration	[96]
	L-arginine, Methacrylated hydroxyapatite nanoparticles		Cell metabolic function, Angiogenesis, Osteogenesis	rBMSCs, HUVECs	Rat critical-sized calvarial defect regeneration	[4]
Collagen nanofiber	–	–	Osteogenic differentiation, Inhibits fibroblast invasion	BMSCs	Biomimicking structure	[46]
Chitosan nanofiber	–	–	Osteogenic differentiation	Luc-ASCs	Periosteum mimic	[62]
GelMA hydrogel	E7 peptide, Wharton's jelly microparticles	–	Cell metabolic function	BMSCs	SD rat skull defect bone regeneration	[58]
	Magnesium-ion-modified black phosphorus nanosheets	–	Vascularization, Neurogenesis, Bone regeneration	BMSCs, HUVECs, NSCs, PC12	SD rat calvarium defect regeneration	[57]
	Human mesenchymal stem cells	Topographical	Cell migration, Angiogenesis, Osteogenesis	hMSCs, HUVECs	BALB/c mice skull defect regeneration	[97]
SilMA hydrogel	PLLA microspheres, CaO_2 nanoparticles	–	Macrophage phenotypic transition, Osteogenesis, Angiogenesis	BMSCs, M2 macrophages, HUVEC	C57BL/6 male mice skull defect regeneration	[11]
PVA hydrogel	Curcumin, Phytic acid	Topographical	Antibacterial, Anti-inflammatory, Osteogenesis	BMSCs, RAW 264.7	Biomimicking structure	[98]
Fibrin hydrogel	Polydopamine-g-C_3N_4 nanosheets	Mechanical	Immunomodulation, Angiogenesis, Osteogenesis	hEMSCs	Mouse bone regeneration under oxidative stress	[99]
Gelatin + calcium alginate hydrogel	Diselenide	–	Angiogenesis, Osteogenesis	BMSCs, HUVECs	SD rat critical-size bone defect regeneration	[100]
Alginate + GelMA hydrogel	Whitlockite nanoparticles	Mechanical, Electrical	Osteogenesis, Angiogenesis, Neurogenesis	BMSCs	SD rat calvarial bone	[101]
Acrylamide + GelMA hydrogel	–	–	Immobilize bone graft	BMSCs	New Zealand rabbit tibial defect regeneration	[102]
PNIPAM + GelMA hydrogel	Gold nanorod-porous silicon, Bone-forming peptides, Angiogenic peptides	Mechanical	Angiogenesis, Stem cell migration, Osteogenesis	HUVECs, hBMSCs	SD rat tibial defect regeneration	[103]
PEGS hydrogel	CaP nanoparticles		Cell metabolic function, Proangiogenesis	HUVECs, BMSCs	SD rat distraction osteogenesis	[104]
PEGPLADM hydrogel	Mesenchymal stem cells	–	Vascularization, Endochondral bone formation	mMSCs	C57BL/6 mice bone allograft healing	[35]
3D printed collagen	$Ca(NO_3)_2{\cdot}4H_2O$, $K_2HPO_4{\cdot}3H_2O$	–	Osteogenic differentiation	BMSCs	Biomimicking structure of periosteum and bone	[46]
3D printed GelMA, Methacrylated silk fibroin, GelDA	Graphene oxide nanosheet, Bone marrow mesenchymal stem cells	Topographical	Osteogenic differentiation	–	Biomimicking structure	[76]
PCL scaffold	rhBMP-2, Periosteum-derived mesenchymal cells	-	Cell migration, Osteoinduction	rPMSCs, hPMSCs	SD rat critical-size femoral defect regeneration	[105]

Table 1 (continued)

Composition	Biochemical Cues	Biophysical Cues	Mechanism	In vitro model	Therapeutic application	References
Collagen porous scaffold	Osteogenic cells	–	Osteogenic differentiation	-	Rat open fracture	[47]
Gelatin porous scaffold	rhBMP-2	–	Endochondral ossification, Recruit periosteum derived stem cells	PTDCs	C57BL/6 mice cranial defect regeneration	[106]
PCL + Chitosan-Xanthan porous scaffold	–	–	Osteogenic differentiation	Dental pulp stem cells	Biomimicking structure	[63]
PLGA porous scaffold	Baicalein	–	Inhibit soft tissue infiltration, Cell metabolic function, Angiogenesis, Osteogenesis	BMSCs, HUVECs	Rat craniomaxillofacial bone defects	[71]
Polyurethane membrane	*Cissus quadrangularis* extract	–	Osteogenic differentiation	MC3T3-E1, HUVEC, rBMSCs, Saos-2	Rat critical bone tibial defect	[107]
PLA membrane	Carbon nanotubes	Topographical	Cell alignment, Osteogenesis	hBMSCs	SD rat periosteal defect	[72]
PLGA membrane	–	Topographical	Cell alignment, Osteogenesis	ADMSC	Biomimicking structure	[65]
Gelatin membrane	Hydroxyapatite nanoparticles	Topographical	Cell alignment, Angiogenesis, Osteogenesis	rMSCs,	SD rat vascularized bone regeneration	[75]
Collagen membrane	$BaTiO_3$, Multiwalled carbon nanotubes	Mechanical, Electrical	Immunomodulation, Osteogenesis	BMSCs	C57/BL6 mice cranial defect regeneration	[83]
Elastic wood membrane	Pamidronate disodium, Deferoxamine	Topographical	Cell adherence, Angiogenesis, Osteogenesis	mBMSCs, HUVECs	SD rat critical-sized skull defect	[64]
Eggshell membrane	Nanoceria	Topographical	Cell metabolic function, Angiogenesis	hOMFs, Macrophages, SC cells	C57BL/6 J mice cranial defect regeneration	[108]
PDMS membrane	–	Topographical	Inhibit fibroblast infiltration, Cell alignment, Angiogenesis, Osteogenesis	rMSCs, HUVECs	SD rat critical-sized calvarial defect	[109]
PHBV membrane	PHA, Barium titanate	Electrical	Osteogenesis, Immunomodulation	BMSCs, RAW 264.7	Rat critical-sized cranial defect	[110]
Cuttlebone-derived organic matrix membrane	–	Topographical	Cell metabolic function	rBMSCs and HUVECs	SD rat calvarial defect	[111]

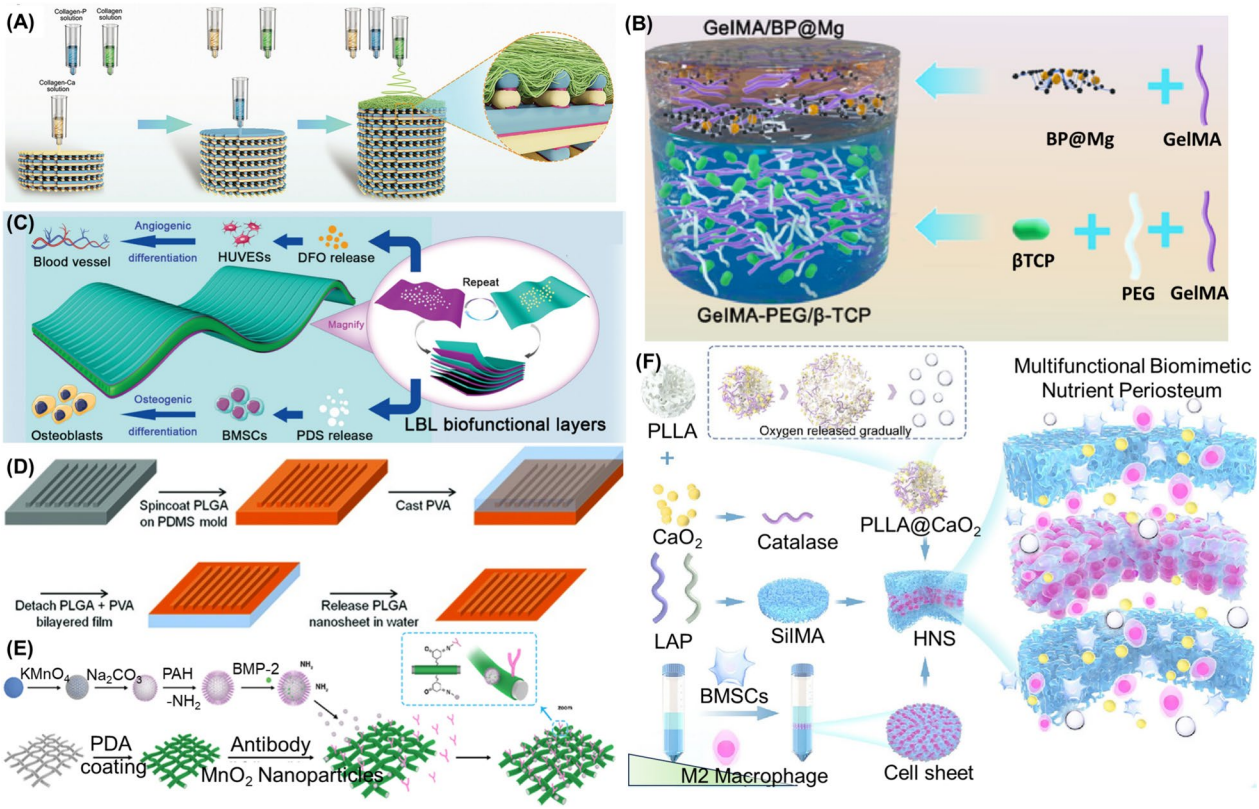


Fig. 4 Different structural periosteum-mimicking designs. **A** Schematic for continuous manufacturing of 3D-printed and top-layered electrospun scaffold. Reproduced with permission from John Wiley & Sons [46]. **B** Bilayered GelMA hydrogel exhibiting periosteal and bone repair layers. Reproduced with permission from Elsevier [57]. **C** Elastic wood-derived layer-by-layer assembly of membranes for artificial periosteum. Reproduced with permission from John Wiley & Sons [64]. **D** Microgroove patterned PLGA sheets. Reproduced with permission from John Wiley & Sons [65]. **E** PLA nanofibrous membrane loaded with nanoparticles. Reproduced with permission from American Chemical Society [66]. **F** Hamburger-like structure comprising a middle cell sheet layer to mimic the natural periosteum. Reproduced with permission from Elsevier [11]

and osteoinduction [55]. Angiogenesis and neurogenesis are essential processes in bone regeneration [56]. While numerous studies have focused on biomaterial implants that replicate the structure and function of native bone tissue, the reconstruction of the neurovascular network has largely been overlooked in biomaterial design. Hydrogel has been used for periosteal regeneration by using methacrylated gelatin in a bilayer form, with the upper layer serving as the periosteal repair layer and the bottom as the bone repair layer (Fig. 4B) [57]. In this study, a bilayer hydrogel system mimicking the periosteum is employed to investigate its potential in enhancing bone regeneration by stimulating both angiogenesis and neurogenesis. The proposed hydrogel platform integrates black phosphorus (BP) nanosheets modified with magnesium ions (BP@Mg) to facilitate neurovascularized bone tissue repair. The upper layer consists of BP@Mg incorporated into a gelatin methacryloyl (GelMA) hydrogel, while the lower layer comprises a double-network hydrogel formed by two interpenetrating polymer matrices of GelMA and polyethylene glycol diacrylate (PEGDA), reinforced with β-tricalcium phosphate (β-TCP) nanocrystals. The magnesium ion modification strategy was specifically designed to improve the stability of BP nanosheets and enable the sustained release of therapeutic ions. A biocompatible artificial periosteum hydrogel membrane was fabricated using modified gelatin to chemotactically recruit bone marrow stem cells for better cell proliferation and differentiation [58].

Fibrin is a biopolymer synthesized by the aggregation of fibrinogen and used as a proficient healing material. It not only works as a hemostatic material but is also actively involved in cell proliferation, adhesion, migration, differentiation, angiogenesis, and controlling inflammation [59]. Gassling et al. generated a platelet-rich fibrin periosteum with good cellular metabolic activity and proliferation but low biocompatibility compared to the collagen membrane [60]. Demol et al. investigated the influence of fibrin carriers on the periosteal cells toward osteogenic development [61]. The carrier upregulated

the cell proliferation but reduced the expression of osteogenic markers. Although fibrin can enhance cellular metabolic activities, its uncontrolled degradation and limited mechanical properties limit its use in regenerating the periosteum [3]. Chitosan is a polysaccharide-rich in acetyl groups, having a slow degradation rate and good antibacterial properties. Chitosan-based porous matrices for periosteum showed slow degradation, low thrombogenicity, and improved osteoinductivity [26]. Romero et al. generated a periosteum mimicking freeze-dried porous coating and nanofiber membrane of chitosan, showing enhanced ALP activity and can deliver osteoprogenitor cells [62]. In another study, a chitosan-based bilayer membrane was developed as an osteoinductive periosteal substitute. A highly porous and compressible scaffold with roughness and hydrophilicity is considered suitable for cell growth and migration [63]. Although chitosan presents superior mechanical strength compared to collagen, it still lacks the mechanical strength required to mimic natural periosteum [3].

Apart from the natural polymers, researchers have also used natural components like elastic wood directly from the plants as layer-by-layer assembly for enhanced biocompatibility and regenerative potential (Fig. 4C) [64]. Current artificial periosteum designs face significant limitations, including challenges in replicating the natural anisotropic mechanical and structural properties, insufficient adhesion to tissue, and inadequate coordination of angiogenic and osteogenic processes. Drawing inspiration from natural wood (NW), an elastic, wood-derived artificial periosteum is engineered to replicate both the architecture and functional attributes of native periosteum. This construct integrates an elastic wood (EW) framework, a polydopamine (PDA) adhesive interface, and sequentially assembled biofunctional layers via a layer-by-layer (LBL) approach. The EW, obtained from NW, acts as an anisotropic scaffold that guides cellular alignment and exhibits mechanical properties-such as elastic modulus and flexibility-comparable to those of natural periosteum. To support coordinated vascular and bone tissue formation, the LBL-modified surface functions as a controlled release system, enabling spatially and temporally regulated delivery of pamidronate disodium (PDS) and deferoxamine (DFO), encapsulated within chitosan (CS) and hyaluronic acid (HA) matrices, respectively. Additionally, the synergistic application of PDA coating and LBL bioactive layers significantly enhances the scaffold's adhesion to bone defect sites.

These natural polymers show enhanced cellular metabolic activities, but poor mechanical strength, uncontrolled degradation, and pathogenic impurities limit their clinical applications. One common strategy to address these challenges is to crosslink the polymer and create composite systems that incorporate synthetic polymers and inorganic additives [67, 68]. Synthetic polymers like poly (lactide-co-glycolide) (PLGA), polylactic acid (PLA), and PCL can be implicated in the development of artificial periosteum due to their excellent mechanical properties [3]. Tariq et al. used an interdisciplinary approach to fabricate a multilayer electrospun periosteum [69]. The researchers generated a hybrid material comprising poly(ε-caprolactone) (PCL) as a support layer, PCL/gelatin/magnesium-doped zinc oxide as a vascular layer, and gelatin/bioactive glass/COD liver oil as an osteoconductive layer. The materials, having different properties, when integrated into the scaffold, enhance cell proliferation, adhesion, and growth, thereby successfully mimicking the native periosteum. An artificial PLGA periosteum membrane was developed for treating femoral fractures [70]. The nanofiber membrane enhanced the activity and demonstrated better clinical performance, which can be used to treat open fractures. Inspired by the periosteum-bone complex, a porous PLGA scaffold was synthesized using the negative mold and phase separation technique. The scaffold demonstrated an above periosteum-like dense structure, which inhibits fibroblast infiltration, and the bottom porous structure allowed osteogenic cell adhesion, proliferation, and differentiation [71]. Micropatterned structures in the PLGA sheets have also been fabricated using a PDMS mold as a periosteum mimetic scaffold (Fig. 4D) [65].

A 'sticker-like' PLGA nanosheet featuring microgrooved surface patterns is fabricated using a straightforward approach that integrates spin coating with micropatterning techniques. These microstructured PLGA nanosheets exhibit strong physical adhesion to both flat and porous substrates while maintaining stability in aqueous conditions. Additionally, their ability to influence and organize cellular spatial orientation highlights their potential as a biomimetic periosteum for applications in bone tissue regeneration. PLA-based nanofibrous membranes have also been developed to mimic the native periosteum (Fig. 4E) [66]. A functionalized artificial periosteum was engineered using an electrospun scaffold modified with a Leptin receptor antibody (LepR-a) and bone morphogenetic protein 2 (BMP2)-loaded hollow manganese dioxide (h-MnO_2) nanoparticles, facilitated by a polydopamine (PDA)-mediated surface modification strategy. This biomimetic periosteum exhibited appropriate mechanical strength along with excellent biocompatibility, making it suitable for bone tissue engineering applications. PLA inverse opal membrane was synthesized to mimic artificial periosteum to adhere to the defect area and enhance bone regeneration [72]. Xu et al. developed a PLA nanofiber membrane to mimic the bone-periosteum structure,

which provided good biodegradable properties and mechanical support and helped in bone regeneration [73]. Multilayered PCL nanofibrous membranes have been fabricated to mimic the characteristics of native periosteum [69]. The presence of PCL in the matrix of the scaffold demonstrated a uniform degradation rate and excellent mechanical properties. A bi-layered PCL porous membrane with slow degradation properties was synthesized to provide long-term support for cell growth and good mechanical strength [63]. The periosteum, resembling physico-chemical properties, was obtained by fabricating a PCL electrospun membrane, providing high tensile strength and long-term durability [74]. The periosteum plays an essential role in bone regeneration by orchestrating osteoimmunomodulatory responses and promoting neovascularization. Nevertheless, the scarcity of autologous periosteum and the limitations of conventional tissue engineering strategies, which often rely on simple structural designs, hinder the effective replication of periosteal functions. To overcome these challenges, Hao et al. proposed a biomimetic nutrient periosteum featuring a distinctive hamburger-like configuration (Fig. 4F) [11]. This design incorporates a central layer composed of a mixed cell sheet containing millions of bone marrow stromal cells (BMSCs) and M2 macrophages. Flanking this cell sheet are silk protein-based hydrogel layers embedded with porous PLLA microspheres and CaO_2 nanoparticles. The engineered hydrogel layers not only reinforce the mechanical integrity of the construct but also enable a sustained release of oxygen from CaO_2 for up to 30 days, thereby enhancing the viability of the encapsulated cells. This biomimetic strategy successfully recapitulates the structural and functional characteristics of the native periosteum, providing a supportive regenerative microenvironment.

Synthetic polymers having controllable degradation and mechanical properties usually face challenges regarding biocompatibility and bioactivity. Further modulation of the materials with additive and bioactive molecules is highly sought for clinical applications. With the extended research on biogenic periosteum, biomaterials infiltrated with inorganic additives have gained a lot of attention to overcome the limitation of mechanical strength lacking by natural polymers and bioactivity by synthetic polymers. As of now, different inorganic additives like hydroxyapatite, bioactive glass, tricalcium phosphate, graphene oxide, manganese dioxide nanoparticles, and zinc oxide nanoparticles have been used to develop an artificial periosteum [66, 69, 73, 75, 76]. Hydroxyapatite is the natural component of the bone and is the most preferred inorganic additive for bone regeneration. Adding hydroxyapatite to the gelatin membranes can enhance the mechanical strength of the substitute and induce a long-term degradation rate. Also, releasing calcium ions can modulate the microenvironment towards a more osteogenic [77]. Bioactive glass has been reported to have the potential for osteoinduction, osteoconduction, and osteointegration for enhanced bone regeneration [78, 79]. The tissue-engineered periosteum comprising the layer made of gelatin/bioactive glass/COD liver oil was prepared for better osteoconductive properties. Bioactive glass is considered a preferred inorganic additive in bone scaffolds due to its good bioactivity, due to the release of calcium, phosphorus, and silicon, and it has the potential to deposit hydroxyapatite under simulated fluids [69]. In another study, bioactive glass added to the synthetic poly (vinylidene fluoride-trifluoroethylene) polymer promoted angiogenesis and proliferation of osteoblasts by providing calcium and silicon ions for enhanced bone regeneration [80]. Adding nanoparticles to the polymer matrices can also significantly influence the biomaterial's physical properties as well as the cellular responses [11, 69, 81–84]. The presence of manganese dioxide nanoparticles on the surface of the PLA bionic scaffold significantly eradicated the inflammation-induced oxidative stress, as evidenced by the increased cell viability and reduced expression of COX2 [66]. Apart from the structure and composition of the biomaterials, the involvement of biochemical factors like cells and growth factors, as well as the biophysical factors like topographical architecture, and mechanical and electrical stimulation, are equally essential for modulating the bone regeneration mechanism of the periosteum.

Biochemical factor-based strategies

During a fracture, numerous biomolecules are involved in the healing process, playing critical physiological roles in the regeneration of bone tissue. These biomolecules, such as whole cells, cell sheets, growth factors, cytokines, signaling molecules, peptides, phytoconstituents, amino acids, synthetic drugs, nanomaterials, and ions, regulate key stages of healing, including cell proliferation, differentiation, vascularization, ROS modulation, antibacterial, migration, and immunomodulation (Fig. 5A). Their coordinated actions are essential for restoring bone integrity and function [112]. Biocomposite materials offer advantageous physicochemical properties for tissue regeneration, such as mechanical strength and structural support. However, they often lack inherent bioactivity, which limits their ability to actively promote cellular interactions and tissue healing. To overcome this, bioactive components, such as growth factors or biologically functionalized molecules, are typically incorporated into these materials to enhance their regenerative potential and mimic the natural bioactivity of tissue environments [113, 114].

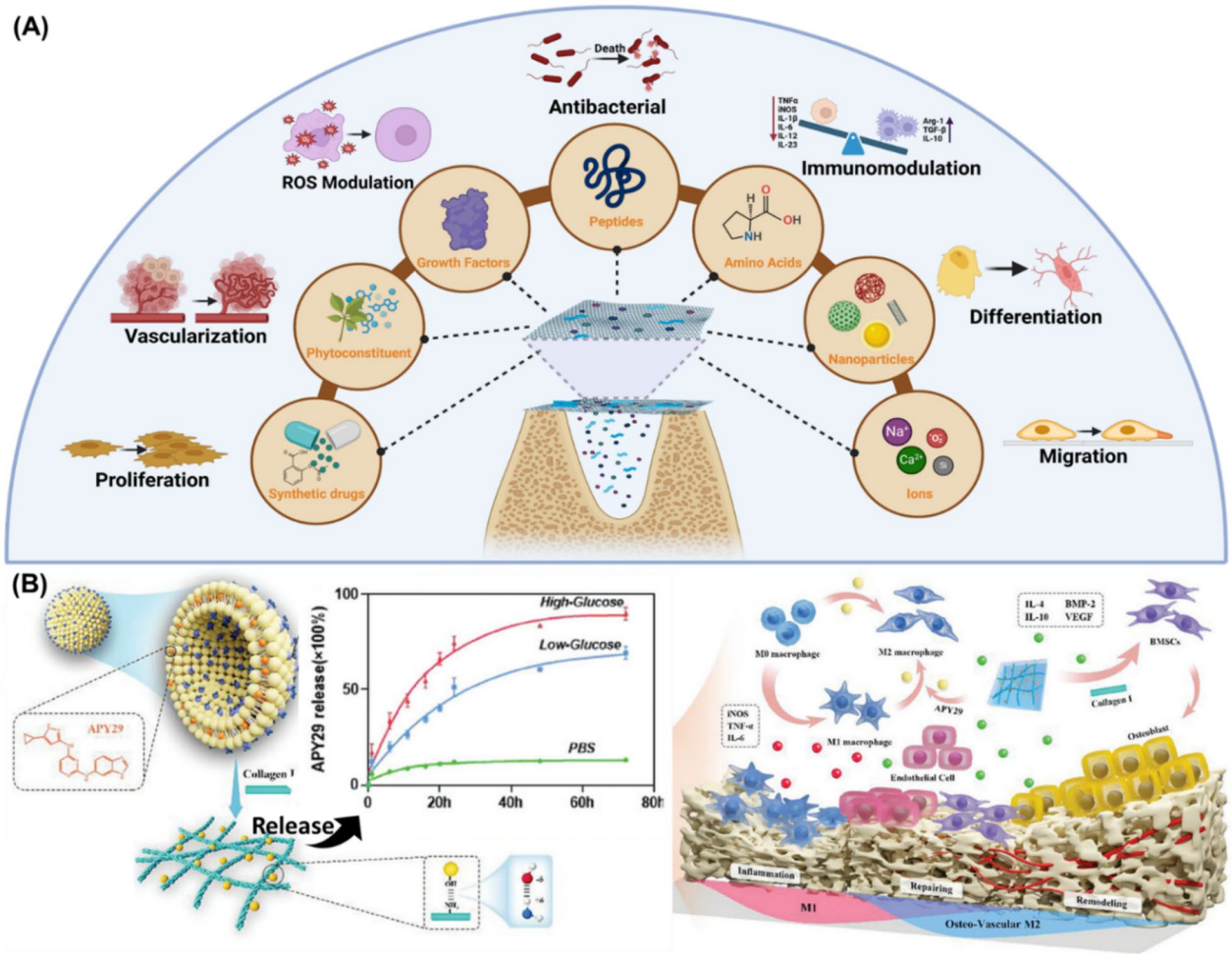


Fig. 5 Biochemical factors-based strategies for the artificial periosteum. **A** Schematic illustration of different biochemical factors that can be incorporated into the periosteum matrix and can modulate the cellular activities by their sustained/controlled release into the defect milieu. **B** An illustrated image representing the surface functionalization of the nanomaterial containing APY29, an immunomodulator, onto the polymeric periosteum matrix modulating macrophage polarization and osteogenesis by its sustained release. Reproduced with permission from John Wiley & Sons [92]

The periosteum, a highly vascularized tissue, serves as a critical niche for a variety of cell types essential for bone healing and regeneration. The cells for biomimicking the periosteum can be used differently: first, the cells can be added directly to the polymeric scaffolds, and second, they can be used to develop cell sheets and then be used directly at the defect site. Transplanting osteoblasts accelerated bone formation and biomineralization, as observed through the enhanced expression of ALP and OCN [115]. Incorporating MSCs in biomimetic periosteum also upregulated the expression of osteogenic genes for improved bone formation potential [35, 116]. MSCs can release signaling molecules in the microenvironment, which can stimulate the resident cells to repair the tissues further. Bone marrow MSCs loaded with gelatin-based bioink were developed for 3D-printed artificial periosteum. The cell-laden 3D-printed substitute effectively induced enhanced in vitro and in vivo osteogenesis [76]. The periosteum is a highly vascularized tissue that provides a nutrient and oxygen supply to the underlying bone cells for efficient functionality. Using endothelial cells in the artificial periosteum is advantageous for forming new blood vessels and providing therapeutic effects to the newly forming bone [117]. Research has also advanced the use of scaffold-free cell sheets containing their native ECM as an artificial periosteum layer providing a similar biological microenvironment. Under this technique, normally, the confluent and viable layer of the cells are picked without using proteolytic enzymes, rendering cell-to-cell junction and the deposited basal ECM

[118]. These cell sheets can provide better cell retention, high vascularization, and juxtacrine interaction with the host cells at the defect area [119]. A biomimetic cell sheet periosteum was generated using human amniotic MSCs, which was further used to develop a double cell sheet for bone healing [120]. The double cell sheet comprising the osteogenic and vascular cell sheet successfully promoted bone formation and rat calvarial defect repair. The synergistic effect of both the cell sheets showed higher bone mineral density after 12 weeks as compared to the individual cell sheets. A mixed cell sheet comprising bone marrow stem cells (BMSCs) and M2 macrophages was used as the middle layer in a hamburger-like structure to mimic the periosteum, effectively replicating the regenerative microenvironment [11]. The cell sheet layer allowed simultaneous angiogenesis, osteogenesis, and osteoimmunomodulation. Although cell sheets have been proficiently used to mimic the periosteum, poor mechanical properties, easy fragmentation during cell transfer, and inadequate nutrient supply within dense cell sheets can generate hypoxic conditions, thereby limiting their clinical applications. Moreover, using cells with biomaterial poses difficulty in maintaining cell viability, and the limited cell source requires more advancements in stimulating cells in the defect area.

Further, to make the artificial periosteum polymer matrices functional, the effective way is to incorporate growth factors like bone morphogenetic protein (BMP), fibroblast growth factor (FGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF) to regulate the signaling pathway for enhanced bone regeneration [121]. BMPs have very high osteoinductive potential, showing great therapeutic effects in the treatment of bone disorders. Growth factors like BMP2 and BMP7 act as direct stimulators of osteoblast differentiation [122]. Similarly, newer therapeutic candidates targeting the Wnt signaling pathway also function as direct osteoinductive agents. In contrast, certain signaling molecules primarily act as mitogens, promoting the proliferation of bone-forming cells or enhancing vascularization [123]. This role is exemplified by factors such as Platelet-Derived Growth Factor (PDGF) and FGF. VEGF holds a particularly significant role, as it not only stimulates angiogenesis but also indirectly influences osteoblast activity by promoting BMP production through endothelial cells [124]. Beyond the traditional pathways focused on bone formation, emerging therapeutic strategies aim to target additional aspects of the bone healing process, including the modulation of inflammation, enhancement of vascularization, and facilitation of cell migration to the fracture site. BMP2 decorated onto the surface of biomimetic periosteum PLA scaffolds significantly enhanced the proliferation of MSCs, further increasing osteogenic differentiation and mineralization [66]. VEGF attached to the micropattern of the artificial periosteum membrane allowed enhanced cell differentiation and vascularization [75]. Wu et al. loaded the fibrous bionic periosteum layer with the vascular endothelial growth factor to induce the endogenous cambium layer for enhancing the regeneration of bone [95]. Although growth factors have high efficacy, they have a very short shelf life and are very expensive, thereby limiting their use for clinical applications.

Small biomolecules, besides cells and growth factors, can also stimulate cellular activities and activate cell signaling pathways for replicating the biomolecular functions of native periosteum [125, 126]. They can be loaded within the polymeric matrix or decorated onto the surface to modulate cell activities through their sustained/controlled release. Small molecules have emerged as promising alternatives to growth factors in bone regeneration, offering advantages such as lower costs, improved stability, and reduced side effects [127]. Both natural and synthetic small molecules have demonstrated osteoinductive properties by activating various signaling pathways. Melatonin, resveratrol, and purmorphamine influence MAP kinase, BMP, Wnt, Sirt1, and Hedgehog pathways to promote bone formation and osteogenic differentiation [127]. Plant-derived compounds like decalpenic acid, flavonoids, and quinones have shown potential in bone regeneration [128]. These molecules can act as co-activators of the BMP2 pathway, activate Wnt signaling, or inhibit the NF-κB pathway. Recent research has focused on developing delivery strategies for naturally-derived small molecules in bone regenerative engineering applications [129]. Additionally, small molecule-based approaches have been explored for craniofacial and dentoalveolar bone reconstruction, addressing challenges in complex medical cases [130]. Icariin, a phytocompound, has been considered effective in bone regeneration by creating a balance between the differentiation of osteoblasts and osteoclasts [85]. *Cissus quadrangularis,* commonly known as bone setter, has also proven excellent bone regenerative properties and prevents bone loss under oxidative stress [131]. Incorporation of the optimized fraction when loaded in the artificial periosteum membrane provided better cell differentiation and bone formation under compromised bone due to osteosarcoma [107]. When incorporated in the polymeric membrane, Baicalein, a flavonoid, showed enhanced pro-osteogenic properties by increased expression of ALP, type I collagen, OCN, and formation of calcium nodules [71]. Different antibiotics like vancomycin, moxifloxacin hydrochloride, rifampicin, curcumin, and ceftazidime have been used during bone regeneration to overcome the issues of infections becoming the main

cause of implant failures after surgical procedures or bone loss during osteomyelitis [85, 98, 132, 133]. Modification of the polymeric matrix with the RGD peptide and amino acids enabled enhanced cell spreading and adhesion of the osteoblasts from the periosteum for proper bone regeneration [134–136]. Any fracture is associated with the inflammatory responses as the initial phase of tissue repair. Modulation of the initial inflammatory response to pro-regeneration is important for bone healing. Immune cells like T cells and macrophages act indirectly in bone healing by secreting cytokines into the osseous niche. Macrophages are the major players in regulating tissue regeneration by polarizing into different phenotypes [137]. After the injury, M1 macrophages are recruited towards the injury site and secrete pro-inflammatory cytokines to clear the damaged cells and further recruit stem cells. However, the prolonged inflammatory phase can be detrimental, and hence, it is critical for the macrophages to be polarized to the M2 anti-inflammatory phenotype. An immunomodulator, APY29, is used to polarize the macrophages towards M2 and further stimulate the secretion of osteogenic and angiogenic markers (Fig. 5B) [92]. Under diabetic conditions, fluctuating glucose levels and increased inflammatory responses create a hostile environment that significantly hinders periosteal regeneration. Effective modulation of the immune response within injured tissues is essential for maintaining a stable immune microenvironment, as well as supporting osteogenesis and angiogenesis. To address the challenges presented by the hyperglycemic conditions at acute injury sites, a glucose-responsive composite scaffold-comprising polylactic acid (PLA), type I collagen (COL I), and liposome-encapsulated APY29 (PCLA)-was developed. The presence of self-assembled type I collagen on the scaffold surface facilitates osteogenic differentiation, while the system's glucose sensitivity enables localized release of APY29-loaded liposomes. This targeted release induces macrophage polarization toward the pro-regenerative M2 phenotype, suppresses pro-inflammatory cytokine production, enhances the bone immune microenvironment, and promotes both osteogenesis and angiogenesis. Other anti-inflammatory biomolecules like lidocaine, COD liver essential oil, phage P11, and phytic acid have been incorporated into the biomaterial matrix for effective regenerative properties [96, 98, 132, 138].

Biophysical factor-based strategies

Recently, biophysical factors have been equally important in influencing periosteum function and bone regeneration. These factors, like topographical, mechanical, and electrical stimulation, help in the physical resemblance to the natural periosteum for improved bone healing. Surface topography at the micro/nanoscale can evoke specific cellular responses, influencing cell growth, differentiation, and matrix deposition [139]. The periosteum shows prominent topographical changes during the healing process. After the injury, the periosteal reactions lead to the thickening of the inner cellular layer of the periosteum [3, 140]. The proliferating progenitor cells then show a beam-like structure, followed by the wavy topography due to callus formation. The developed periosteum has longitudinally oriented cells and the extracellular matrix that further regulates the formation of aligned collagenous fibers, cell alignment, and direction of bone development [65]. Therefore, topography plays a significant role in biomimicking the periosteum to modulate osteogenesis. A sticker-type PLGA sheet with microgroove patterns was generated using a PDMS mold [65]. A Janus artificial periosteum with a top micropatterned surface and a lower microfibrillar adhesive surface was fabricated [109]. The bottom microfibrillar pattern was inspired by the Gecko satae for better interaction with the bone surface. The top micropatterned surface with different grooves of 40–120 µm can influence cell adhesion differently and modulate the phenotypic orientation of the macrophages. Such phenotypic polarization of macrophages for osteo-immunomodulation was also achieved by using aligned nanofibers [92, 96]. Zhao et al. have generated a bi-layered biomimetic periosteal membrane with different orientations of nanofibers [54]. The top aligned fibers mimic the outer fibrous layer, while the bottom random layer mimics the inner cambium layer of the periosteum. The scaffold patterns for the bionic periosteum can also be modified by varying the pore sizes of the scaffolds to regulate different cell functionality. Zhang et al. have shown how varying the pore sizes of the PLGA scaffolds can influence the behavior of different cells for different layers of the periosteum, ranging from inhibiting the cell intrusion due to small pores to protein adhesion and cell differentiation by large pore sizes [71]. An anisotropic scaffold for the periosteum has also been generated, inspired by the elastic wood pattern, to resemble the physico-chemical properties of the periosteum [64, 98]. The surface roughness provided by the layer-by-layer arrangement of the scaffolds also controls cell proliferation and growth [69, 95]. The micro/nanopattern on the surface of the artificial periosteum also influences the functions of the cells. Patterns generated by carbon nanotubes [72], Mg-doped ZnO nanoparticles [69], hydroxyapatite nanoparticles [75], graphene oxide nanosheets [76], and bioactive glass particles [80] have recently been surging to mimic the periosteum-like physical characters. These topographical patterns can create remarkable niches to mimic periosteum characters and induce bone regeneration with or without any biochemical factors. In a recent study, a biomimetic nanoceria

mineralized eggshell membrane was developed [141]. The incorporation of nanoceria was intended to mimic the surface topography of the periosteum. When compared to the natural periosteum, this biomimetic membrane provided similar pore size, fiber orientation, and diameter, suggesting the role of nanoceria in maintaining the topographical surface of the periosteum. Topography plays a crucial role in regulating cell behavior, influencing various aspects such as morphology, adhesion, migration, and proliferation. Both micro- and nano-scale features can affect cellular responses, with recent studies indicating impacts on gene expression and differentiation [142]. The extracellular matrix (ECM) mediates these interactions, with nanotopography influencing protein adsorption and subsequent cell responses [143]. Mechanotransduction pathways, including focal adhesion signaling and Rho/ROCK activity, are involved in these biomechanically driven cellular responses [144]. The physical architecture of the substrate alone can modulate pathways like MAPK, potentially regulating cell proliferation [145]. Synthetic nanotopography has been used to control intricate cellular mechanisms such as stem cell lineage commitment and tissue architecture formation [146].

The periosteum is the outer covering of the bone with viscoelastic properties ranging between 920 and 1930 kPa, stabilizing the bone [4]. The layers of the periosteum are designed to control the dynamic mechanical tension and pressure experienced between the muscles and bone [41, 147]. Periosteum layers comprising collagen and elastin fibers with Sharpey's fibers attached to the bone surface at an angle of 45 degrees provide unique mechanical properties [41]. Providing mechanical stimulation to artificial periosteum can be beneficial in bone repair. It has been observed that mechanical unloading can lead to bone resorption, while increasing the mechanical load can upregulate the expression of c-*fos,* thereby increasing bone mineral density [148]. Moreover, mechanical loading can change the flow of interstitial fluid, providing biological stress in the cytoskeleton of the major cells of the periosteum, thereby regulating bone formation [149]. Watanabe-Takano et al. identified osteocrin, a secretory peptide derived from periosteal-osteoblast, as a mechanotransducer involved in mechanical load-induced bone growth [150]. It is also reported that increasing the mechanical load induces the expression of the osteocrin gene by suppressing the Forkhead box protein O1 transcription factor. Hence, biomimicking the mechanical environment can modulate the periosteal cell fate, which is beneficial in bone formation. Studies are advancing in the area of understanding the effect of pore geometries of the scaffolds on the micro-mechanical environment, like mechanical strain and fluid-induced wall shear stress of the cells when loaded in the bioreactor [151]. Looking into the mechanobiological environment of the periosteum, it is clear that the periosteum-derived stem cells face many shape and volume-changing stresses throughout life, starting from the early stages of development [41]. The cytoskeleton of the periosteum-derived stem cells organizes and assembles to regulate their internal tension and control the related pathways for maintaining bone growth [152, 153]. Such mechanical stimulations can be advantageous in regulating the functions of the stem cells while developing the tissue-engineered periosteum. Researchers have also explored the photodynamic potentials of the nanomaterials to stimulate the osteogenic effect in the developed bionic periosteum membrane. Photo-active gold nanorods are incorporated within the polymer matrix, wherein the release of bioactive compounds is controlled at different time points through light irradiation (Fig. 6A) [103]. An adaptive periosteal system with dual-responsive capabilities was developed to provide stage-specific stimulation of angiogenesis and osteogenesis aligned with the inflammatory and anabolic phases of bone healing. The system leverages the enzymatic sensitivity and photoreactivity of gold nanorod–porous silicon nanocarriers to enable controlled release of bioactive peptides: angiogenic peptide QK is released in response to matrix metalloproteinase-2 (MMP-2) activity, while the osteogenic peptide BFP is released upon near-infrared (NIR) light activation. These nanocarriers, embedded within a hydrogel matrix, form a biomimetic periosteum capable of dynamically adapting to different phases of bone repair. Compared to control groups, this engineered system demonstrated over a twofold enhancement in angiogenesis, stem cell recruitment, and osteogenic differentiation.

In the human body, bioelectric cues are crucial for tissue stimulation and regeneration. Electrical stimulation (ES) significantly enhances the regeneration of nerves, bones, cardiovascular tissues, and wounds [154]. However, the use of conventional devices with stimulating metal electrodes is invasive and requires external batteries. Consequently, electrically active materials with excellent biocompatibility have attracted attention for their applications in stimulation and regeneration in tissue engineering. To fully exploit the potential of these materials, biocompatibility, operating mechanisms, electrical properties, and even biodegradability should be carefully considered. The electrically active biomaterials hold great potential for advancing the field of tissue engineering, and their demonstrated success underscores the importance of continued research in this field [155]. Out of the diverse strategies explored for bone regeneration, electrical stimulation has garnered considerable interest due to

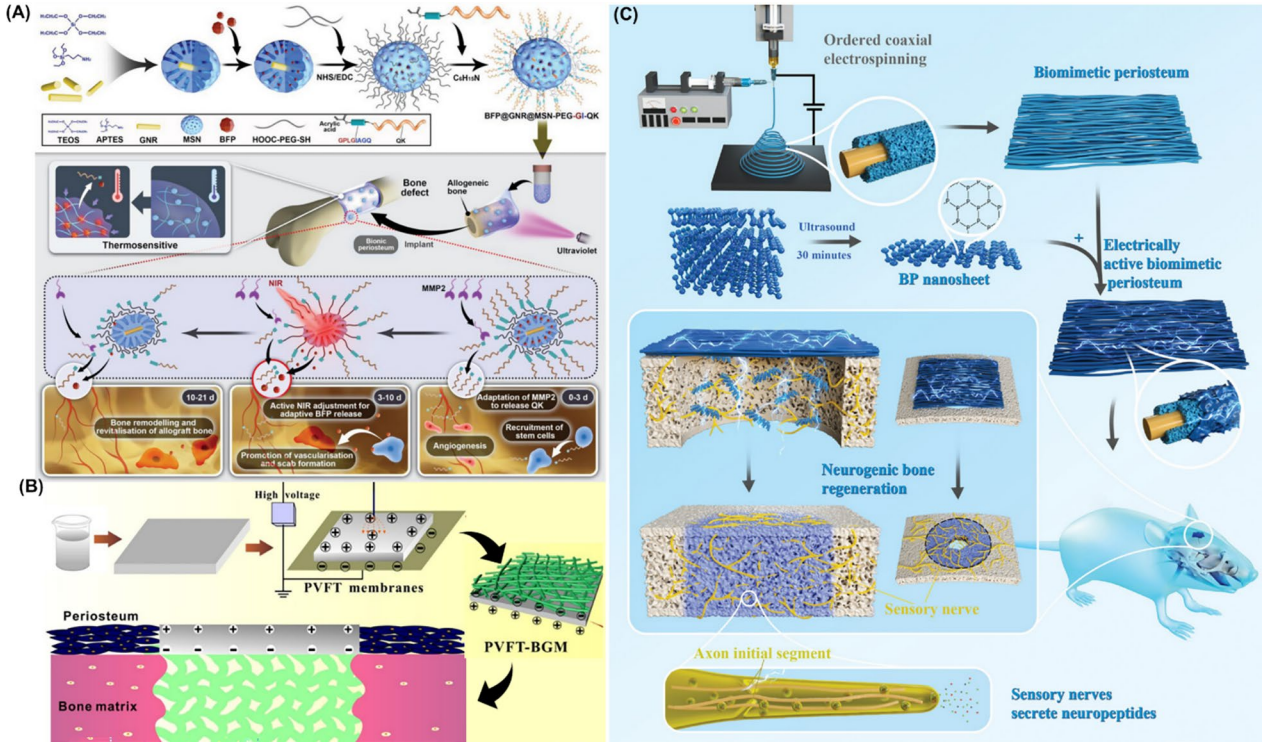


Fig. 6 Biophysical factors-based approach for the generation of artificial periosteum. **A** Schematic illustration of the enzyme and photodynamic adaptive artificial periosteum. Reproduced with permission from John Wiley & Sons [103]. **B** Generation of electrically active PVFT-BGM artificial periosteum with piezoelectric signals to electrically induce the regeneration mechanism. Reproduced with permission from Elsevier [80]. **C** An electro-responsive periosteum membrane comprising black phosphorus for innervated bone regeneration. Reproduced with permission from John Wiley & Sons [88]

its cost-effectiveness and notable therapeutic efficacy, leading to the development of a wide range of electroactive biomaterials. ES activates various signaling pathways, including BMP, MAPK, ERK, and p38, ultimately leading to the expression of RUNX2, the master regulator of osteogenic differentiation [156]. The calcium/calmodulin pathway is involved in ES-induced elevation of TGF-β1 mRNA in osteoblastic cells, contributing to their proliferation [157]. ES affects multiple cellular processes, including adhesion, pro-migration, proliferation, and differentiation of bone-related cells. Electroactive biomaterials, such as conductive and piezoelectric materials, are advancing in bone tissue regeneration applications. ES based approaches span from conventional techniques that utilize electroconductive materials coupled with external power supplies for electrical stimulation, to advanced self-powered systems incorporating piezoelectric materials and nanogenerators. Since the discovery of piezoelectric properties, researchers have focused on understanding the importance of electrical stimulus in bone remodeling [158]. ES and osteogenesis are related via bone piezoelectricity. The mechanism by which electrical stimulation promotes bone regeneration is related to the piezoelectric properties of bone [159]. Electroactive biomaterials can restore the electrical microenvironment to facilitate bone regeneration. It can mimic the natural physiological environment, including electrical, biochemical, and mechanical signals, to promote the functional recovery of bone tissues. ES can regulate biological processes and enhance extracellular matrix synthesis, accelerating bone regeneration. Under electrical stimulation, the membrane potential is highly polarized, significantly affecting cell recruitment, adhesion, proliferation, differentiation, the early inflammatory phase, and angiogenesis by controlling ion channels, signal transduction pathways, and reactive oxygen species [156, 160–162]. The stimulation through electrical signals to the artificial periosteum can be helpful in the rejuvenation of the hampered electrical signaling pathway after periosteum damage. Studies have reported the use of piezoelectric materials like poly(vinylidene fluoride-trifluoroethylene), Germanium Selenide (GeSe), black phosphorus, barium titanate, and carbon nanotubes (Fig. 6B-C) [73, 80, 83, 88, 110, 163]. Inspired by the nanostructure and piezoelectric properties of bone as well as the structure and function of the periosteum,

scaffolds that mimic the periosteum's architecture and functionality, featuring a piezoelectric signal-coupled bioactive ion-releasing nanofibrous surface, were developed [80]. The biomimetic scaffolds exhibit a gradient architecture, comprising a piezoelectric polymer layer integrated with a bioactive glass nanofibrous surface. Recent studies have demonstrated the efficacy of piezoelectric materials in promoting osteogenesis, angiogenesis, and neurogenesis. Liu et al. developed a biomimetic periosteum with piezoelectric properties that enhanced bone regeneration through immunomodulation and osteogenic stimulation [110]. Herein, the study introduces an innovative strategy for fabricating a biomimetic periosteum aimed at enhancing bone regeneration through the comprehensive application of functionalized piezoelectric materials. The engineered periosteum, exhibiting strong piezoelectric responsiveness and superior physicochemical properties, was developed using a biocompatible and biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) matrix. Antioxidant polydopamine-modified hydroxyapatite (PHA) and barium titanate (PBT) were incorporated into the matrix via a straightforward one-step spin-coating process to produce a multifunctional piezoelectric periosteum. The inclusion of PHA and PBT significantly improved the scaffold's surface hydrophilicity, roughness, mechanical strength, and degradation profile, while providing consistent and favorable endogenous electrical stimulation-key factors for promoting bone repair. Leveraging both the piezoelectric cues and bioactive components, the resulting periosteum exhibited excellent biocompatibility, osteoinductivity, and immunomodulatory potential in vitro. It effectively supported mesenchymal stem cell (MSC) adhesion, proliferation, and osteogenic differentiation, while simultaneously promoting M2 macrophage polarization and mitigating inflammation induced by reactive oxygen species (ROS). Similarly, a 3D-printed scaffold combining piezoelectricity with sustained release of Mg^{2+} ions, for innervated and vascularized bone formation [164]. Whitlockite, a natural magnesium-containing calcium phosphate, has recently gained significant attention for its potential in bone formation due to its unique piezoelectric properties following sintering and its ability to sustain the release of magnesium ions (Mg^{2+}). In this study, a composite scaffold, referred to as the PWH scaffold, was fabricated through 3D printing using piezoelectric WH (PWH) and PCL to fulfill the physiological requirements for regenerating neurovascularized bone tissue, specifically by generating an endogenous electric field at the site of injury. The controlled release of Mg^{2+} from the PWH scaffold demonstrates multiple beneficial biological effects, working synergistically with its piezoelectric properties to inhibit osteoclast activation while promoting the neurogenic, angiogenic, and in vitro osteogenic differentiation of BMSCs. Qian et al. showed that piezoelectric zinc oxide nanogenerator scaffolds accelerated peripheral nerve repair and motor function recovery [165]. In this study, using 3D injectable method, a piezoelectric nanogenerator scaffold is created. This scaffold demonstrates desirable physical and mechanical properties, such as high elasticity, stiffness, aligned porosity, and surface energy. It supports proliferation and attachment of Schwann cell and angiogenesis, as indicated by the elevated expression of critical functional proteins like NGF and VEGF. The scaffold generates a biomimetic electrically conductive microenvironment while avoiding significant toxicity to vital organs, thereby facilitating peripheral nerve regeneration through its multifunctional properties. Consequently, this mechano-responsive biomimetic piezoelectric scaffold holds significant potential for neuroengineering applications in regenerative medicine. Although ES has demonstrated potential as an osteoinductive cue, there is a lack of homogeneity in research approaches, making it challenging to draw definitive conclusions about optimal stimulation parameters and cell responses [156, 166]. There are numerous biochemical and biophysical stimulation strategies to resemble the characteristics of natural periosteum. However, the clinical situation and the location of the bone defect govern the actual periosteum design to be patient-specific. The biomechanics of non-load-bearing cranial and load-bearing tibial bones are different; thus, the artificial periosteum needs to be designed specifically for a particular purpose. Moreover, the combination of different factors on the same platform is specific to the patient's demand for enhanced therapeutic effect.

Multifunctional roles of engineered scaffolds for periosteum regeneration

Structural cues

As already discussed, periosteum consists of an outer aligned fibrous layer and an inner cellular layer in a random arrangement. Different periosteal-mimicking biomaterials have been fabricated comprising different osteo-inductive cues and osteogenic cells but still lack complete regeneration potential [76]. Therefore, biomaterials that mimic periosteum need to have the required features of surface topographical arrangement, which influences cell adhesion, proliferation, and differentiation [4]. The structural cues can help imitate the natural periosteum to obtain efficient bioactivity for advanced bone healing. The structural resemblance in the form of aligned structures in the periosteal membrane, topography through nanomaterial coating, patterning on the membrane, different pore architectures, and stimulation

through electrical and mechanical cues are proficiently under play for developing an artificial periosteum.

The researchers used carbon nanotubes (CNTs) to provide nano topography to the PLA inverse opal membrane [72]. CNTs are considered the biomimetic equivalent of fibrin, making the material more effective for its application [167]. The nano topography by CNTs provided the mechanical strength and directional orientation of the MSCs. The nanopatterning allowed the cells to grow in the direction and showed better osteogenic differentiation compared to the random arrangement on the scaffolds. Nanotubes ensured the integrity of the membrane when stretched due to their high tensile flexibility. The nano topographical alignment can mimic the collagen membrane of the periosteum. The layer-by-layer arrangement of the scaffold allowed better cell growth and proliferation due to surface roughness provided by the structural topographic arrangement of the layers [69]. This topography promoted cell proliferation and adhesion with better biocompatibility and bioactivity. A hierarchical porous structure was developed in the PLGA scaffolds [71]. The uppermost surface of the scaffold having a pore size of 1 to 6 μm is beneficial to mimic the periosteum-like barrier to prevent the intrusion of cells from the soft tissues to the bone defect area. The bottom surface has dual macropores: smaller than 10 μm for better protein adsorption and cell adhesion, while the larger pores of 100 μm and above help in cell migration and subsequent differentiation. Using decellularized periosteum extracellular matrix in the coaxial PCL nanofibers allowed better cell proliferation, migration, and tissue mineralization compared to the normal electrospun PCL nanofibers [74].

An artificial periosteum with micro-grooved patterns was studied for the alignment and proliferation of MSCs and HUVEC for osteogenic and angiogenic effects [109]. Yang et al. have developed the biomimetic periosteum membrane by generating micropatterns using printed hydroxyapatite nanoparticles onto the surface of the membrane [75]. The topographical arrangement allowed cell migration and induced cell differentiation. MSCs presented highly aligned organization due to these micropatterns, which induced angiogenesis and osteogenesis (Fig. 7A–D). Rat MSCs cultured on the biomimetic membrane demonstrated a well-aligned organizational structure, which subsequently promoted angiogenesis and osteogenesis. The biomimetic membrane with biomineralized micropatterns significantly enhanced vascularized bone regeneration and accelerated new bone formation. This micropatterned biomimetic membrane could be a promising alternative to using a patient's periosteal tissue for bone regeneration, with the potential for clinical translation in orthopedics. A 3D-printed periosteum biomimetic construct comprising gelatin-dopamine infiltrated with graphene oxide nanosheets was developed [76]. The developed material showed good physico-chemical properties and high cell adhesion to the membrane. An electrospun scaffold functionalized with leptin receptor antibody (LepR-a) was generated, which helped in the enhanced recruitment of the skeletal stem cells through the attachment to the LepR-a compared to the non-functionalized periosteum membrane [66]. This functionalized membrane recruited more Prx1-EGFP cells in the cranial defect model compared to the control. The nanostructured morphology of the native bone is also mimicked by the incorporation of 400–500 nm-sized bioactive glass particles for the recruitment of the bone precursor cells and the formation of periosteum-like tissues [80]. The presence of nanoparticles in the scaffold matrix also activated the calcium-sensing channels of the osteoblasts by depositing calcium ions. The utilization of Mg-doped ZnO nanoparticles into the PCL/gelatin vascular layer of the tissue-engineered periosteum showed enhanced properties by increasing the elastic modulus due to the presence of Mg and antibacterial properties due to ZnO [69]. An elastic wood skeleton for the artificial periosteum was developed to be used as an anisotropic skeleton inspired by natural wood. The anisotropic character of the material resembles the elastic and flexible nature of the natural periosteum while simultaneously guiding cells for their directional behavior [64].

Chen et al., have also developed a wood-inspired anisotropic PVA hydrogel that exhibits good mechanical properties and sustained delivery of the drugs alongside the anisotropic characters [98]. The synergistic effect of the biochemical factor and topography in artificial periosteum allowed sequential immunomodulation for efficient bone repair [96]. This further helped in osteogenic differentiation and neovascularization. The hierarchical micro/nanostructure due to the self-assembly of collagen with the nanofibers mimicked the microenvironment of the extracellular matrix of the natural periosteum, providing better structural support for cell adhesion, proliferation, and differentiation [95]. Alignment of the nanofibers provides multifunctionality like glucose response, immunomodulation, angiogenesis, and osteogenesis [92]. The synergistic effect of the topographical arrangement and the release of growth factors from the membrane allowed enhanced in vivo angiogenesis and osteogenesis [75].

There is an increased use of piezoelectric materials to mimic the piezoelectric nature of the osseous tissues for better bone regeneration [168, 169]. These materials can significantly induce the migration of stem cells under electrical stimulation. The piezoelectric poly(vinylidene fluoride-trifluoroethylene) polymer-based biomaterial showed better osteogenic potential

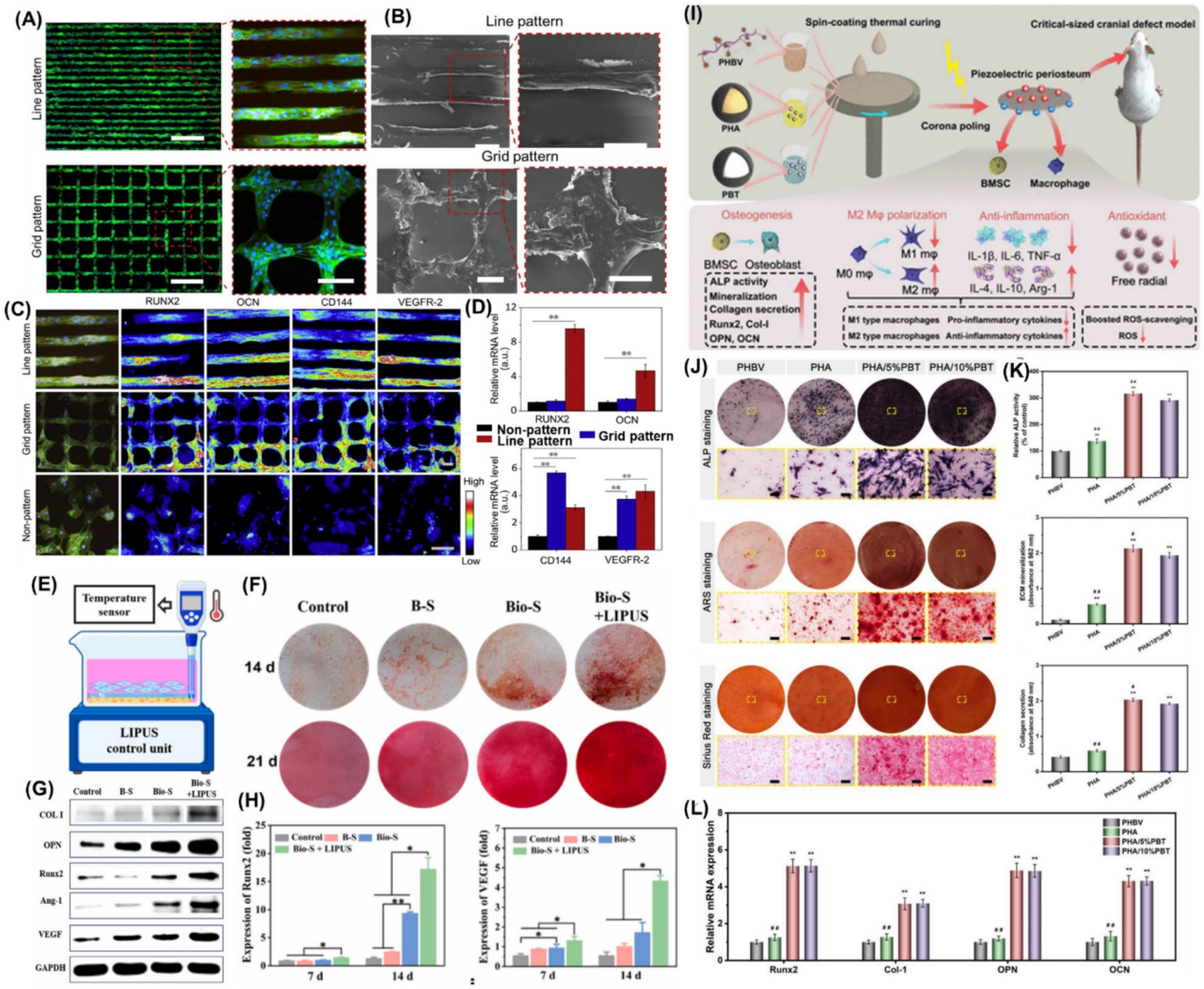


Fig. 7 Role of structural cues in periosteum mimicking biomaterials for functional bone regeneration. **A** Fluorescence and **B** SEM images of rMSCs alignment grown online-patterned and grid-patterned periosteum biomimetic membrane. **C** Qualitative and **D** Quantitative analysis of gene expression for osteogenic markers (RUNX2 and OCN) and angiogenic markers (CD144 and VEGFR-2) on different micropatterned surfaces. Reproduced with permission from Elsevier [75]. **E** Schematic representing cell culturing under LIPUS-assisted stimulation. **F** Alizarin red S-stained calcium nodules. **G** Western blotting and **H** mRNA expression of osteogenic and angiogenic marker proteins and genes after 14 days of electrical stimulation. Reproduced with permission from Elsevier [101]. **I** Schematic representation of bionic piezoelectric periosteum. **J** Osteogenic activity was evaluated through ALP, ARS, and Sirius Red staining. **K** Quantitative evaluation of ALP activity, matrix mineralization, and collagen secretion. **L** Relative mRNA expression of osteogenic genes (RUNX2, OPN, OCN, type 1 collagen). Reproduced with permission from American Chemical Society [110]

by recruiting stem cells by imitating the physiological microenvironment of the periosteum [163]. PLA nanofibers were co-doped with the piezoelectric Ge-Se to promote innervated osteogenesis. Ge-Se is considered degradable under physiological conditions and, therefore, allows better degradation of the membrane for ion release [73]. The presence and release of the ions also allowed photothermal therapy for osteosarcoma. The effect of electrical stimulation on neuronal bone regeneration was also determined under the TCP-PLA/Ge-Se nanofiber membrane [73]. The Schwann cells grown on the surface of the membrane showed upregulated gene expression under stimulation by ultrasound compared to the non-stimulated groups. It was demonstrated that the electrical stimulation activated the calcium signaling, PI3K-Akt, and Ras pathways. It further enhanced the osteogenic differentiation of MSCs under the stimulation medium. Su et al. have also studied the effect of electrically active biomimetic periosteum on neuronal bone regeneration [88]. The

black phosphorus present in the biomimetic membrane stimulated Schwann cells by the Fanconi anemia pathway, promoted the axon initial segment, and regulated dense core vesicle transport, neurotransmitter release, and subsequent osteogenesis by MSCs. Currently, researchers are using non-invasive low-intensity pulsed ultrasound (LIPUS), an FDA-approved method, for electrical stimulation. Zhao and group studied the effect of LIPUS-assisted electrical stimulation with the bionic periosteum membrane [101] (Fig. 7E-H). After stimulating the BMSCs by LIPUS, significantly high osteogenic, angiogenic, and neurogenic effects were observed. The calcium deposition by the cells was evaluated through alizarin red staining, representing the very high amount of calcium deposited by the cells due to enhanced cell differentiation under LIPUS stimulation compared to the non-stimulated group. Further evaluation of osteogenic marker proteins showed very high expression of OPN, RUNX2, and type 1 collagen. Also, there was about 1.8 times higher expression of RUNX2 genes by BMSCs under stimulation compared to the control. Apart from the osteogenic effect, angiogenesis was also validated by the increased expression of Ang-1 and VEGF. An increase of VEGF gene expression by 4.3 times under LIPUS stimulation suggested the great potential of using electrical signals for functional bone regeneration by bionic membrane. The synergistic effect of the piezoelectric property of the polymer and nanostructured pattern could enhance the periosteum function for better bone regeneration [80]. Jiang et al. have used LIPUS as a source of synergistic effects of mechanical and electrical stimulation [83]. $BaTiO_3$/multiwalled-carbon nanotubes/collagen membranes having ideal piezoelectric properties were fabricated as an artificial periosteum. After the stimulation with the LIPUS, the local electro-microenvironment of the bone was restored. The electrical and mechanical signals activated Ca^{2+} influx through Piezo1 and helped in the modulation of macrophage phenotype. Coating layer-by-layer membranes with polydopamine allowed better adhesion of the composite membrane to the damaged bone area and promoted bone formation more efficiently [64]. The polydopamine coating on the surface of the PLA membrane helped in the adhesion of the membrane to the bone surface [72]. In another study, the piezoelectric effect of $BaTiO_3$ in the nanocomposite periosteum membrane was evaluated (Fig. 7I-L) [110]. The osteoinductive effect was revealed by the enhanced stained area observed through ALP, ARS, and Sirius red staining under piezoelectric membrane than the control. The membrane also presented enhanced ALP activity, mineral deposition, and collagen secretion. Similar to the staining, mRNA expression analysis also confirmed the enhanced expression of RUNX2, OPN, OCN, and type 1 collagen genes due to the stimulatory effects of the piezoelectric material.

ROS modulation

Regeneration of the periosteum is a complicated process comprising several stages, and the effectiveness of these stages can be greatly affected by the increased content of ROS [170]. These reactive molecules (such as $O_2^{\bullet}$, H_2O_2, and $^{\bullet}OH$), generated from normal cellular metabolism, play a crucial role in this context, displaying a dual nature that can both support and hinder cellular functions [171]. In pathological conditions like trauma, osteoporosis, diabetes, infections, and aging, the negative impact of elevated ROS on periosteum regeneration becomes more severe [172, 173]. These situations not only increase ROS levels but also reduce the ability of the body to neutralize them, thereby intensifying oxidative stress. For example, in diabetic patients, hyperglycemia significantly increases ROS levels within mitochondria and the cytoplasm, disrupting all phases of bone healing, including periosteum regeneration [174]. Excessive ROS can inhibit the proliferation of osteoprogenitor cells, thereby reducing the cell niche available for the development of bone [170]. Additionally, oxidative stress can disrupt the normal differentiation process of osteoblasts, leading to impaired mineralization and bone matrix deposition [175]. It is known that oxidative stress directly impairs the function and survival of periosteal MSCs by activating p53-mediated apoptosis and inhibiting mitochondrial biogenesis [176]. Furthermore, oxidative stress suppresses key regenerative signaling pathways, oxidizing cysteine residues on β-catenin inhibits its nuclear translocation, thereby blocking Wnt/β-catenin signaling [177]. Oxidative stress also impairs BMP/Smad signaling by reducing receptor sensitivity and interfering with Smad phosphorylation [178]. Angiogenesis is similarly affected, as oxidative stress destabilizes HIF-1α and reduces VEGF transcription, leading to vascular regression and impaired oxygen supply [179]. In parallel, oxidative stress activates pro-inflammatory transcription factors such as NF-κB and AP-1, upregulating TNF-α, IL-1β, and IL-6, which prolong inflammation, promote osteoclastogenesis via RANKL upregulation, and inhibit osteoblast differentiation [180]. Additionally, oxidative stress-induced activation of MAPK pathways, particularly p38 and JNK, further contributes to periosteal dysfunction by modulating gene expression profiles toward catabolic and inflammatory phenotypes [181]. The net result is a hostile periosteal niche characterized by chronic inflammation, cellular senescence, impaired osteogenesis, and defective angiogenesis conditions that severely hinder bone repair [182]. Therefore, managing

excess ROS in diabetic patients is crucial for minimizing oxidative stress, enhancing stem cell activity, and encouraging osteogenic differentiation. Hormonal imbalances can significantly contribute to osteoporosis, particularly in women. A crucial role is played by estrogen in bone remodeling ensuring bone strength [183]. In addition to its role in bone health, it also has antioxidant properties, helping to neutralize harmful ROS. However, this balance is disrupted after menopause, leading to increased bone resorption and an increase in oxidative stress [184].

Trauma, such as bone fractures or injuries, also triggers an immediate inflammatory response that is essential for initiating the healing process. Moreover, trauma-induced ROS can aggravate inflammation, which further impairs the regenerative capacity of the periosteum [185]. Additionally, aging and persistent inflammation raise systemic ROS levels, disrupting the ability of the periosteum to support bone regeneration, leading to delayed or impaired healing [186]. The natural antioxidant systems of the body, which include both enzymatic defenses and non-enzymatic defenses, are essential for protecting bone health by neutralizing ROS [187]. However, when ROS levels increase due to factors like injury, inflammation, or metabolic disorders, natural defense system dysfunction and hence supplementing with external antioxidants becomes crucial [188]. By reducing oxidative damage, these external supplements help boost osteogenic activity in the periosteum, thereby strengthening bone matrix formation and eventually promoting efficient healing, making them a valuable aid in periosteum regeneration therapies [189].

Various artificial periosteal constructs made from biological and polymeric materials have been utilized for both preclinical and clinical applications [190]. However, their primary roles are largely restricted to acting as physical barriers against soft tissue ingrowth and providing scaffolding that facilitates osteoblast migration to the defect site, thereby promoting bone regeneration. Additionally, these constructs offer antibacterial properties and signaling cues that encourage osteo-induction. Despite these advancements, there has been limited research into the oxidative stress environment at the defect site. Evidence shows that oxidative stress can significantly hinder bone formation by reducing the viability and differentiation of stem and progenitor cells into osteoblasts [191]. This can lead to cell damage and apoptosis, adversely affecting both the quantity and quality of new bone formation. Furthermore, oxidative stress may trigger osteoclastogenesis, increasing bone resorption and complicating effective bone repair. Therefore, developing periosteal constructs with strong antioxidant properties holds promise for improving bone regeneration [192]. Most of the biomaterial-based therapeutic strategies (hydrogels, scaffolds, or functional coatings) aim to restore the balance between ROS production and antioxidant defenses, thereby promoting a favorable environment for periosteum regeneration [193, 194]. Biomaterials designed to perform as an artificial periosteum come in various forms. Artificial periosteum can be made from various materials, each offering unique advantages. Those based on naturally derived materials are biocompatible and inherently bioactive, making them ideal for applications. Synthetic biomaterials are also frequently used for their customizable mechanical properties and ease of functionalization [195]. Additionally, hybrid biomaterials that combine natural and synthetic components show great promise in achieving an optimal balance of mechanical strength, bioactivity, and antioxidant properties [196].

In a recent study, Wan et al. utilized biomimetic mineralization techniques to develop nanoceria (nCe) (Ce^{3+} Ce^{4+}) mineralized eggshell membranes (nCe-ESMs) as artificial periosteum for bone tissue engineering [141]. These ESMs, designed to mimic the periosteum, were produced by integrating nCe into high-molecular-weight polyacrylic acid (HPAA)-conjugated ESMs **(**Fig. 8A-F**).** The incorporation of nCe deposits enhanced the mechanical properties and improved the bioactivity of the membranes. nCe is recognized for its ability to stimulate the migration, proliferation, and differentiation of MSCs and endothelial progenitor cells. Its capacity to shift between Ce^{3+} and Ce^{4+} oxidation states in response to oxidative stress grants nCe attractive biocatalytic properties and immunomodulatory effects that help to regulate the tissue regeneration microenvironment [197–200]. In a recent study, Zhao et al. developed a biomimetic periosteum via electrospinning to replicate the natural periosteum and enhance bone regeneration by leveraging endogenous healing mechanisms [201]. This engineered periosteum exhibits a bilayer structure comprising an aligned electrospun layer of PCL-gelatin and deferoxamine (DFO) that mimic the outer layer of the periosteum and the other random layer made up of PCL-gelatin and aspirin (ASP) in the shell and silicon nanoparticles (SiNPs) in the core. This bilayer configuration enables the controlled release of loaded compounds, thereby precisely modulating the inflammation, angiogenesis, and osteogenesis. ROS can be efficiently scavenged by the random inner layer, enhancing cellular function. However, the outer layer facilitates vascularization and prevents the infiltration of fibroblasts.

Shi et al. successfully fabricated a graphitic carbon nitride (g-C_3N_4)/polydopamine (PDA) composite nanosheet (PDA@g-C_3N_4) through the in-situ polymerization of PDA on the surface of g-C_3N_4. An artificial periosteum was developed using the composite with the

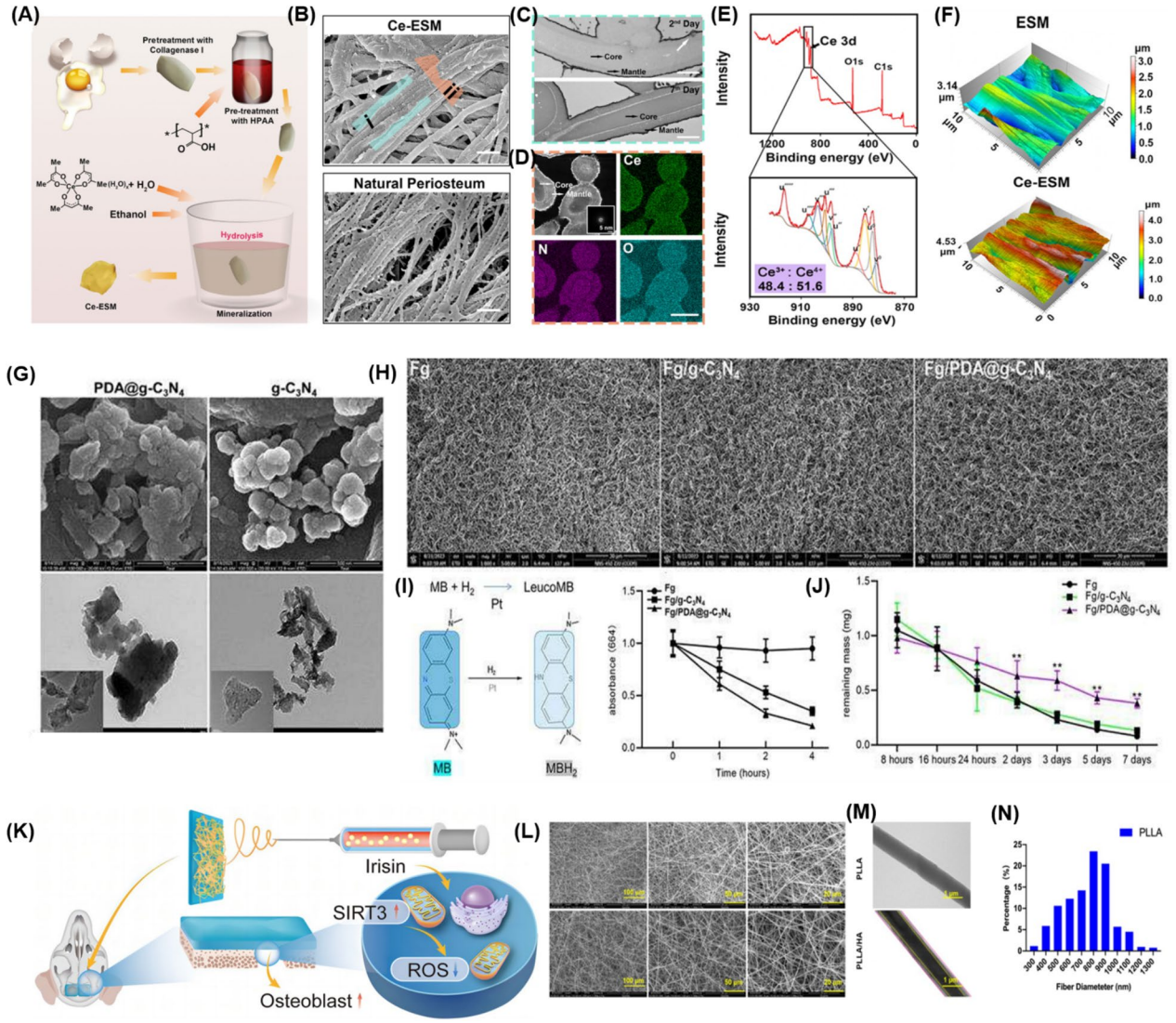


Fig. 8 Development of multifunctional ROS-responsive artificial periosteum. **A** Schematic representation of the fabrication process of nCe-ESMs. **B** SEM images comparing the surface morphology of nCe-ESMs and natural periosteum. **C** TEM images illustrating the mineralization progression of nCe-ESMs on days 2 and 7. **D** STEM-EDX images of nCe-ESMs. **E** Wide-scan XPS spectrum and high-resolution Ce3d spectra of nCe-ESMs. **F** 3D reconstruction of AFM analyses of nCe-ESMs. Reproduced with permission from the American Chemical Society [141]. **G** SEM and TEM images depicting the morphology of PDA@g-C3N4, employed in the synthesis of Fg/PDA@g-C3N4 and engineered composite scaffolds. **H** SEM analysis of the porous microstructure of the scaffolds. **I** Reaction equation of H_2 with the MB-Pt reagent and the corresponding UV–vis absorbance spectra of MB-Pt following H_2 reduction. **J** In vitro enzymatic degradation assay of the composite scaffolds. Reproduced with permission from Elsevier [99]. **K** Schematic illustration of the biomimetic periosteum design incorporating Irisin with ROS-responsive properties. **L** SEM representation of the hybrid fibers. **M** Representative TEM images of fibers. **N** Pore size distribution of the PLLA membranes. Reproduced with permission from Oxford University Press [202]

crosslinking of fibrin hydrogel, especially for elder diabetic patients [99] (Fig. 8G-J)**.** Delayed healing in elderly populations is linked to an increased pro-inflammatory M1 macrophage gene within fracture calluses, accompanied by elevated ROS levels due to prolonged M1 macrophage activity. Consequently, for effective bone regeneration, the development of a multifunctional periosteum capable of modulating macrophage behavior and mitigating excessive ROS production is important. In this study, the synthetic periosteum, upon NIR stimulation, facilitated the controlled release of hydrogen and oxygen. Under hypoxic and oxidative stress conditions, the released ions resulted in the enhanced growth and osteogenesis of ectodermal stem cells isolated from human nasal mucosa (hEMSCs). Furthermore, the artificial periosteum exhibited multiple reparative effects on

aged hEMSCs, including the regulation of the secretion of cytokine and the upregulation of proteins associated with angio- and osteogenesis. These therapeutic effects were validated in vivo using a cranial defect model in aged rats. Overall, these findings highlight the potential of the engineered periosteum enriched with hEMSCs as a promising strategy for bone defect repair in EDPs, particularly under conditions characterized by excessive ROS levels [99]. Hua and colleagues developed a nanofibrous membrane composed of poly (l-lactic acid) (PLLA) and HA using the coaxial electrospinning technique [202]. By incorporating Irisin into the core–shell structure of nanofibers made of PLLA and HA, a controlled release of bioactive molecules can be maintained. Irisin is a potent antioxidant phytomolecule that prevents cell apoptosis induced by oxidative stress [203]. This study demonstrated that Irisin activated superoxide dismutase 1 (SOD1) and glutathione peroxidase 1, leading to a reduction in ROS levels in bone marrow MSCs (BMMSCs) while safeguarding mitochondrial integrity from oxidative damage (Fig. 8K-N) [202]. Furthermore, irisin enhances the expression of Sirtuin 3, a key regulator of various antioxidant enzymes. In vitro studies demonstrated that loaded artificial periosteal membranes showed excellent biocompatibility, increased ALP activity, and calcium deposition for improved osteogenesis of BMMSCs. Mechanistically, the presence of Irisin significantly enhances the functioning of mitochondria, thereby activating sirtuin antioxidant signaling. To determine the therapeutic potential, Irisin functionalized membranes were implanted into critical-sized calvaria defects in rats. Observations at 4- and 8-week post-surgery revealed that the hybrid membranes effectively promoted vascularized bone regeneration, as evidenced by increased bone matrix synthesis and new blood vessel development. These findings indicate that the nanofibers exhibit properties typical of a biomimetic periosteum, underscoring their potential for periosteum regeneration and effective treatment of critical bone defects by regulating homeostasis and improving mitochondrial function in osteoprogenitor cells [202].

Bioactive factor and ion delivery

Many traumatic conditions and metabolic disorders can damage the periosteum layer and disrupt the normal regenerative potential of the bone. Tissue engineering strategies have led to the development of advanced biomaterials comprising multiple elements for functional bone regeneration. Bioactive factors (proteins, growth factors, ions, flavanoids) coupled with the biomaterial matrix provide desired physical, chemical, and biological properties for tissue repair. The bioactive factors incorporated into the biomaterial function as a multifaceted bioactive system promoting bone regeneration by their sustained and sequential release. The degradation of polymeric matrices allows the sustained delivery of bioactive molecules like growth factors, drugs, and phytocompounds. COD liver oil is an essential oil rich in vitamins A and D, and has very high anti-inflammatory properties [138]. The sustained release of COD liver oil from the tri-layered artificial periosteum helped better cell proliferation and differentiation [69]. The permeability, degradation, and mineralization of the membrane allowed the sustained release of COD liver oil. The addition of gelatin to the membrane significantly influenced the release compared to the normal periosteal membrane, increasing the release from 21.6% to 81% after day 7. The growth factor is attached to the mineralized micropattern of the artificial periosteum membrane. The subsequent long-term release of the calcium phosphate ions from the biomineralized membrane and the growth factors allowed enhanced vascularization and accelerated bone formation [75]. Controlled release of BMP2 from the hollow manganese dioxide nanoparticles decorated on the PLA scaffolds enhanced the ALP expression and calcium deposition by the skeletal stem cells [66]. A nutrient periosteum with a hamburger-like structure was generated, comprising PLLA microspheres and CaO_2 nanoparticles in the top and bottom layers, to provide proper nutrient supply to the middle cell sheet layer [11]. The slow release of oxygen from CaO_2 enhances the viability of the cells present in the cell sheet layer. PLA nanofibers were coated with tricalcium phosphate to generate an organic–inorganic scaffold to mimic bone-periosteum structure [73]. The long-term release of calcium and phosphorus from the porous tricalcium phosphate helps in the spatiotemporal support for bone regeneration [204]. Similarly, another study developed a nanoceria-incorporated eggshell membrane to mimic the natural periosteum [141]. Under the influence of this biomimetic membrane, the macrophages were observed to be differentiated into TRAP + pre-osteoclasts majorly stimulated by the release of cerium. These cells further upregulated the secretion of VEGF, Slit3, and PDGF-BB, stimulating the attachment, migration, and neurotrophic capability of Schwann cells, leading to enhanced bone formation.

Vascularization is very crucial for efficient bone regeneration, development, and homeostasis. Blood vessels provide oxygen, nutrients, growth factors, and osteogenic progenitor cells essential for bone formation [205, 206]. Angiogenesis precedes and couples with osteogenesis during bone repair, with VEGF acting as a key regulator in this process [207]. VEGF stimulates endothelial cell migration and proliferation while indirectly promoting osteogenesis through paracrine signaling [208]. Impaired blood flow can lead to compromised fracture healing

and bone-related disorders [209]. In tissue engineering strategies, ensuring adequate vascularization of scaffolds is critical for successful bone regeneration [210]. A GelMA-based periosteum mimicking bone aid (PMBA) containing methacrylated hydroxyapatite nanoparticles and L-arginine-based unsaturated poly(ester amide) was developed by Yang and group [4] (Fig. 9A). The material is considered the proof of the concept demonstration for advanced biomaterials for functional bone regeneration achieved by the sustained release of degradation products (Ca^{2+} and L-arginine). It was demonstrated that the hydroxyapatite nanoparticles present in the polymer matrix can decompose and release Ca^{2+} for subsequent physiological activity. The released Ca^{2+} ion can further activate NOS in rBMSCs and HUVEC, stimulating the production of NO by catalytic degradation of L-arginine (Fig. 9B-C). The increased expression of NO can further induce synergistic activation of osteogenesis and angiogenesis required for functional bone regeneration. The L-arginine and Ca^{2+} can be sustained release from the PMBA membrane for 30 days, showing no initial burst release (Fig. 9D-E). L-arginine is the precursor of NO, and Ca^{2+} is known for regulating the activity of NOS [211]. The synergistic release of L-arginine and Ca^{2+} was further analyzed for the production of NO. After 7 days of incubating cells with PMBA, cells showed higher NOS expression with Ca^{2+} release compared to the non-functionalized GelMA hydrogel (Fig. 9F-G). There was a dose-dependent increase in the NOS expression level in rBMSCs and HUVECs, mainly tuned by Ca^{2+}. The release of the bioactive agents from the biomaterial matrix warranted the activation of the NO–cGMP pathway for an orchestrated osteogenic–angiogenic effect. The presence of Baicalein worked synergistically with the structural arrangement of the PLGA scaffolds by providing pro-angiogenic and pro-osteogenic properties [71]. The active groups of Baicalein exposed due to the controlled release from the polymer matrix are mainly responsible for its multifunctionality. There was a slow-release pattern for up to 5 days with a later sustained release for up to 23 days due to the low aqueous solubility of Baicalein. The loaded membranes also provided enhanced cell adhesion on the surface. There was enhanced tube formation in the conditioned media of the functionalized scaffold within 4 h of incubation. There was also an enhanced expression of VEGF and angiopoietin-1, suggesting the role of Baicalein in vascular structure formation. The release of Baicalein further induced the pro-osteogenic effect by showing the enhanced expression of osteogenic markers like ALP, OCN, type 1 collagen, and calcium nodule formation. In one of the studies, VEGF was encapsulated in a hyaluronan-PLLA core–shell nanofibrous membrane, stimulating the controlled release from the outer layer of the periosteum and the bone defect region for efficient vascularization and ossification [95]. Layer by layer, bio-functional membranes were generated for the spatiotemporal release of bioactive agents like pamidronate disodium and deferoxamine, showing synergistic osteogenesis and angiogenesis [64]. Currently, more focus is shifting towards generating bionic periosteum with multifunctional potential for angiogenesis and neurogenesis. Li et al. generated a double-layer periosteum with an angio-neurogenesis coupling effect [90]. Whitlockite, an ion source, and abundant bone inorganic material was introduced into the bionic periosteum membrane. The release of magnesium ions promoted angiogenesis and neurogenesis while releasing calcium and phosphate ions enhanced the osteogenic effects. Introducing ionic groups enhanced collagen fiber development, calcium deposition, and expression of OCN and type I collagen. The ion release enhanced the expression of eNOS, CD31, and VEGF, suggesting the angiogenic potential compared to the control, which had no source of ions. Similarly, the ion-functionalized group enhanced the pro-neural effect by showing an increased expression of neural growth factor. The study speculated that ions play a significant role in enhancing functional bone regeneration by showing a multifunctional effect of osteo-angio-neurogenesis. Mao et al. have also generated an ion-incorporated bionic periosteum membrane by introducing zinc and magnesium

(See figure on next page.)

Fig. 9 Biochemical factors released from the biomimetic periosteum influence functional bone regeneration. **A** Schematic representation for the application of periosteum mimicking bone aid (PMBA) functionalized with Ca^{2+} ions (from hydroxyapatite nanoparticles) and L-Arginine. **B** The mechanism involved in the osteogenic and angiogenic role of released Ca^{2+} and L-Arginine. **C** Schematic showing the synergistic role of released Ca^{2+} and L-Arginine in the activation of NO/cGMP signaling pathway. Release pattern of **D** Ca^{2+} and **E** L-Arginine from PMBA. **F** Representative western blot and **G** quantitative expression analysis of NOS and p-NOS in rBMSCs and HUVECs for enhanced osteogenesis and angiogenesis. Reproduced with permission from John Wiley & Sons [4]. A biomimetic wood-derived hydrogel (WH) was functionalized with Cur and PA, showing sustained release of **H** Cur at different pH conditions and **I** PA for treating infectious bone. **J** Anti-bacterial mechanism by functionalized membrane and **K** Images representing inhibition zone against *S. aureus* and *E. coli* by different functionalized and non-functionalized membranes. **L** Mechanism deduced for osteogenic activation effect of WH-Cur-PA. **M** ALP staining of BMSCs in the presence of different samples. Gene expression analysis for **N** OCN and **O** RUNX2 under different functionalized hydrogels. Reproduced with permission from American Chemical Society [98]

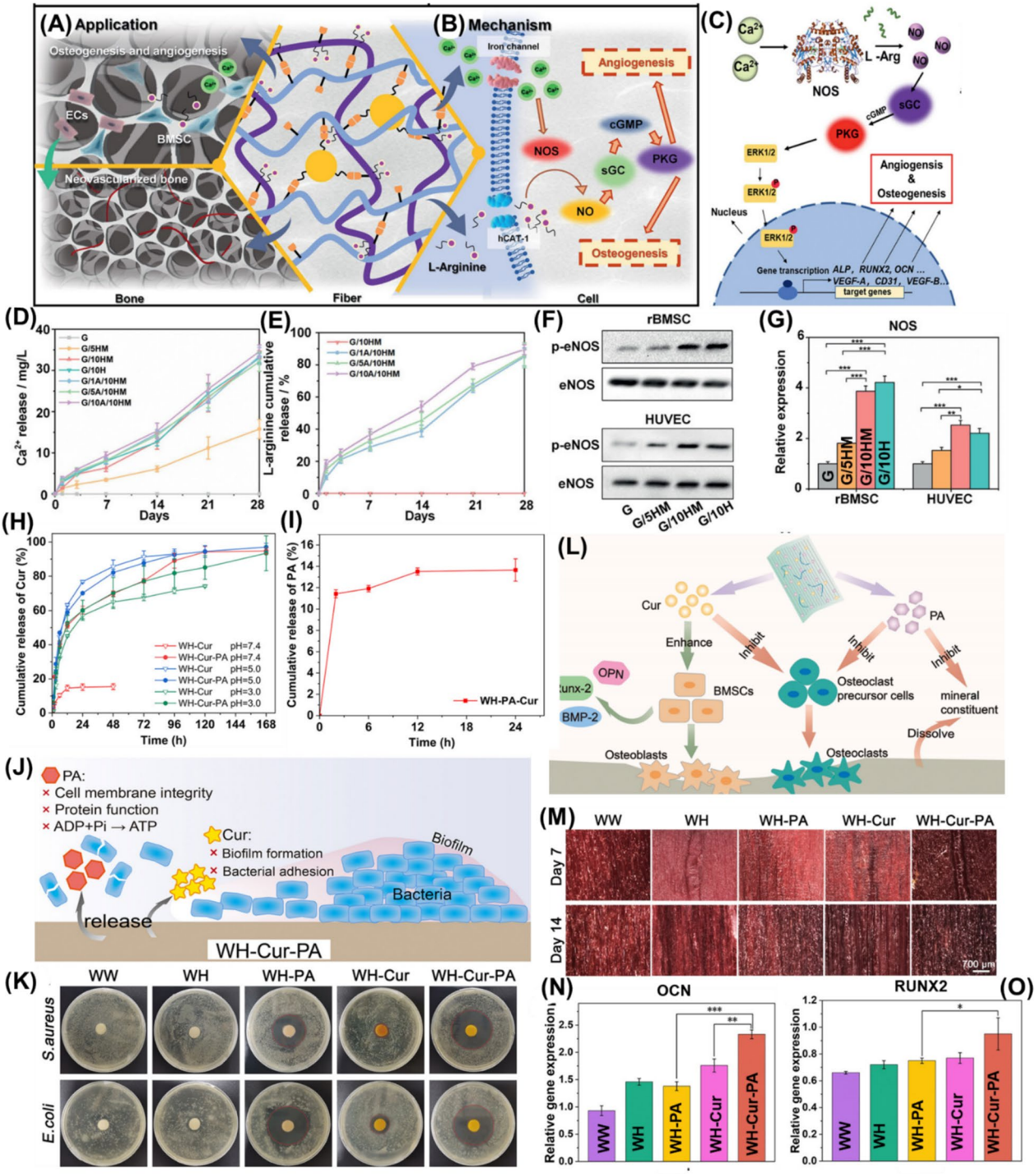


Fig. 9 (See legend on previous page.)

ions into the polymeric matrix [94]. The incorporation of ions changed the phase separation and transition in pore architecture, which further influenced the rapid release of ions. Further, the ions led to the formation of negatively charged tetrahedral units, which subsequently inhibited the burst release of positively charged ions. The release of ions stimulated the polarization of macrophages to the M2 phenotype, establishing a conducive immunogenic osteodifferentiation. The sustained release of ions enhanced osteogenic differentiation via the JAK/

STAT pathway. The release of magnesium, zinc, and calcium simultaneously enhanced the expression of type I collagen, BMP2, OPN, and RUNX2 compared to the individual ion effect [94]. The ion-incorporated bionic membrane showed the highest calcium deposition and ALP-positive cells compared to the control.

Bacterial infections and immune responses are one of the major clinical challenges faced during bone injuries. Infection control plays a crucial role in bone regeneration, particularly in contaminated defects. Rapid proliferation and adhesion of bacteria can recur repeatedly for a long time [212]. Treating infected bone defects is a significant clinical challenge that requires innovative approaches [213]. Recent research has focused on developing dual-function biomaterials that can simultaneously promote bone healing and control infection. The intersection of antibiotic delivery and tissue regeneration is an area that requires further investigation to develop effective strategies for addressing infection control and initiating bone regeneration simultaneously. Recent research also highlights the crucial role of immunomodulation in bone regeneration. Inflammation, once seen as purely defensive, is now recognized as essential for supporting regenerative processes [214]. Macrophages and T-cells interact with progenitor cells and bone-forming osteoblasts, making them potential targets for immune-modulatory interventions. The release of bioactive factors influences immune cell function and bone accrual, emphasizing the importance of osteoimmunology in osseointegration [215]. Maintaining immune homeostasis is crucial, as excessive inflammation can hinder bone regeneration. Recent research in the area of osteoimmunology regulating scaffolds is increasing for bone regeneration [216]. Hydrogel-based delivery systems offer promising localized immunomodulatory strategies [217]. Recently, white wood (WW) derived hydrogel (WH) functionalized with curcumin (Cur) and phytic acid (PA) was prepared to mimic the natural periosteum [98]. Dual drug loading into the hydrogel matrix can synergistically work for an efficient regenerative effect. Cur is a very potent antibacterial plant compound. However, the bioavailability of Cur is of great concern, leading to the advancements toward dual drug loading [218]. The photo- and thermal stability of Cur leads to increased bioavailability when used along with PA. The incorporation of PA into the WH membrane alongside Cur not only improved Cur bioavailability but also functioned as an antibacterial, anti-inflammatory, and osteogenic agent. The sustained co-delivery of Cur and PA from a dual drug-loaded hydrogel composite membrane demonstrated superior efficacy in inhibiting bacterial growth and inflammation while stimulating the differentiation of MSCs towards osteogenic lineage compared to membranes without drug loading. The release profile of Cur from the functionalized membranes was evaluated under varying pH conditions. The cumulative and sustained release of Cur in the presence of PA was greater than that of individual release for up to 168 h (Fig. 9H). The release kinetics were significantly influenced by pH, with the highest Cur release observed at pH 5 compared to pH 3 and 7.4. The inclusion of PA contributed to maintaining a lower pH, rendering WH-Cur-PA less sensitive to pH fluctuations than WH-Cur. PA played a crucial role in stabilizing Cur in an acidic environment, thereby enhancing its bioavailability and prolonging its release. The PA release profile exhibited an initial burst release within the first 12 h, followed by a sustained release phase (Fig. 9I). The effect of WH composite membranes was checked against *S. aureus* and *E. coli,* the most common infection-causing bacteria. WH-Cur-PA revealed the most prominent anti-bacterial effect with the maximum zone of inhibition (Fig. 9K). The mechanism behind the anti-bacterial effect is presented in Fig. 9J. Bacteria normally form biofilm on the implant surface, aggregated in the extracellular matrix of polymer substances [219]. PA can damage the bacterial cell membranes by chelating with metal ions, misfunctioning proteins, and nucleic acid [220]. However, Cur is involved in inhibiting the formation of biofilm and bacterial quorum sensing [221]. The presence of Cur in the functionalized membrane may be involved in the inhibition of biofilm formation, thereby further allowing PA to contact the bacterial cells and disrupt the cell membrane and organelles for an efficient anti-bacterial effect. The presence of Cur and PA also induced osteogenesis as observed by the increased ALP staining in the WH-Cur-PA group compared to the others (Fig. 9M). The synergistic release of Cur and PA stimulated the secretion of ALP from the cells for early osteogenic differentiation. RT-PCR results further showed an increased expression of OCN and RUNX2 in BMSCs in WH-Cur-PA compared to the control (Fig. 9N-O). The mechanism for the osteogenic effect by the released Cur and PA can be speculated as shown in Fig. 9L. The antibiotic-loaded PLGA nanofibers allowed the long-term release of vancomycin, ceftazidime, and lidocaine for 35 days, increasing the rate of bone union [132]. Moxifloxacin, an antibacterial drug, and icariin, a phytocompound, have been introduced into the periosteal-mimicking nanofibers. The dual drug sustained delivery of the biomolecules stimulated the membrane to represent antibacterial and osteo-inductive properties [85]. An artificial periosteum was developed and loaded with filamentous phage P11, which helped preserve inflammatory signals in macrophages and promoted the recruitment of MSCs [96]. The sustained release of APY29, an immunomodulator, by a change in hydrophilicity of liposomes under diabetic periosteum

regeneration has successfully improved tissue regeneration [92].

Therapeutic roles and clinical relevance of periosteum-mimicking biomaterials scaffolds

ECM scaffolds combined with biochemical factors for periosteum regeneration

The presence of periosteum is highly required for proper bone healing. An artificial periosteum can, therefore, become crucial to enhance bone regeneration due to damage to the natural periosteum. To study the effect of an artificial periosteum made of GelMA functionalized with E7 peptide, Zhang et al. generated a rat skull defect model [58]. After 4 and 8 weeks, the healing of the defect was maximum in the groups treated with functionalized hydrogel. In the control group, a large defect area was present compared to the groups with the functionalized scaffold. The presence of E7 peptide allowed the formation of a large area of the new bone. An increased BV/TV, trabecular thickness, trabecular number, and decreased trabecular separation were evaluated in the artificial periosteal membrane compared to the control. No inflammation or infection was observed after 8 weeks of material implantation, suggesting the safe application of the scaffold. The histological analysis showed different amounts of new bone formation and different-sized bone marrow cavities formed under different groups. The thickness of new bone tissues was highest in the scaffolds with E7 peptide compared to the non-functionalized one. The staining presented new periosteal tissues to cover the bone after 8 weeks. Similarly, more collagen deposition and a higher number of mature bones were present in the periosteal functionalized membranes. It was deduced that the addition of E7 peptide and its localized delivery from the hydrogels and interaction with the osteogenic cells can enhance the osteogenic effect. The simultaneous delivery of E7-BMP-2 peptide and alendronate through calcium chelation to nanofiber membrane demonstrated improved alveolar bone formation in rat maxillae [222]. Functionalization of silk fibroin electrospun scaffolds with polydopamine and E7 peptide enhanced BMSC recruitment, proliferation, and osteogenic differentiation, promoting bone regeneration in a rat calvarial defect model [223]. Additionally, the use of a heptaglutamate (E7) domain to couple bioactive peptides, such as DGEA and BMP2-derived peptides, to allograft bone resulted in significantly improved peptide loading, retention, and potential for delivering osteoinductive signals [224]. The E7 peptide, a heptaglutamate domain, plays a crucial role in enhancing bone cell activity by facilitating the coupling of bioactive peptides to hydroxyapatite-containing materials and allograft bone. Studies have shown that E7-modified peptides, such as E7-DGEA and E7-BMP2pep, exhibit significantly improved binding and retention on various bone substrates compared to unmodified peptides [224, 225]. This enhanced coupling leads to increased osteoblastic differentiation of mesenchymal stem cells and improved bone formation in vivo [226]. The E7 domain also demonstrates potential for targeted drug delivery to bone tissue. Notably, E7-BMP2pep conjugated to an organic bovine bone showed comparable or superior bone regeneration to recombinant BMP2 without the associated complications, suggesting a safer alternative for bone regeneration therapies.

Biomimicking the natural structure of the periosteum, an asymmetrically structured nanofibrous membrane is generated with an aligned layer mimicking the outer fibrous layer and a random layer mimicking the inner cambium layer [54]. The outer layer is functionalized with deferoxamine, which is required for the angiogenic properties, while the inner layer was co-axially spun and loaded with aspirin in the shell for anti-inflammatory properties and silicon nanoparticles in the core for osteogenic effect. Deferoxamine (DFO), an iron chelator with angiogenic properties, has been shown to increase osteocyte proliferation and bone volume fraction in mandibular distraction osteogenesis [227]. A rat skull defect model was generated to check the effect of the developed artificial periosteum without incorporating fillers in the defect area (Fig. 10A). The functionalized membrane presented maximum healing of the bone after 8 weeks compared to the non-functionalized membrane and the commercial Heal-all collagen membrane, as confirmed by micro-CT imaging (Fig. 10B). The micro-CT analysis suggested significantly higher fractional bone volume (BV/TV), bone mineral density (BMD), and trabecular number (Tb.N) in the functionalized membrane, attributed to the sustained release of the biochemical factors to the defect milieu (Fig. 10D-F). The histological analysis showed that a very small amount of bone was formed in the control and commercial groups after 8 weeks. A more thickened bone was observed in the bilayer membranes (Fig. 10C). Immunofluorescence imaging further validated the potential of the bilayer membrane toward bone and periosteum regeneration. There was an elevated expression of OCN, OPN, VEGF, CD31, CD206, and Periostin, while decreased expression of TNF- α, iNOS, and TRAP in the functionalized bilayer membrane compared to other groups postoperatively. The increased expression of osteogenic and angiogenic genes was related to the stimulatory effect of deferoxamine and Si ions toward angiogenesis and osteogenesis. The release of aspirin further stimulated the polarization of macrophages and controlled inflammatory responses. The decreased TRAP level associated with the osteoclasts is reported to be the effect of deferoxamine release from the membranes. This

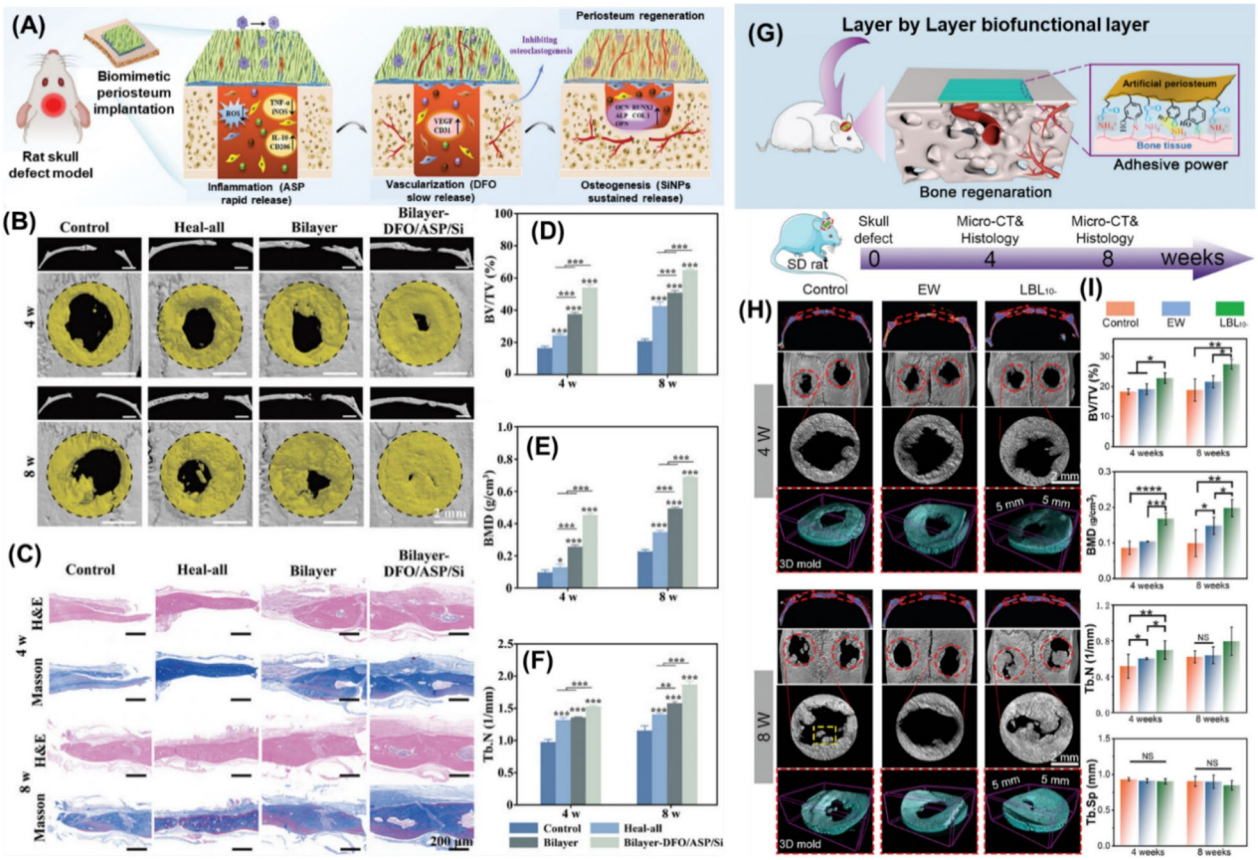


Fig. 10 Therapeutic role of biomimicking periosteum membranes functionalized with biochemical factors. **A** Schematic representing the synthesis of nanofibrous bi-layered membranes functionalized with deferoxamine, aspirin, silicon nanoparticles, and the mechanism for periosteum regeneration by controlled release of the molecules. **B** Micro-CT images, **C** Histological analysis and micro-CT quantitative analysis of bone formation after implanting bi-layered membranes in vivo, representing **D** BV/TV, **E** BMD, and **F** Tb.N. Reproduced with permission from John Wiley & Sons [54]. **G** Schematic for the synthesis and application of wood-inspired LBL periosteum membranes functionalized with pamidronate disodium and deferoxamine. **H** Micro-CT reconstruction images show newly formed bone areas and empty defect gaps. **I** Quantitative evaluation of BV/TV, BMD, Trabecular Number, and Trabecular Separation. Reproduced with permission from John Wiley & Sons [64]

programmed multifunctional effect of the membrane was mainly recognized due to the synergistic effect of the bioactive molecules after their sustained release.

A natural wood-inspired bio-mimicked periosteum was designed to replicate its anisotropic structure [64]. A bio-functional layer-by-layer (LBL) sustained release platform was generated for the sequential release of pamidronate disodium and deferoxamine (Fig. 10G). A rat cranial defect model was used to evaluate the effect of this artificial periosteum on osteogenesis. The micro-CT evaluation showed a significantly larger new bone area in the LBL compared to other groups. The bone repair from the periphery to the center was higher in the LBL membrane group, leaving a small empty gap compared to the non-functionalized and control groups, which had comparatively larger empty defect gaps (Fig. 10H) [64]. Moreover, under a control group, unconnected bone was formed, which restricted the formation of new bone. The quantitative analysis for the new bone formation also showed the same pattern (Fig. 10I). In the LBL group, 26.79% of BV/TV was observed with respect to 18.74% in the control group after 8 weeks. Similarly, BMD was also about 2 times higher in the LBL group compared to the control. The highest trabecular number and the lowest trabecular separation values were obtained in the LBL compared to others. Such an increase in bone formation was majorly attributed to the long-term release of the drugs from the membrane. The histological images revealed the bone-promoting potential of LBL as a large amount of new bone was formed with a high osteoblast number, while the control group was majorly covered with fibrous tissues, and no significant bone formation was observed. Immunofluorescence staining of angiogenic protein CD31 and osteogenic protein OCN was also evaluated to determine the effect of LBL. CD31 expression shows the newly formed vasculature more

prominently in the LBL group compared to the other. The result suggested LBL provided adequate blood supply for osteogenesis. Similarly, the high expression of OCN in the LBL group implies the release of drugs that promote osteogenesis. The LBL assembly of the membrane allowed the sequential release of the drugs from the matrix after implanting in vivo, wherein deferoxamine stimulated angiogenesis at the early stage to provide adequate nutrient supply, and then the subsequent release of pamidronate disodium can guide cell adherence, proliferation, and differentiation [64]. DFO promotes osteogenesis by activating β-catenin signaling in mesenchymal stem cells [228]. While DFO inhibits osteoblast proliferation at high concentrations, it increases alkaline phosphatase activity, indicating enhanced cellular differentiation [229]. In vivo studies show that DFO enhances bone regeneration in mandibular distraction osteogenesis by increasing osteocyte numbers and bone volume fraction [227]. However, some research suggests that DFO may negatively affect certain bone development markers and downregulate genes crucial for bone formation, such as Msx1 and Cx43 [230]. These findings highlight the complex role of DFO in bone cell regulation, demonstrating its potential to enhance osteogenesis and bone regeneration while also indicating possible adverse effects on certain aspects of bone development.

Recently, Wan et al. adopted a method of biomineralization to incorporate cerium oxide nanoparticles into the eggshell membrane [231]. This periosteum-like membrane regulated immune response, stimulated neuro-vascularization, and modulated early-stage bone regeneration in the cranial defect model. This biomimetic membrane allowed cranial defect repair by filling the defect area and replacing the gradually degrading membrane without infiltration of soft tissues. The functionalized membrane group distinctly showed bilayer morphology as that of the natural periosteum. The Ce-ESM group showed a thicker soft tissue layer than the control group at 4 weeks post-surgery. Immunofluorescence staining also provided evidence of new periosteum regeneration by significantly higher expression of periostin in the Ce-ESM group. The 3D reconstructed images of micro-CT revealed more bone regeneration due to the presence of nanoceria in the membrane matrix. Higher values of trabecular thickness, bone mineral density, and bone volume/total volume were observed in the Ce-ESM group. Further, von kossa histological staining revealed complete bridging of the newly formed bone due to nanoceria. However, the control group showed the filling of the defect area majorly by the fibrous connective tissue and limited bone regeneration. Immunofluorescence staining further evidenced the innervation in the Ce-ESM group by high expression of CD31 compared to the control, suggesting the angiogenic potential of nanoceria. The functionalization of the biomimetic membrane with nanoceria proved to be advantageous in enhancing osteogenesis and neo-vascularization for regenerating periosteum-like tissues and bone [231].

Biophysical properties and surface features for periosteum regeneration

The periosteum designs without surface structures lack adhesion-centric design, poor inhibition of soft tissue infiltration, and less osteogenesis. Currently, a lot of research in the area of adhesion surfaces and surface patterns is advancing to mimic the structure and function of the periosteum. The unique mechanical properties of the periosteum and its role as a stem cell niche contribute to its smart material characteristics [40]. Surface features like roughness and hydrophilicity of implants can mimic native bone properties, promoting osseointegration [232]. Patterned scaffolds provide a powerful tool for investigating and controlling cell-substrate interactions in tissue engineering applications. Research has shown that patterned scaffolds significantly influence protein adhesion and subsequent cellular responses. Nanofibrous scaffolds selectively enhance protein adsorption, particularly fibronectin and vitronectin, leading to improved osteoblast attachment [233]. Surface microtopography affects protein distribution, with fibrinogen preferentially adsorbing on protrusion flanks and valleys, influencing platelet and cell adhesion patterns [234]. Engineered protein motifs can be precisely displayed on surfaces to regulate osteoblast adhesion and differentiation, with cell response dependent on the density of adhesive motifs [235]. Nanopatterned fibronectin surfaces control integrin clustering and adhesion site size, affecting endothelial cell spreading and proliferation. These studies demonstrate that local ligand density, rather than average density, is crucial for cell behavior [236]. Recently, Yang et al. generated a bi-layered Janus periosteum with an exterior microgroove patterned surface and an interior adhesive layer made of gecko-inspired fibrillar setae arrays coated with mussel-inspired poly (dopamine methacrylamide-co-hydroxyethyl methacrylate) (PDMH) (Fig. 11A) [109]. This artificial periosteum provided adhesiveness under dry and wet conditions and good shear properties. The membrane also modulated the cell functions towards osteogenesis and angiogenesis without any biochemical factor [56]. The group having the microgroove with PDMH coating provided the best cell adhesion. It was also seen that the maximum cell alignment of MSCs and HUVECs was obtained in 40 μm micro-grooved patterns with an arrangement of 0°–10°. Also, the expression of ALP, OCN, VEGF-A, and eNOS increased on the 40 μm micro-grooved pattern compared to the flat

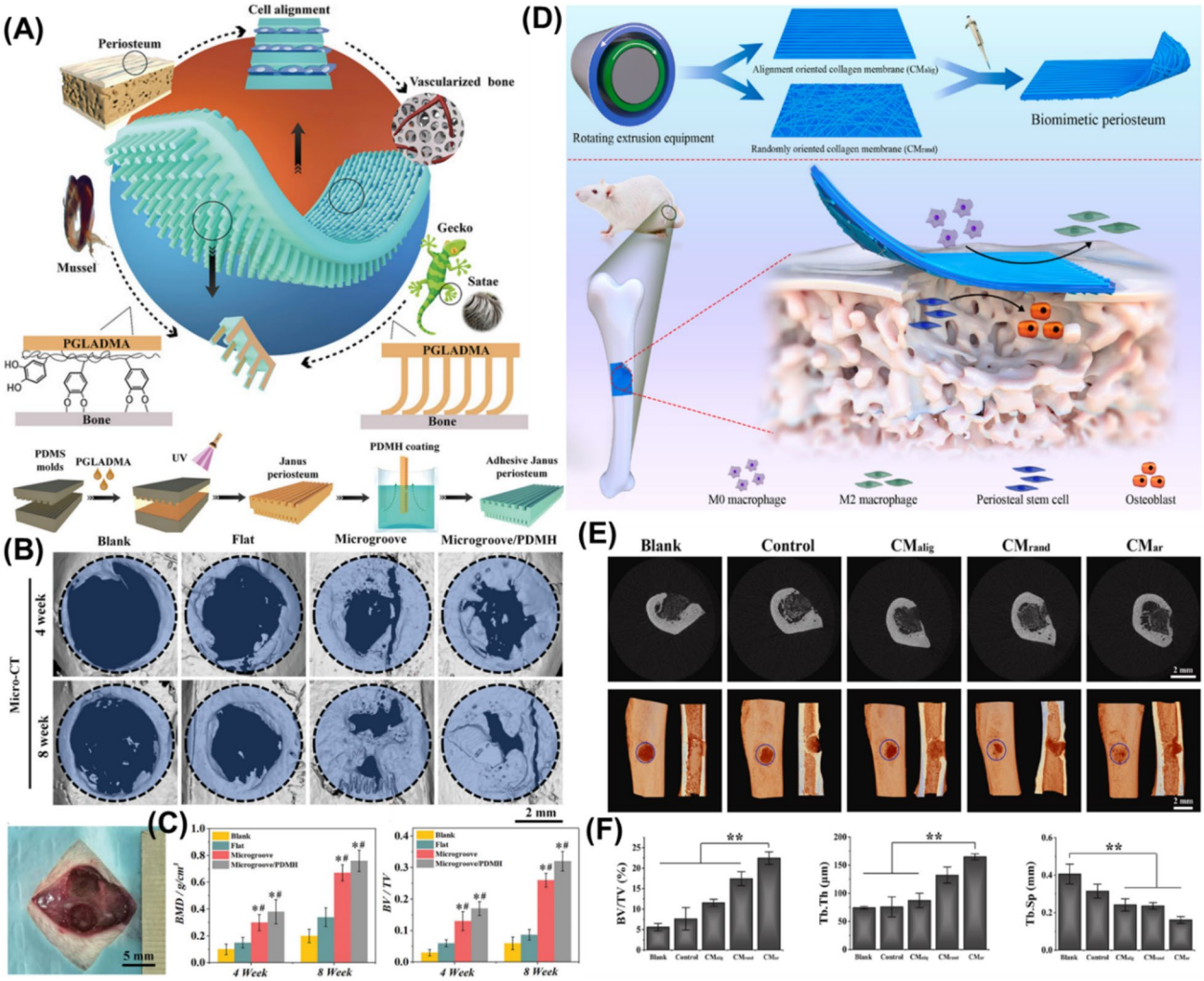


Fig. 11 Therapeutic role of periosteum mimicking scaffold designs with surface topography. **A** Schematic representing bi-layered Janus periosteum with exterior microgroove patterned surface and interior adhesive layer made of gecko-inspired fibrillar setae arrays coated with mussel-inspired PDMH. In vivo therapeutic effect of Janus membrane represented through **B** Micro-CT reconstruction images and **C** quantitative analysis. Reproduced with permission from John Wiley & Sons [109]. **D** An illustration showing a hierarchical biomimetic periosteum with an aligned collagen membrane (CM_{alig}) mimicking the outer fibrous layer while a randomly oriented membrane (CM_{rand}) resembles the inner cambium layer of the natural periosteum. **E** Micro-CT and **F** histological analysis in the nude mice ectopic bone model. Reproduced with permission from Elsevier [237]

surface, suggesting high bone formation and vascularization potential. Further, a rat critical-sized calvaria defect model was generated to determine the therapeutic efficacy of the Janus periosteal membrane. Micro-CT analysis presented a larger new bone area, bone mineral density, and bone tissue volume/total tissue volume in the microgroove/PDMH group than only micropatterned or flat groups (Fig. 11B-C) [109]. The results suggested the synergistic action of micropatterned surface and PDMH coating for accelerated bone regeneration. The histological analysis showed maximum woven bone structure after 4 weeks and lamellar bone structure after 8 weeks with the microgroove/PDMH group compared to the fibrous connective tissue formation on a flat surface. The immunofluorescence staining revealed that the defect site having microgroove/PDMH showed more α-SMA and OCN-positive cells compared to other groups [109]. These results suggested the promising potential of biophysical cues of anatomical patterns to effectively modulate cell function and induce angiogenesis and osteogenesis, providing great prospects for the clinical treatment of bone fractures.

A hierarchical biomimetic periosteum with differently aligned membranes and immunomodulatory and

osteogenic potential for bone healing was developed [237]. An aligned collagen membrane (CM_{alig}) mimicked the outer fibrous layer, while a randomly oriented membrane (CM_{rand}) resembles the inner cambium layer of the natural periosteum (Fig. 11D). CM_{alig} was considered immunomodulatory as it facilitated the polarization of M1 macrophage to M2 morphology. The heterotrophic ossification was observed more in CM_{rand} under the rat Achilles tendon defect model, as verified through histological staining after 8 weeks, showing tendon-like tissue formation. The degree of ossification was further confirmed by increased expression levels of the RUNX2 and OSX proteins in the CM_{rand} group. The ectopic bone model was also generated by implanting membranes subcutaneously in nude mice. The micro-CT and histological analysis showed more enhanced bone formation in the CM_{rand} group than the CM_{alig} group, suggesting the influence of surface topography on osteogenesis (Fig. 11E-F) [237]. A rat femur defect model was also generated to evaluate the effect of periosteal membranes on bone formation. After 6 weeks of implantation, more dense bone was observed in groups having CM_{ar} (CM_{alig} and CM_{rand} joined together) compared to the individual membranes, as evidenced through the micro-CT, quantitative analysis, and histological staining. Similarly, more periostin-positive cells were observed in the CM_{ar} group, suggesting the potential for neo-periosteum formation in the hierarchical membrane [237]. Topography can precisely manipulate osteoimmunomodulation by tuning surface properties, affecting immune cell behavior and osteogenic differentiation [238]. The interplay between implant surface topography and immune cells is crucial for osseointegration, with surface engineering being utilized to improve bone formation in various microenvironments [239]. Studies have shown that integrating surface nanotopography and chemistry can significantly influence the osteoimmune environment, impacting inflammatory cytokine expression, osteoclastic activities, and osteogenic factors [238]. Furthermore, a combinatorial approach using chemistry-microtopography screening has identified materials capable of directing mesenchymal stem cell fate and modulating macrophage phenotype simultaneously, offering promising alternatives for bone-regenerative applications [240]. Nanotopography can also regulate the immunomodulatory phenotype of MSCs by decreasing intracellular tension and increasing oxidative glycolysis [241]. The high plasticity of immune cells allows for precise manipulation of the osteo-immune environment through nanotopography, a concept termed "nano-osteoimmunomodulation" [238].

The surface topography generated by different pore diameters also influences the bone regenerative effect in artificial periosteum. A PLGA-based periosteum scaffold was generated with a hierarchical porous structure using a phase separation technique and negative mold method [71]. The above layer mimics the natural periosteum structure, which inhibits fibroblast infiltration, while the bottom layer has a dual pore structure for protein adsorption, cell adhesion, and osteogenesis. The scaffolds were tested in a calvaria critical-sized defect model for 8 weeks. Limited bone formation was observed in membranes having no pores, while the porous membranes showed significantly high bone formation, as evidenced by the micro-CT analysis. The histological staining also supported the results as a high thickness of newly formed tissue was observed in porous membranes comparable to the native tissues, while the non-porous membrane represented a very thin tissue layer. The immunohistochemical staining for OCN and VEGF further corroborated the results [71]. The hierarchical porous architecture of the membranes mimicked the native microenvironment of the periosteum and bone for effective angiogenesis and osteogenesis.

A nerve-rich periosteum is also vital for proper bone regeneration. An electrically active periosteum was generated for neuronal bone regeneration with an endogenous electric field generated through black phosphorus [88]. In the rat calvaria bone defect, the biomimetic electrically active periosteum membrane was placed to observe the neuronal growth-mediated bone repair, where nerves were damaged using capsaicin. Micro-CT analysis done after 8 weeks postoperatively showed more bone defect repair with a reduced defect size by 22.19% after loading black phosphorus to the periosteum membrane. The histological analysis reported more calcium and collagen deposition (3.82-fold higher) in the periosteum membrane, having a decellularized nerve matrix with the polymer [88]. The deposition further increased with the addition of electroactive black phosphorus nanosheets in the matrix. This electrically active periosteum proved to have potential clinical application through neurogenic bone regeneration. In another study, piezoelectric whitlockite containing PWH scaffold was tested in a rat calvarial defect [164]. The scaffolds demonstrated exceptional efficacy in promoting new bone formation, accompanied by abundant neurogenic and angiogenic activity. The piezoelectric scaffold, featuring sustained Mg^{2+} release, effectively facilitated the regeneration of neurovascularized bone tissue in vivo, providing new perspectives for advancements in regenerative medicine.

ECM scaffolds and cell-based strategies for periosteum regeneration

The interaction between cells and the ECM is a dynamic process involving complex molecular mechanisms. Integrins, key cell-surface receptors, mediate bidirectional signaling between cells and the ECM, influencing cellular behavior and tissue architecture [242]. This interaction is regulated by protein conformational changes, which can be triggered by mechanical stress, ligand binding, and post-translational modifications [243]. The ECM not only provides physical support but also transmits environmental signals that affect cell proliferation, differentiation, and survival [244]. Cells actively shape the ECM around them, creating a feedback loop that influences their behavior [245]. This cell-ECM crosstalk plays crucial roles in various biological processes like periosteum regeneration and bone formation. Decellularized periosteum ECM can promote bone defect regeneration and ectopic ossification, serving as a template for mineral crystal formation [36]. ECM-based scaffolds, including ECM-modified biomaterials and decellularized ECM, show promise in bone tissue engineering due to their osteoinductive and osteoconductive properties [246]. Natural ECM sheets derived from human dermal fibroblasts enhance osteogenic differentiation of mesenchymal stem cells by increasing growth factor binding and calcium deposition [247]. Periostin, a key ECM component of the periosteum, is involved in various stages of bone repair, including stem cell activation, cartilage and bone deposition, and reconstitution of the stem cell pool [248]. Decellularized ECM (dECM) from the periosteum has shown promising potential in bone and periodontal tissue regeneration. Studies have demonstrated that periosteum dECM can promote bone-like apatite formation and critical bone defect regeneration [36]. The decellularization process effectively removes cellular components while preserving the native ECM properties, including collagen, glycosaminoglycans, and mechanical characteristics [249]. Periosteum-derived scaffolds from different anatomical locations, such as the tibia and calvarium, exhibit varying angiogenic and osteogenic activities, offering tissue-specific options for bone regeneration [250]. dECM can be used directly or as injectable hydrogels, promoting stem cell proliferation, migration, adhesion, and differentiation [251]. These scaffolds have demonstrated biocompatibility both in vitro and in vivo, making them promising candidates for bone tissue engineering and clinical applications [249, 251].

Despite allografts being the gold standard for treating bone defects, their long-term clinical failure has necessitated the development of an alternative strategy. The use of the periosteum stem cell population with scaffolds as a tissue engineering periosteum has intrigued researchers, especially because of its potential to regenerate bone more effectively than grafts. For example, Hoffman et al. developed MSCs-laden hydrogel as a periosteum biomimetic and tested it on the murine segmental femoral graft model against allograft [35]. Bone callus formation was analyzed through Micro-CT for up to 16 weeks post-implantation. The tissue-engineered periosteum showed a fourfold increase in the bone volume compared to the allograft after 16 weeks. Further analysis revealed increased bridging of the bone with the engineered periosteum, although the bridging was incomplete. Histological results also showed more callus formation in the engineered periosteum and more material resorption than in the allograft. In addition, it also represented increased production of cartilaginous matrix for bridging the gap. More intensive examination of this matrix further revealed hypertrophic condensation of the cells similar to that observed during the chondrogenic differentiation of MSCs [35]. The transplanted GFP^+ mMSCs were examined through immunohistochemical analysis, showing more positively labelled GFP^+ mMSCs at the cartilaginous bridging matrix after 16 weeks. The histomorphometry analysis further quantified the bridging callus, exhibiting a 4.5-fold (7.6 mm^2) increase after 16 weeks compared to the allograft. A 4.9-fold (4.8 mm^2) and 31.0-fold (1.5 mm^2) increase in the mesenchyme callus area and total cartilage callus area, respectively, was also found after 9 weeks. The total woven callus area was also increased by 2.2-fold (3.7 mm^2) under engineered periosteum. This bottom-up cell-laden biomaterial approach has proved to be more effective compared to the clinically used allografts, with the potential for further increase in efficacy by incorporating inductive cues into the matrix. Another group demonstrated the role of periosteum-derived MSCs as a cellular therapy for functional bone regeneration. It was evidenced that periosteum-derived MSCs are more effective than bone marrow-derived MSCs. Further profound effects can be attained by using cells with biochemical cues, mainly those secreted by the seeded cells during the early weeks [252]. Further studies were conducted to generate a bionic periosteum membrane with integrated rat periosteum-derived MSCs in two ways [105]. First, seeding the cells to the mimetic periosteum 24 h before the implantation into rats (PI) and another way to directly seed cells in the surgical room just before the implantation (DS) (Fig. 12A). 10 weeks post-implantation, the PI group showed no healing, while the DS group showed about 67% healing as determined through radiographic analysis, suggesting less cellular potential by preincubation into the scaffold (Fig. 12B). 3D reconstruction images by micro-CT and quantitative analysis also showed negligible new bone in the PI group compared to

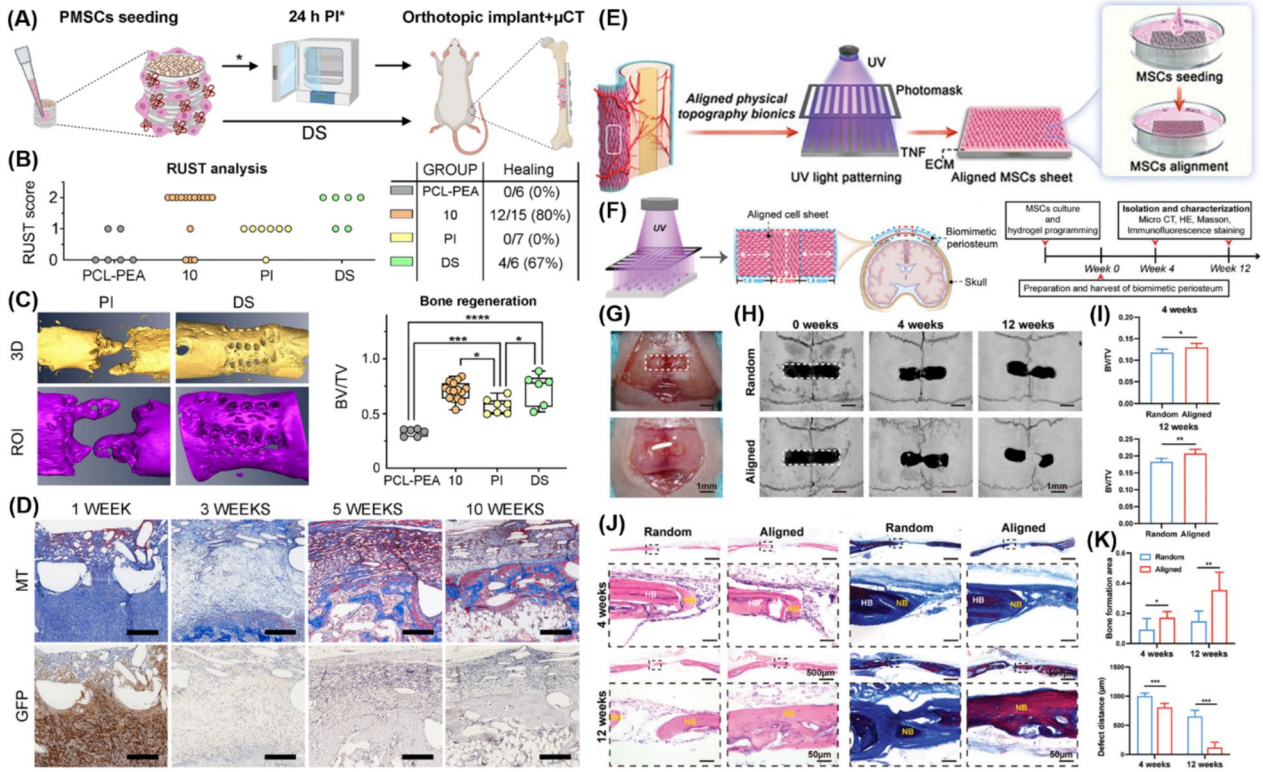


Fig. 12 Therapeutic role of cell-laden periosteum mimicking scaffold designs. **A** Schematic representation of experimental design by two ways of cell seeding PI (pre-incubation) and DS (direct-seeding) before implantation **B** Radiographic score and healing evaluation of bone under PI and DS cell seeding **C** 3D reconstruction images and quantitative analysis of bone regeneration **D** histological and immunohistochemical analysis of the bone healing under direct cell seeding with the bionic periosteum membrane. Reproduced with permission from Nature [105]. **E** Method of preparing aligned MSC cell sheet as the inner layer of the hydrogel **F** Experimental design and **G** biomimetic periosteum implantation in critical-sized skull defect model **H** Micro-CT images and **I** Quantitative analysis of newly formed bone in the center of the calvaria defect **J** Histological images and **K** Quantification of bone formation and distance from the defect for tissue healing after the implantation of the aligned cell sheet. Reproduced with permission from John Wiley & Sons [97]

the DS group (Fig. 12C). However, the cellular treatment showed enhanced BV/TV compared to the non-functionalized scaffold. DS group was subsequently studied by immunohistochemistry at different time points during tissue repair. The seeded cells were found inside the scaffold rather than on the surface, elucidating active cell migration 1 week after the implantation (Fig. 12D) [105]. Another study reported the use of aligned MSC cell sheets as the inner layer of the bi-layered stimuli-responsive hydrogel (Fig. 12E) [97]. This biomimetic periosteum layer exhibited high osteogenic differentiation potential along with cell migration and angiogenesis. This bionic periosteum biomaterial was implanted in the critical-sized skull defect to determine its osteogenic potential. (Fig. 12F-G). The Micro-CT results confirmed the newly formed bone in the aligned group with the thicker bone after 4 weeks compared to the cell-free group. The center of the defect was also observed to be completely filler after 12 weeks in the presence of an aligned cells group compared to the control (Fig. 12H-I). More bone regeneration was further evidenced through the histological images. The host tissue was also found to be well integrated into the newly formed bone with an aligned cell layer, suggesting accelerated bone healing (Fig. 12J-K) [97]. After week 4, early vasculature development around the host defect in the aligned group could provide nutrients for significantly high bone regeneration in the defect area. The number of capillaries further increased after 12 weeks in the aligned cell group. The intensity of the OSX-positive cells, essential for bone growth and remodeling, was comparatively higher at the new bone site in the aligned group. Lamin A/C staining further confirmed the presence of human cells in the defect region after the implantation of biomimetic periosteum, with the intensity persisting for about 3 months [97]. Altogether, this personalized biomimetic periosteum scaffold maintained the osseous microenvironment for its clinical application in repairing bone defects.

Conclusion and prospects

The restoration of bone defects, particularly in the context of chronic injuries and complex fractures, demands advanced therapeutic strategies that go beyond conventional grafting techniques. Among the critical elements of successful bone regeneration is the periosteum which is a dynamic and vascularized membrane integral to osteogenesis and bone repair. As this review highlights, it is quite evident now that the periosteum plays a critical role in bone repair and regeneration. Considering the significance of the periosteum to the bones, it is an indispensable part of tissue-engineered materials and grafts. The osteoprogenitor cells present in the periosteum layer are differentiated into osteogenic linear cells for proper healing of the fracture under different healing phases. In a clinical setting, periosteum implantation, such as autologous periosteum transplantation, to cover and heal bone defects to promote neo-bone formation has been attempted, but its application remains limited. One major challenge is the limited availability of autologous periosteum, particularly in patients requiring extensive bone repair. Moreover, factors such as age and the location of the bone injury can affect the osteogenic capacity of periosteum. In older patients, the ability of periosteum to generate new bone tissue is often diminished, complicating the healing process. Additionally, the risk of excessive scarring and fibrosis at the injury or graft site can hinder its effectiveness in promoting bone healing. Periosteum has an orchestrated and complex organization due to the presence of multiple layers, numerous components, structural arrangement, and their combinations thereof. To mimic the microarchitecture and function of the natural periosteum, the native composition and organization need to be considered to generate an engineered artificial periosteum. Researchers have explored different strategies for developing an effective biomimetic periosteum. These approaches aim to recapitulate the structural, biochemical, and biomechanical cues inherent to the periosteum, thereby creating a conducive microenvironment for orchestrated tissue repair.

In this review, a comprehensive study of the periosteum structure and function is presented to understand the native composition of the periosteum for proper designing of a bionic periostea. Along with that, state-of-the-art in the field of artificial periosteum in terms of composition, design, therapeutic role, and future potential research directions in regenerating periosteum and bone are also well summarized. The key to developing an ideal biomimetic periosteum is selecting proper scaffold materials and simulating the physical, chemical, and biological characteristics of a natural periosteum. Although different biomimicking periosteum have been developed so far, none of them have completely met all the conditions. Every biomaterial is still at the stage of in vitro and preclinical studies and further needs to be tested in the clinical setting. The development of microfabrication personalized patient-specific periosteal constructs that take into account individual variations in bone structure, healing capacity, and systemic health represents another promising yet complex goal. The integration of advanced technologies such as 3D bioprinting, nanotechnology, and external stimuli-responsiveness offers new strategies to fine-tune scaffold architecture and functionality [253]. Additionally, an emerging area of interest is the role of the nervous system in bone regeneration. Recent studies indicate that neuronal signals can significantly influence bone homeostasis and repair, suggesting that future biomimetic periosteum may need to incorporate neurotrophic factors or even neuron-mimicking elements to enhance regenerative outcomes [254]. Also, with the advancements in stem cell biology, a deeper understanding of cellular behavior in the presence of periosteal biomaterial architecture is highly desired. Such advancements continue to contribute valuable insights into cell-scaffold interactions, differentiation pathways, and the modulation of the periosteal niche. Understanding how various cell types respond to different periosteum-mimicking biomaterials will be key to refining scaffold designs and ensuring successful integration with host tissue.

Despite these advances, several challenges must be addressed to transition biomimetic periosteal scaffolds from bench to bedside. One of the main barriers is the translation of preclinical results into clinical efficacy due to variability in animal models, scale-up processes, and regulatory hurdles [255]. Standardized evaluation protocols and robust large-animal studies are urgently needed to better predict human responses. Furthermore, while current scaffolds mimic periosteal structure to a degree, they often fall short of fully replicating the dynamic cell–matrix interactions and mechano-transductive feedback mechanisms of native tissue [40, 256]. Another limitation lies in achieving optimal integration of scaffolds with host tissue, particularly in cases where local vascularization is impaired. Future designs must prioritize vascular integration, potentially through pre-vascularization techniques or stem cell incorporation. Moreover, the immunomodulatory aspects of biomaterials are gaining attention, highlighting the need to balance pro-healing inflammatory responses while avoiding chronic inflammation or fibrotic encapsulation.

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Data availability
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Declarations

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Competing interests
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