

Review

Multi-Layered Electrospun Scaffold Design Strategies Targeting Osteosarcopenia

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Abstract

Osteosarcopenia, the concurrent deterioration of bone density and skeletal muscle mass and function, represents a growing clinical challenge in ageing populations worldwide. Conventional single-tissue therapeutic approaches fail to address the intricate biochemical and mechanical crosstalk between bone and muscle at their shared interface. Electrospun nanofibrous scaffolds offer a versatile platform capable of recapitulating the hierarchical architecture of the musculoskeletal extracellular matrix (ECM). This review critically examines current strategies for designing multi-layered electrospun scaffolds that simultaneously target bone and muscle regeneration within the context of osteosarcopenia. We discuss the pathophysiological basis of bone–muscle crosstalk, highlighting the shared roles of the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis and chronic inflammation. To effectively mimic native tissues, we evaluate aligned compliant fibers (e.g., poly(ϵ -caprolactone) (PCL)-gelatin) for myogenic differentiation and random, stiffer mineralized fibers (e.g., poly(lactic-co-glycolic acid) (PLGA)/nano-hydroxyapatite (nHA)) for osteogenic differentiation. Furthermore, incorporating a gradient transition zone is strongly emphasized to minimize interfacial stress under physiological loading. Advanced electrospinning techniques—coaxial, emulsion, and melt electrowriting—are reviewed for spatially controlled and sequential delivery of vital growth factors like IGF-1 and bone morphogenetic protein-2 (BMP-2). Preclinical evaluation models are addressed, identifying a critical gap regarding the lack of aged and ovariectomised (OVX) composite defect animal models specific to osteosarcopenia. Finally, translational barriers, including manufacturing scalability and regulatory complexities, are evaluated. This review proposes a unified design framework for osteosarcopenia-targeted scaffolds and identifies critical knowledge gaps requiring future investigation.

Keywords: Osteosarcopenia, electrospinning, multi-layered scaffold, bone–muscle interface, growth factor delivery.

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Introduction

The global demographic shift towards an ageing population has brought musculoskeletal disorders to the forefront of biomedical research. Among these, osteosarcopenia—defined as the simultaneous presence of osteoporosis (or osteopenia) and sarcopenia—has emerged as a distinct clinical entity with prevalence estimates ranging from 5 to 37% in community-dwelling older adults aged 65 years and above, escalating to 17–96% in hip fracture cohorts [1,2]. In Asian populations, where rapidly ageing demographics intensify the clinical burden, recent cohort studies report an osteosarcopenia prevalence of approximately 6–7% in community-dwelling Chinese and Japanese older adults [3,4] and approximately 29% in a Korean hip-fracture cohort [5], broadly consistent with the wider ranges documented in Western populations [1]. Unlike isolated osteoporosis or sarcopenia, osteosarcopenia carries a synergistically elevated risk of falls, fractures, institutional-

isation, and mortality, making it a significant burden on healthcare systems worldwide [6]. The concurrent decline of both tissues reflects shared pathophysiological mechanisms including dysregulated growth hormone/insulin-like growth factor-1 (GH/IGF-1) signalling, chronic low-grade inflammation, vitamin D insufficiency, and mechanical disuse [7,8] (Fig. 1). Epidemiological studies consistently demonstrate that individuals with osteosarcopenia exhibit worse functional outcomes—including lower gait speed, reduced grip strength, and higher frailty index scores—than patients with either condition alone, underscoring the need for integrated therapeutic strategies that address both tissues concurrently. A meta-analysis of eight cohort studies (19,836 participants) confirmed that osteosarcopenia significantly increases the risk of fracture (pooled odds ratio (OR) 2.46, 95% confidence interval (CI) 1.83–3.30), falls (OR 1.62, 95% CI 1.28–2.04), and mortality (OR 1.66, 95% CI 1.23–2.26) relative to non-osteosarcopenic older adults

[9].

Current pharmacological interventions for osteoporosis (bisphosphonates, denosumab) and sarcopenia (resistance exercise, protein supplementation) target each tissue independently, overlooking the tightly coupled bone–muscle unit [10]. This fragmented approach is inherently suboptimal because bone and muscle share developmental origins from common mesenchymal progenitors, a co-regulated vascular supply, and a complex biochemical signalling network mediated by secreted myokines and osteokines. Furthermore, pharmacological agents such as bisphosphonates suppress bone remodelling but have no demonstrated benefit for skeletal muscle mass or function, while anabolic agents like testosterone or selective androgen receptor modulators carry significant cardiovascular and hepatic risks that limit their use in the frail elderly population most affected by osteosarcopenia. Tissue engineering offers an alternative paradigm: the design of biomaterial scaffolds that can simultaneously support osteogenesis and myogenesis within a single implantable construct. Electrospinning, a scalable nanofabrication technique, produces fibrous membranes whose fibre diameter (50 nm–5 μ m), porosity (>80%), and topographical cues closely mimic the native extracellular matrix (ECM) of both bone and muscle [11,12].

Crucially, the bone–muscle interface is not a simple boundary but a graded transition encompassing periosteum, enthesis-like attachments, and a zone of progressively mineralised collagen fibrils [13]. Reproducing this structural complexity demands multi-layered scaffold architectures in which each layer is independently optimised for a specific tissue type, yet the layers are integrated into a mechanically continuous construct. Aligned electrospun fibres provide contact guidance for myoblast fusion into organised myotubes [14], whereas randomly oriented and mineralised fibres support osteoprogenitor adhesion and differentiation [12]. Combining these configurations in a single scaffold through sequential or simultaneous electrospinning is the central engineering challenge addressed by this review. The inherent versatility of the electrospinning process—allowing independent tuning of polymer composition, fibre morphology, mineral content, and incorporated bioactive agents across different scaffold layers—positions it as an ideal fabrication platform for this demanding application.

Despite the growing body of literature on electrospun scaffolds for individual tissue regeneration, a comprehensive framework for osteosarcopenia-targeted multi-layered designs remains absent. Existing reviews have addressed electrospun scaffolds for bone or muscle independently, or have focused on tendon-to-bone interface engineering in the context of sports injuries, but none have systematically considered the unique pathophysiological context of osteosarcopenia—characterised by systemic hormonal deficiency, chronic inflammation, and impaired regenerative capacity—as a design constraint for scaffold devel-

opment. This review bridges that gap by synthesising knowledge from musculoskeletal biology, materials science, and scaffold engineering. Section (Pathophysiology of Osteosarcopenia and the Bone–Muscle Interface) summarises the pathophysiology of osteosarcopenia and the structural requirements of the bone–muscle interface. Section (Electrospinning Strategies for Musculoskeletal Tissue Engineering) evaluates electrospinning parameters and advanced techniques relevant to musculoskeletal tissue engineering. Section (Multi-Layered Scaffold Design for the Bone–Muscle Interface) presents design strategies for multi-layered scaffolds, including material selection, mineralisation, growth factor delivery, and preclinical evaluation models. Section (Challenges, Future Perspectives, and Conclusions) identifies translational challenges, emerging technologies, and future directions, and draws overall conclusions.

Pathophysiology of Osteosarcopenia and the Bone–Muscle Interface

Molecular Crosstalk Between Bone and Muscle

Before discussing the molecular crosstalk that motivates dual-tissue scaffold design, it is important to anchor the term to its clinical diagnostic criteria so that materials scientists can clearly identify the target patient population. Sarcopenia is currently defined by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) as the simultaneous presence of low muscle strength (hand-grip <27 kg in men, <16 kg in women, or chair-stand time >15 s for five rises) and low muscle quantity or quality (appendicular skeletal muscle mass index <7.0 kg/m² in men and <5.5 kg/m² in women), with severity staged by reduced physical performance such as gait speed $\leq$ 0.8 m/s [15]. Osteoporosis is defined by the World Health Organization (WHO) as a bone mineral density T-score $\leq$ –2.5 at the femoral neck or lumbar spine, with osteopenia covering T-scores between –1.0 and –2.5 [16,17]. Osteosarcopenia denotes the concurrent fulfilment of both criteria in the same individual, and international consensus statements emphasise its identification as a distinct geriatric syndrome that confers synergistically elevated risk of fragility fracture and disability [9,18,19]. Scaffold design strategies discussed in subsequent sections are therefore intended for patients meeting these dual diagnostic thresholds, who typically present with low-energy fractures, impaired healing capacity, and a chronic inflammatory milieu rather than acute traumatic injury.

Bone and skeletal muscle are endocrine organs that communicate through a repertoire of secreted factors collectively termed myokines and osteokines [20,21]. Muscle-derived interleukin-6 (IL-6), irisin, myostatin, and β -aminoisobutyric acid (BAIBA) regulate osteoblast and osteoclast activity, whereas bone-derived osteocalcin, sclerostin, and receptor activator of nuclear factor kappa-B ligand (RANKL) modulate muscle metabolism and insulin

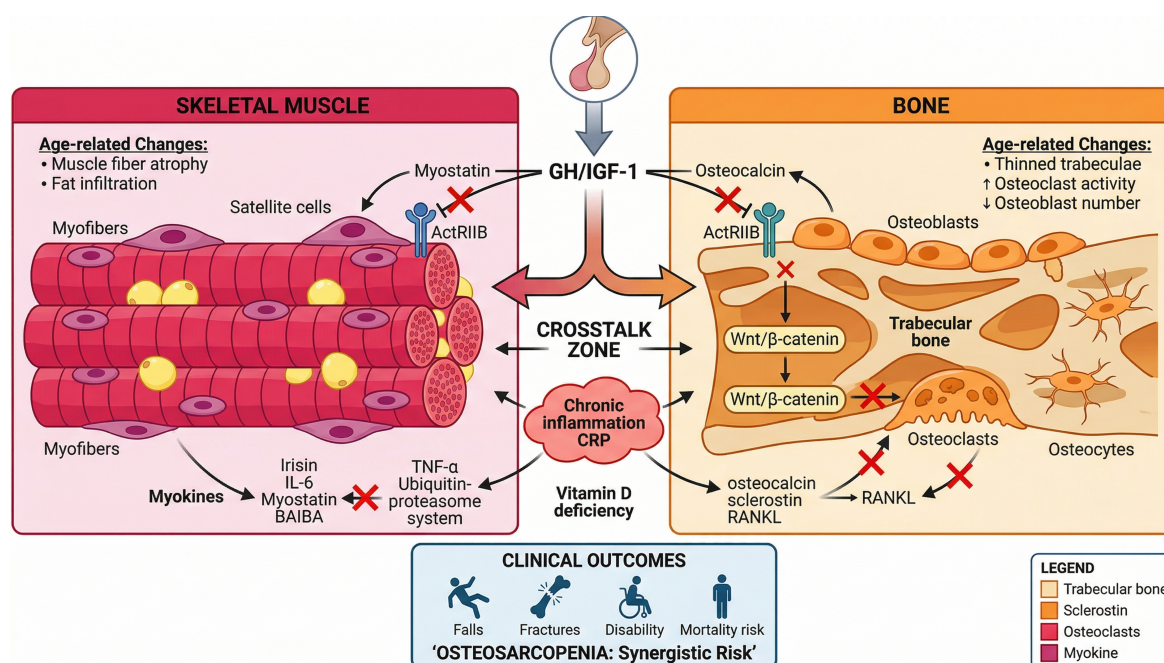


Fig. 1. Pathophysiology of osteosarcopenia and bone–muscle molecular crosstalk. The diagram illustrates the shared molecular mechanisms underlying the concurrent decline of bone and skeletal muscle, including dysregulated GH/IGF-1 signalling, chronic inflammation (elevated TNF- α , IL-6), vitamin D insufficiency, and impaired myokine–osteokine paracrine loops. Myostatin overexpression suppresses both satellite cell proliferation and osteoblast differentiation via ActRIIB signalling, while sclerostin inhibits Wnt/ β -catenin-dependent myogenesis. Abbreviations: GH, growth hormone; IGF-1, insulin-like growth factor-1; TNF- α , tumour necrosis factor-alpha; IL-6, interleukin-6; ActRIIB, activin receptor type IIB; RANKL, receptor activator of nuclear factor kappa-B ligand. Figure created using Google Gemini.

sensitivity [22,23]. In osteosarcopenia, this paracrine loop is disrupted: elevated myostatin suppresses both satellite cell proliferation and osteoblast differentiation via activin receptor type IIB (ActRIIB) signalling, while sclerostin overexpression from osteocytes inhibits Wnt/ β -catenin-dependent myogenesis [24,25]. The bidirectional nature of this crosstalk implies that restoring function in one tissue may positively impact the other, providing a strong biological rationale for dual-tissue therapeutic strategies.

The GH/IGF-1 axis serves as a master anabolic regulator for both tissues. Age-related decline in circulating IGF-1 reduces mammalian target of rapamycin (mTOR)-mediated protein synthesis in muscle and impairs osteoblast survival through phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) attenuation [26,27]. Similarly, chronic inflammation characterised by elevated tumour necrosis factor-alpha (TNF- α) and C-reactive protein (CRP) accelerates both muscle proteolysis via the ubiquitin–proteasome pathway and osteoclastic bone resorption through upregulation of RANKL and suppression of osteoprotegerin (OPG) [28]. These converging molecular pathways provide rational therapeutic targets for dual-tissue scaffold design, as locally delivered growth factors or anti-inflammatory agents could simultaneously rescue osteogenesis and myogenesis [29]. Exosome-mediated intercellular communication between bone and muscle cells has recently emerged as

another layer of crosstalk, with muscle-derived exosomes shown to transfer microRNAs that regulate osteoblast differentiation, further underscoring the intimate biochemical connection between these tissues [25]. In the context of osteosarcopenia, circulating exosomal microRNA (miRNA) profiles are significantly perturbed. For instance, aged muscle tissue releases exosomes enriched in miRNAs such as miR-34a, which induces senescence in bone marrow mesenchymal stem cells (BMSCs) and effectively suppresses osteoblastogenesis. Conversely, protective bone-derived exosomal miRNAs, such as the Wnt-activating miR-218, are reduced during aging, while muscle-derived factors like myostatin upregulate osteocyte sclerostin secretion, further disrupting the muscle–bone axis. To therapeutically target this pathological crosstalk, recent tissue engineering strategies utilize electrospun scaffolds designed to mimic the native bioelectric and biochemical microenvironment. By incorporating bioactive cues or engineered exosomes, these functionalized scaffolds serve as controlled delivery platforms to modulate exosomal miRNA networks, thereby holding promise for concurrent bone and muscle regeneration [25,30,31]. Additionally, mechanical loading—a stimulus shared by both tissues during physical activity—activates piezo1 mechanosensory channels in osteocytes and stretch-activated channels in myofibres, coordinating adaptive responses through shared intracellular signalling

cascades involving calcium, calcineurin, and nuclear factor of activated T-cells (NFAT). The convergence of these pathways in osteosarcopenia creates a vicious cycle: reduced muscle mass diminishes mechanical loading on bone, accelerating bone loss, which in turn increases fracture risk, leading to immobility and further muscle wasting. Breaking this cycle requires interventions that can simultaneously restore anabolic signalling in both tissues—a goal that locally delivered growth factors from dual-tissue scaffolds are uniquely positioned to achieve.

Structural Complexity of the Bone–Muscle Interface

The attachment of skeletal muscle to bone is mediated through tendons, periosteum, and in some anatomical locations, direct fascial insertions [32]. The enthesis—the insertion site of tendon into bone—comprises four histologically distinct zones: tendon proper (aligned type I collagen), uncalcified fibrocartilage, calcified fibrocartilage, and subchondral bone [33]. Each zone exhibits a gradient in mineral content (0–65 wt% hydroxyapatite (HA)), collagen fibre orientation (highly aligned to random), and elastic modulus (200 MPa to 20 GPa) [34,35] (Fig. 2). This graded structure has evolved to minimise stress concentrations at the junction between mechanically dissimilar tissues and to provide a smooth transfer of tensile loads from compliant muscle to rigid bone. The tidemark—the boundary between calcified and uncalcified fibrocartilage—serves as a critical mechanical transition point, and its disruption in degenerative conditions leads to interface failure.

For scaffold design targeting osteosarcopenia, the relevant interface extends beyond the classical enthesis to include the periosteal surface where muscle forces are transmitted to the bone cortex. This interface demands a scaffold with at least two functionally distinct layers: (i) an aligned, compliant layer mimicking the parallel fibre architecture of skeletal muscle and its endomysial connective tissue, and (ii) a stiff, mineralised layer reproducing the randomly oriented, calcium phosphate-rich matrix of woven bone [36,37]. A transitional zone between these layers, featuring graded mineralisation and intermediate fibre alignment, is desirable to avoid stress concentrations and delamination under physiological loading conditions [38]. Finite element analyses of biological entheses have demonstrated that gradient mineralisation reduces peak interfacial stresses by 30–50% compared with abrupt transitions, providing a biomechanical rationale for gradient scaffold design. The vascular architecture of the interface zone also merits consideration: the periosteum is highly vascularised and serves as a conduit for nutrient exchange between bone and muscle, suggesting that scaffold designs should incorporate porosity or channel structures that permit vascular ingrowth at the interface. Moreover, the periosteum contains a population of skeletal stem/progenitor cells with both osteogenic and chondrogenic potential, and recent evidence suggests that periosteal cells can be recruited by

muscle-derived signals to contribute to bone repair. Scaffold designs that create a permissive environment for periosteal cell migration at the bone–muscle interface may therefore enhance regenerative outcomes by leveraging this endogenous progenitor cell reservoir. The complexity of this interface—spanning mechanical gradients across three orders of magnitude in stiffness, compositional gradients from pure collagen to 65 wt% mineral, and cellular gradients from myoblasts to osteoblasts—poses formidable challenges for scaffold engineers but also provides clear design specifications that can guide material selection and fabrication strategies.

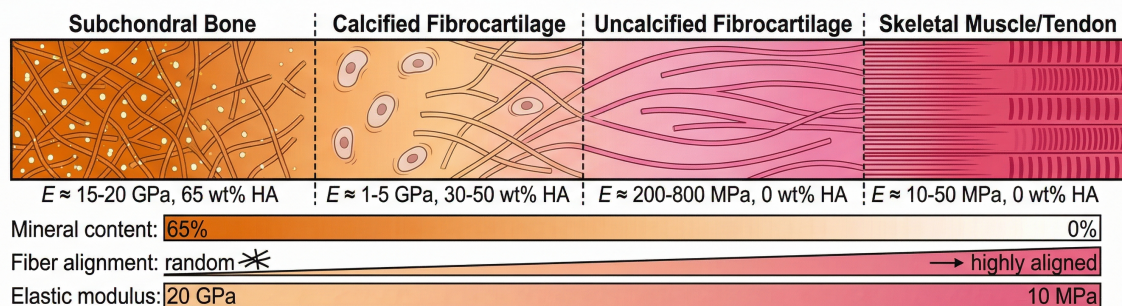
Electrospinning Strategies for Musculoskeletal Tissue Engineering

Aligned Fibres for Skeletal Muscle Regeneration

Skeletal muscle is a highly anisotropic tissue in which parallel arrays of multinucleated myofibres are bundled within connective tissue sheaths (endomysium, perimysium, epimysium) [39]. Recapitulating this unidirectional architecture is critical for functional muscle regeneration, as myotube alignment directly determines the direction and magnitude of contractile force generation. Electrospun fibres collected on a high-speed rotating mandrel (≥ 1500 rpm) exhibit preferential alignment with angular deviations typically below 15° from the collection axis [14,40]. Studies using poly(ϵ -caprolactone) (PCL), poly(lactic-co-glycolic acid) (PLGA), and silk fibroin have demonstrated that aligned substrates significantly enhance C2C12 myoblast elongation, fusion index, and myotube length compared to randomly oriented controls [41,42]. The degree of fibre alignment achievable depends on the interplay between electrostatic field geometry and mandrel surface velocity: at peripheral speeds exceeding 10 m/s, fibre alignment approaches 90–95% as measured by fast Fourier transform analysis of scanning electron micrographs.

Fibre diameter further modulates myogenic response. Sub-micrometre fibres (300–800 nm) most closely approximate the dimensions of native endomysial collagen fibrils (50–500 nm diameter) and promote superior myotube maturation relative to micrometre-scale fibres [43]. This diameter-dependent effect is attributed to the increased surface curvature of thinner fibres, which enhances focal adhesion assembly and cytoskeletal tension along the fibre axis through integrin clustering and Rho/ROCK signalling pathway activation. The incorporation of conductive nanomaterials such as graphene oxide or polypyrrole into aligned fibres introduces electrical stimulation capability, synergistically enhancing myosin heavy chain expression and sarcomeric organisation [44,45]. Electrically conductive scaffolds are particularly relevant for osteosarcopenia applications, as age-related denervation contributes to muscle atrophy and impaired neuromuscular junction function; providing electrical cues may partially compensate for this loss. Recent work combining aligned PCL–gelatin electrospun

A Native Bone-Muscle Interface Architecture



B Scaffold Design Requirements

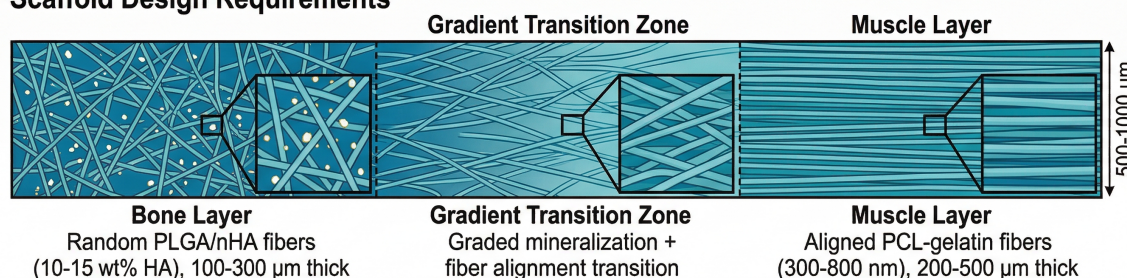


Fig. 2. Structural complexity of the bone–muscle interface. (A) Schematic representation of the four-zone enthesis architecture showing the gradient in mineral content (0–65 wt% hydroxyapatite), collagen fibre orientation (aligned to random), and elastic modulus (200 MPa to 20 GPa) from muscle/tendon to bone. (B) Corresponding design requirements for a multi-layered electrospun scaffold, indicating the aligned fibre layer (muscle-facing), gradient transitional zone, and mineralised random fibre layer (bone-facing). Abbreviations: HA, hydroxyapatite; ECM, extracellular matrix. Figure created using Google Gemini.

scaffolds with cyclic mechanical stretching in bioreactors has achieved myotube densities approaching 70% of native tissue, suggesting that the combination of topographical guidance with dynamic mechanical cues is essential for generating functional muscle constructs. Surface modification of aligned fibres through plasma treatment, aminolysis, or biomolecule conjugation further enhances myogenic outcomes. Laminin coating of aligned PCL fibres, for example, increases myoblast adhesion by 40–60% and promotes earlier onset of differentiation by activating integrin $\alpha 7 \beta 1$ signalling, which is the principal laminin receptor on skeletal muscle cells. For osteosarcopenia applications, where the regenerative capacity of aged satellite cells is diminished, such bioactive surface modifications may be critical for achieving sufficient myogenic response.

Random and Mineralised Fibres for Bone Regeneration

Bone ECM consists of randomly interwoven type I collagen fibrils mineralised with carbonated HA nanocrystals, a hierarchical structure spanning from the nanoscale to the organ level [46]. Conventional electrospinning onto a stationary or slowly rotating collector produces non-woven mats with random fibre orientation that mimic this isotropic collagen network. The high surface-area-to-volume ratio of electrospun fibres provides abundant nucleation sites for mineral deposition, either through direct incorporation of HA nanoparticles into the spinning solution or through post-fabrication mineralisation in simulated body fluid (SBF)

[47,48]. Random fibre orientation is also advantageous for osteogenic applications because it permits isotropic cell spreading, allowing osteoblasts to adopt their characteristic polygonal morphology rather than the elongated phenotype induced by aligned substrates.

Direct blending of nano-HA (nHA) into PCL or PLGA solutions at loadings of 5–15 wt% increases scaffold stiffness (elastic modulus from 8 to 45 MPa), enhances protein adsorption through increased surface roughness and hydrophilicity, and upregulates osteogenic markers (alkaline phosphatase (ALP), osteocalcin, Runt-related transcription factor 2 (RUNX2)) in mesenchymal stem cells (MSCs) [49,50]. However, nHA concentrations exceeding 20 wt% compromise fibre uniformity due to nozzle clogging and bead formation, and may introduce brittleness that reduces scaffold handling properties. Alternative mineral phases, including β -tricalcium phosphate (β -TCP) and strontium-substituted HA (Sr-HA), have shown promise in stimulating both osteogenesis and anti-osteoclastic activity, which is particularly relevant for the osteoporotic bone environment characteristic of osteosarcopenia [51,52]. Strontium ions exert a dual mechanism by promoting osteoblast proliferation through activation of the calcium-sensing receptor while simultaneously suppressing osteoclast differentiation via the RANKL/OPG pathway. This dual action makes Sr-HA a particularly attractive mineral phase for osteosarcopenia scaffolds, where the goal is not merely to form new bone but to do so in a metabolically compromised environ-

ment with elevated osteoclast activity.

Post-fabrication mineralisation strategies, including alternating immersion in calcium and phosphate solutions or electrodeposition of calcium phosphate coatings, achieve conformal mineral layers on individual fibres without disrupting scaffold architecture [53,54]. These approaches enable independent control of fibre morphology and mineral content, a key advantage for multi-layered scaffold fabrication where the mineralised layer must interface seamlessly with an unmineralised muscle-facing layer. SBF immersion for 7–14 days produces apatite layers 1–3 μm thick that closely resemble the carbonated HA found in native bone, whereas electrodeposition allows more rapid (minutes to hours) and tunable mineral coating with precise control over thickness and crystallinity. The choice between direct blending and post-fabrication mineralisation involves trade-offs: direct blending is simpler and ensures mineral distribution throughout the fibre volume, but post-fabrication methods preserve optimal fibre morphology and allow spatial patterning of mineral content through masking techniques. For osteosarcopenia scaffolds, a combined approach may be optimal: direct blending of nHA at moderate loadings (5–10 wt%) to establish baseline osteoconductivity, followed by selective post-fabrication SBF immersion of the bone-facing surface to create a mineral-rich superficial layer that promotes rapid osteoblast attachment while maintaining the fibre flexibility needed for handling and surgical placement.

Advanced electrospinning techniques

Beyond conventional single-nozzle electrospinning, several advanced configurations have been developed to enhance scaffold functionality (Fig. 3). Coaxial electrospinning employs a concentric dual-nozzle system to produce core-shell fibres, enabling encapsulation of bioactive molecules (growth factors, drugs) within a protective polymer core while maintaining a cell-interactive shell [55,56]. This architecture shields labile proteins from organic solvents during fabrication and provides a diffusion barrier for sustained release over weeks to months. Bone morphogenetic protein-2 (BMP-2) and IGF-1 co-loaded coaxial fibres have demonstrated sustained release over 28 days with preserved bioactivity, promoting both osteogenic and myogenic differentiation in co-culture systems [57]. The shell polymer composition and thickness can be independently tuned to control release kinetics: thicker shells of slowly degrading polymers such as PCL extend the release duration, while thinner shells of faster-degrading PLGA produce earlier and more complete release profiles.

Emulsion electrospinning offers a single-nozzle alternative in which aqueous protein solutions are dispersed as droplets within an organic polymer phase, yielding fibres with protein-rich domains that release cargo in a diffusion-controlled manner [58,59]. This technique reduces the initial burst release to approximately 25%, compared with

>40% for surface-adsorbed growth factors, thereby improving therapeutic efficacy and reducing the risk of growth factor overdose at the implant site. The simplicity of emulsion electrospinning—requiring only a single nozzle and standard equipment—makes it more amenable to clinical-scale production than coaxial systems, though the encapsulation efficiency is generally lower (60–80% versus 80–95% for coaxial).

Melt electrowriting (MEW), a solvent-free variant, deposits molten polymer in precisely controlled patterns to create scaffolds with tuneable pore geometry and fibre spacing [60,61]. Although MEW fibres are typically larger (20–100 μm) than solution-electrospun fibres, the technique offers unmatched architectural control that is particularly valuable for creating scaffolds with defined macropore sizes (100–500 μm) optimised for cell infiltration and vascular ingrowth. Hybrid approaches combining MEW for structural frameworks with solution electrospinning for nanofibrous infill have produced scaffolds with both macro-scale mechanical integrity and nano-scale bioactivity [62,63]. These multi-scale constructs more faithfully recapitulate the hierarchical nature of musculoskeletal tissues, where mechanical loads are borne by micrometre-scale fibrous networks while cell signalling is mediated at the nanometre scale. For osteosarcopenia applications, MEW-printed PCL grids overlaid with solution-electrospun nanofibre layers could provide the mechanical strength needed for early load-bearing while maintaining the biological cues necessary for both osteogenic and myogenic differentiation. Recent studies in interface tissue engineering have explicitly combined MEW-fabricated micro-scale lattices (fibre diameter 20–40 μm , pore size 200–400 μm) with solution-electrospun aligned or random nanofibre infills (300–800 nm) for bone-muscle and tendon-bone interface reconstruction [64,65], achieving compressive moduli of 2–10 MPa together with cell-instructive nanotopography in a single integrated construct [66,67], and demonstrating concurrent osteogenic and myogenic differentiation in co-culture [68,69].

Multi-Layered Scaffold Design for the Bone-Muscle Interface

Layer Architecture and Gradient Design

The fundamental design principle for an osteosarcopenia-targeted scaffold is the creation of spatially distinct yet mechanically integrated zones that recapitulate the bone-muscle continuum [70,71]. A minimum of two layers is required: a muscle-regenerative layer composed of aligned, compliant fibres and a bone-regenerative layer of random, mineralised fibres. Sequential electrospinning—depositing one layer directly atop the other on the same collector—produces a bilayer construct with an interpenetrating fibre interface that resists delamination under physiological loading [72]. The interfacial bonding strength of sequentially spun

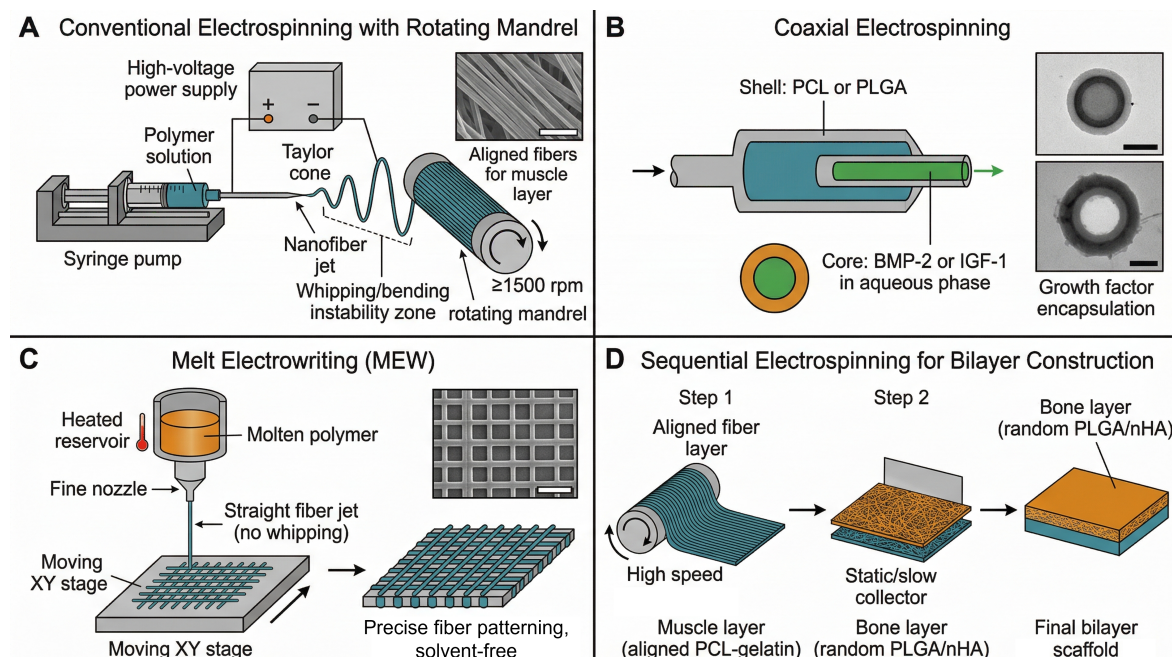


Fig. 3. Advanced electrospinning configurations for multi-layered scaffold fabrication. (A) Conventional single-nozzle electrospinning with rotating mandrel for aligned fibre collection. (B) Coaxial electrospinning producing core-shell fibres for growth factor encapsulation. (C) Melt electrowriting (MEW) for precise fibre patterning. (D) Sequential electrospinning strategy for bilayer scaffold construction, showing transition from high-speed mandrel collection (aligned muscle layer) to static collector (random bone layer) with intermediate gradient zone. Abbreviations: MEW, melt electrowriting; PCL, poly(ϵ -caprolactone); PLGA, poly (lactic-co-glycolic acid). Figure created using Google Gemini.

bilayers depends critically on the residual solvent content of the first layer at the time of second-layer deposition: partially solvated fibres at the interface create polymer chain entanglements that increase peel strength by two- to three-fold compared with fully dried interfaces.

More sophisticated designs incorporate a transitional gradient zone between the muscle and bone layers. This can be achieved through continuous variation of collector speed during spinning (gradually transitioning from high-speed rotation to static collection), compositional gradients (incrementally increasing nHA concentration in the polymer feed via programmable syringe pumps), or post-fabrication mineralisation gradients using masked immersion techniques [73,74]. Finite element modelling has shown that such gradient scaffolds reduce interfacial stress concentrations by 40–60% compared with sharp bilayer interfaces, a finding corroborated by mechanical testing under cyclic tensile loading at physiologically relevant strain amplitudes of 1–5% [75] (Fig. 4). The gradient zone also serves as a biological transition, presenting intermediate cues that may support the differentiation of progenitor cells into fibrocartilage-like phenotypes characteristic of the native enthesis, thereby creating a more physiologically complete reconstruction of the bone–muscle interface. Computational models can guide gradient design by predicting optimal transition zone width and mineralisation profile to minimise stress while maintaining adequate stiffness

for load transfer; parametric studies suggest that a gradient zone spanning 15–25% of total scaffold thickness provides the best compromise between mechanical performance and fabrication feasibility.

Layer thickness is another critical parameter requiring careful optimisation. The muscle-facing layer should be sufficiently thick (200–500 μm) to support three-dimensional myotube formation and accommodate the diffusion of nutrients and oxygen to seeded myoblasts, while the bone-facing layer requires 100–300 μm to provide adequate mineral reservoir and mechanical support for osteoblast colonisation [76]. Total scaffold thickness of 500–1000 μm balances nutrient diffusion limitations with structural requirements. Scaffolds exceeding 1 mm in thickness without perfusable vasculature risk core necrosis within 48–72 hours of implantation, necessitating incorporation of angiogenic factors, pre-vascularisation strategies through co-culture with endothelial cells, or the inclusion of sacrificial fibres (e.g., polyethylene glycol or gelatin fibres that dissolve within hours of implantation) that create interconnected channels for vascular ingrowth and nutrient perfusion throughout the scaffold thickness. Quantitative parameters reported in the literature suggest that sacrificial-fibre-derived channels in the 100–300 μm range are optimal for endothelial cell infiltration and capillary network formation [77,78], with 150–250 μm channels balancing mechanical integrity and vascular ingrowth in bone-facing layers [79],

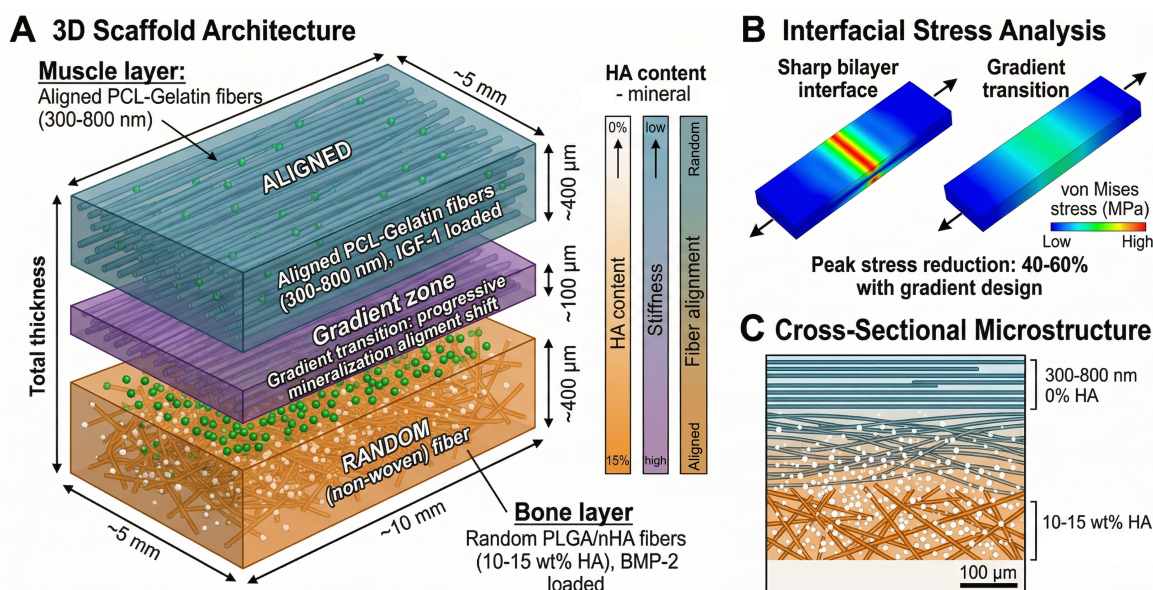


Fig. 4. Multi-layered scaffold design concept with gradient mineralisation. (A) Three-dimensional schematic of the proposed osteosarcopenia-targeted scaffold showing the aligned PCL–gelatin muscle layer (top), gradient transitional zone (middle), and mineralised PLGA/nHA bone layer (bottom). (B) Finite element analysis of interfacial stress distribution comparing sharp bilayer interface versus gradient transition under cyclic tensile loading. The gradient design reduces peak stress by approximately 40–60%. (C) Cross-sectional scanning electron microscopy (SEM) representation showing fibre alignment transition and mineral content gradient. Abbreviations: nHA, nano-hydroxyapatite; FEA, finite element analysis; SEM, scanning electron microscopy. Figure created using Google Gemini.

while 50–150 μm interconnected pores in muscle-facing layers favour myoblast migration and aligned myotube formation [80,81]. Volumetric channel fractions of 20–35% have been associated with the most rapid perfusion onset (within 5–7 days) without compromising scaffold stiffness below physiologically relevant thresholds [82].

Material Selection and Composition

Material selection for osteosarcopenia scaffolds must satisfy divergent biological and mechanical requirements across layers (Table 1). For the muscle layer, compliant and biodegradable polymers with demonstrated cytocompatibility for myoblasts are preferred. PCL (elastic modulus 4–12 MPa as an electrospun mat) offers slow degradation matching muscle remodelling timescales (12–24 months) and is approved by the US Food and Drug Administration (FDA) for implantable devices [83]. Blending PCL with gelatin (70:30 w/w) improves hydrophilicity and cell adhesion by introducing integrin-binding arginine–glycine–aspartic acid (RGD) sequences while preserving fibre alignment capability under high-speed mandrel collection [84]. Silk fibroin represents a natural alternative with excellent biocompatibility, tuneable degradation through control of β -sheet crystallinity, and inherent anti-inflammatory properties, though electrospinning from hexafluoroisopropanol (HFIP) solutions necessitates thorough post-processing to remove residual solvent and to induce water stability through methanol treatment or water vapour

annealing.

For the bone layer, stiffer composites are essential to provide appropriate mechanical cues for osteogenic differentiation. PLGA (85:15 lactide:glycolide) provides faster degradation (6–12 months) appropriate for bone healing kinetics, and its higher glass transition temperature (approximately 55°C) yields stiffer fibres than PCL [85,86]. Incorporating nHA at 10–15 wt% into PLGA raises the composite modulus to 30–50 MPa and provides osteoconductive calcium and phosphate ions upon degradation, creating a locally supersaturated environment that promotes biological apatite nucleation on the scaffold surface [50]. Collagen–HA composites most faithfully replicate bone ECM composition but face challenges in consistent electrospinning due to solution viscosity variations and batch-to-batch variability that complicate regulatory approval [87]. PCL blended with Sr–HA at 10–15 wt% represents an attractive bone-layer candidate that combines the slow degradation and handling properties of PCL with the dual osteoanabolic and anti-osteoclastic activity of strontium ions, as described in Section (Random and Mineralised Fibres for Bone Regeneration) [51,52]. However, while these synthetic composites offer structural support, they must also address the chronic low-grade inflammatory microenvironment of osteosarcopenia. A primary challenge remains the bulk hydrolysis of PLGA, which releases lactic and glycolic acids that can drop the local pH to 5.5–6.5, potentially aggravating inflammation-related

cytokine secretion. To counteract this, incorporating titania (TiO₂) nanoparticles, β -tricalcium phosphate, or Mg-substituted HA as basic fillers can effectively buffer the local environment and enhance osteoblast adhesion [88, 89]. Furthermore, the implementation of a stratified design is essential to match the distinct regeneration kinetics of bone and muscle [90]. This involves extending the bone layer's stability—perhaps through photocrosslinking with methacrylated derivatives like methacrylated poly(ϵ -caprolactone) (PCL-MA)—to align with cortical bridging timescales, while ensuring the muscle layer supports long-term myofibre and neuromuscular junction maturation over 12–18 months [91]. Such precise tuning of degradation profiles, combined with the use of 10 mol% Sr-HA to provide anti-osteoclastic activity in osteoporotic conditions [92,93], creates a more robust foundation for tissue-specific bioactivity.

An emerging strategy employs decellularised ECM (dECM) from bone and muscle tissues as electrospinning additives, leveraging the complex mixture of structural proteins, glycosaminoglycans, and retained growth factors present in native tissue matrices. Bone-derived dECM incorporated into PCL fibres at 5–20 wt% retains native growth factors (BMP-2, transforming growth factor-beta (TGF- β)) and promotes osteogenic differentiation without exogenous supplementation [94,95]. Muscle-derived dECM similarly preserves laminin, fibronectin, and hepatocyte growth factor (HGF), supporting satellite cell activation and proliferation [81,96]. These bio-inspired composites represent a promising direction for achieving tissue-specific bioactivity in each scaffold layer while avoiding the regulatory complexity and high cost associated with recombinant growth factor incorporation [97]. However, dECM sourcing from age-matched donors, decellularisation standardisation to ensure complete removal of cellular antigens while preserving bioactive molecules, and immunogenicity testing in relevant animal models remain hurdles that must be addressed for clinical translation [98, 99]. The use of xenogeneic dECM (e.g., porcine-derived) may offer advantages in supply chain scalability but introduces additional immunological considerations—including residual alpha-gal epitope reactivity and the potential for zoonotic pathogen transmission—that must satisfy FDA xenotransplantation guidance (21 CFR 1271) and European Medicines Agency (EMA) Advanced Therapy Medicinal Product (ATMP) classification requirements before clinical deployment, particularly in the context of elderly, potentially immunocompromised osteosarcopenia patients [100].

Growth Factor Incorporation and Delivery

Spatially controlled delivery of tissue-specific growth factors is arguably the most critical design element for dual-tissue scaffolds, as the biochemical microenvironment ultimately dictates the fate of recruited progenitor cells. For the bone layer, BMP-2 remains the gold standard osteoinduc-

tive agent, with effective doses of 50–100 ng/mL demonstrated *in vitro* [101,102]. However, supraphysiological BMP-2 doses—as used in commercially available collagen sponge carriers (up to 1.5 mg/mL)—are associated with ectopic bone formation, seroma, oedema, and local inflammation, necessitating sustained, low-dose delivery achievable through coaxial fibre encapsulation rather than simple surface adsorption [103]. Vascular endothelial growth factor (VEGF) co-delivery at 10–50 ng/mL promotes angiogenesis essential for bone graft survival and integration with the host vasculature, as bone regeneration is critically dependent on establishment of a functional vascular network within the first two weeks of healing [104,105].

For the muscle layer, IGF-1 (10–50 ng/mL) stimulates satellite cell proliferation and myofibre hypertrophy through mTOR activation, directly counteracting the sarcopenic phenotype characterised by reduced protein synthesis and accelerated proteolysis [26,106]. Myostatin inhibitors, including follistatin-derived peptides and anti-myostatin antibodies, represent a novel class of therapeutic agents that simultaneously promote muscle growth and bone formation by blocking ActRIIB signalling in both tissues, making them particularly attractive for osteosarcopenia applications where dual-tissue anabolism is the therapeutic goal [107]. Coaxial electrospun fibrous membranes co-loaded with BMP-2 and IGF-1 have demonstrated sustained dual release profiles with preserved bioactivity, significantly enhancing osteogenic differentiation of BMSCs compared to single-factor controls [108] (Fig. 5).

The temporal sequence of growth factor presentation is also critical for optimal tissue regeneration: early IGF-1 release (days 1–7) supports rapid myoblast proliferation and initial angiogenesis through synergistic action with VEGF, while sustained BMP-2 release (days 7–28) drives osteogenic commitment of recruited MSCs once an adequate vascular bed is established. Achieving this sequential release profile through differential polymer degradation rates between layers is an area of active investigation. Core-shell fibre architectures offer the additional advantage of decoupling release kinetics from scaffold degradation by varying core polymer molecular weight and shell thickness independently. Future designs may incorporate stimuli-responsive release mechanisms—triggered by pH changes in the healing microenvironment, enzymatic activity from infiltrating cells, or externally applied stimuli such as near-infrared light or magnetic fields—to achieve on-demand growth factor delivery that adapts to the evolving biological needs of the regenerating tissue. Treating osteosarcopenia requires looking past the standard use of BMP-2, VEGF, and IGF-1. Because this condition involves a complex mix of inflammation, poor tissue regeneration, and immune dysregulation, we need a broader range of therapeutic approaches. First, to manage the inflammatory microenvironment, immunomodulatory agents can be utilized. Loading molecules like IL-4 (10–50 ng/mL), IL-10, or re-

Table 1. Summary of material candidates for multi-layered osteosarcopenia-targeted electrospun scaffolds.

Material	Target layer	Elastic modulus (MPa)	Degradation time	Key advantages
PCL	Muscle	4–12	12–24 months	Slow degradation, FDA-approved, excellent spinnability
PCL–Gelatin (70:30)	Muscle	6–15	6–12 months	Enhanced hydrophilicity, improved cell adhesion
Silk fibroin	Muscle	5–17	6–24 months	High biocompatibility, tuneable degradation
PLGA (85:15)	Bone	15–30	6–12 months	Faster degradation matching bone healing
PLGA/nHA (10 wt%)	Bone	30–50	6–12 months	Osteoconductive, enhanced stiffness
Collagen–HA	Bone	20–40	3–6 months	Biomimetic composition, native ECM analogue
PCL/Sr–HA	Bone	25–45	12–24 months	Anti-osteoclastic, pro-angiogenic

solvin D1 into fibrous scaffolds helps shift macrophages toward the tissue-repairing M2 phenotype [109]. Second, targeting the myostatin pathway offers a way to stimulate both bone and muscle simultaneously. Using follistatin-derived peptides or ActRIIB-Fc decoy receptors to locally block the GDF-8/ActRIIB axis fits perfectly with the underlying pathology of osteosarcopenia [110]. Furthermore, ECM-derived bioactive peptides (such as RGD, IKVAV, DGEA, or GFOGER) and dECM hydrogel coatings can provide multifaceted cues [111,112]. These are often easier to navigate from a regulatory standpoint compared to recombinant growth factors. There is also strong potential in vesicle-based delivery. Exosomes—either engineered or derived from MSCs—can be loaded with microRNAs that promote bone (miR-26a, miR-148a) or muscle (miR-1, miR-206, miR-486) growth. Tethering these to scaffold fibers or embedding them in alginate microparticles allows for sustained, bioactive release over 2 to 4 weeks [113]. Finally, non-viral gene delivery offers a practical workaround for the high costs and short half-lives of recombinant proteins. By formulating plasmid DNA or modified mRNA into lipid nanoparticles or polyplexes and embedding them in electrospun fibers, we can ensure localized and sustained protein expression [114,115]. Ultimately, choosing the right approach should depend on the primary driver of the disease in a specific patient group, such as whether they are suffering more from chronic inflammation or anabolic resistance.

Preclinical Evaluation Models

Preclinical assessment of multi-layered scaffolds requires models that capture the complexity of bone–muscle interactions at multiple scales, from cellular behaviour on individual fibres to whole-organ regeneration *in vivo*. Monoculture studies using C2C12 murine myoblasts on aligned layers and MC3T3-E1 murine pre-osteoblasts on mineralised layers provide initial biocompatibility and differentiation data [14,50]. However, co-culture systems more accurately reflect the paracrine signalling environment of osteosarcopenia and are essential for evaluating cross-talk effects between scaffold layers. Recent co-culture studies have demonstrated that myoblast-conditioned medium containing myokines such as IGF-1,

TGF- β , and IL-8 significantly enhances osteogenic differentiation of MSCs, while reciprocal exposure of myoblasts to MSC-derived secretome improves myogenic factor expression, confirming bidirectional paracrine crosstalk between bone and muscle lineage cells [116]. Direct co-culture on bilayer scaffolds represents the most physiologically relevant *in vitro* model, enabling simultaneous assessment of layer-specific differentiation and inter-layer communication through shared culture medium. Perfusion bioreactor culture of such constructs improves cell viability in scaffold cores (from 20% to >70% viable cells at 500 μ m depth) and enhances ECM deposition compared to static culture, making bioreactor-based evaluation an important component of the preclinical workflow [117,118].

Translation to *in vivo* settings requires animal models that recapitulate not only the localized bone–muscle defect but also the systemic metabolic decline characteristic of osteosarcopenia. While subcutaneous implantation of fibrous membranes in rodents (7–30 days) provides valuable initial data on host tissue response and early vascularization [119], it lacks the orthotopic relevance and mechanical loading environment critical for musculoskeletal tissue maturation. Critical-sized segmental bone defects in rat femora (5 mm) or rabbit radii (15 mm) with concurrent volumetric muscle loss (VML) of 20–40% of the affected compartment closely model the clinical scenario of composite musculoskeletal injury. However, to truly reflect the underlying disease pathology, these surgical defects should ideally be superimposed on established systemic osteosarcopenia models. Recent advances highlight the utility of natural aging models, such as the *Octodon degus* rodent [120], or chemically/surgically induced models like ovariectomised combined with dexamethasone (OVX+DEX) in rats [121], which accurately mimic concurrent bone and muscle deterioration. To our knowledge, multi-layered electrospun scaffolds have rarely, if ever, been evaluated specifically in such comprehensive, systemically compromised composite osteosarcopenia models, representing a significant gap in the literature.

Rather than merely identifying this gap, we propose specific, standardized *in vivo* models to better evaluate therapies. First, we recommend a combined OVX and hindlimb suspension model in 12-month-old female

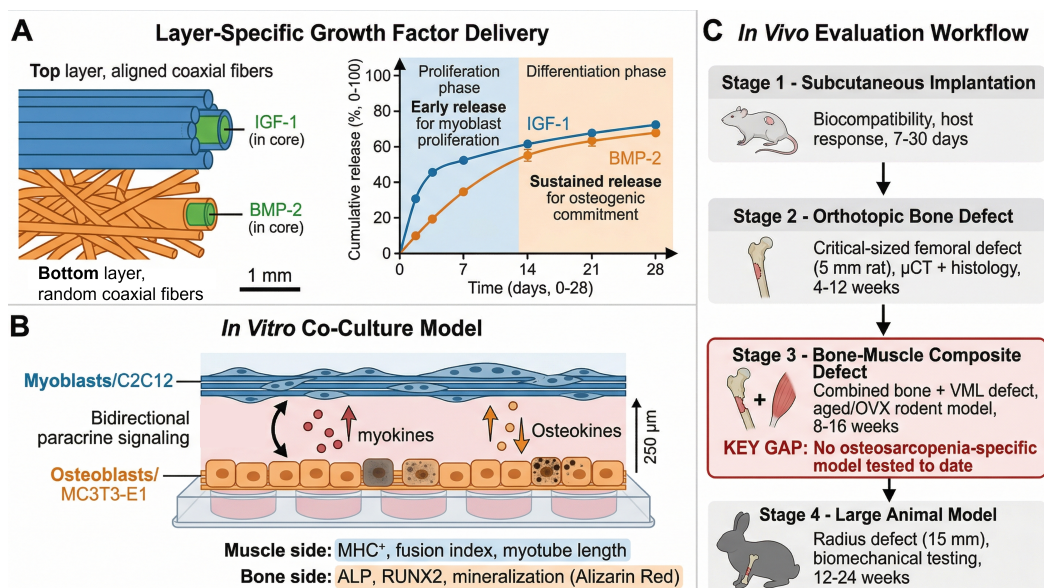


Fig. 5. Growth factor delivery strategy and preclinical evaluation pathway for osteosarcopenia-targeted scaffolds. (A) Layer-specific growth factor loading: IGF-1 encapsulated in coaxial aligned fibres (muscle layer) for early release (days 1–7) and BMP-2 in coaxial mineralised fibres (bone layer) for sustained release (days 7–28). (B) *In vitro* co-culture model showing osteoblasts on the mineralised layer and myoblasts on the aligned layer with bidirectional paracrine signalling. (C) Proposed *in vivo* evaluation workflow from subcutaneous implantation to orthotopic bone–muscle defect model in aged/OVX rodents. Abbreviations: IGF-1, insulin-like growth factor-1; BMP-2, bone morphogenetic protein-2; OVX, ovariectomised; MSC, mesenchymal stem cell. Figure created using Google Gemini.

Sprague–Dawley rats. This involves bilateral OVX at week 0, followed by a 28-day tail-suspension starting at week 4 to induce concurrent disuse atrophy. At week 8, a critical-sized 5-mm femoral defect combined with a 30% VML is created, with outcomes evaluated at 4, 8, and 12 weeks post-implantation. Second, senescence-accelerated mouse-prone (SAMP) substrains 6 and 8 aged 8–10 months offer a valuable model for spontaneous bone loss and sarcopenia without surgical intervention, accommodating 3-mm calvarial or 2-mm femoral defects. Third, a glucocorticoid-induced model (subcutaneous dexamethasone at 1.5 mg/kg/day for 8 weeks in 6-month-old rats) is ideal for capturing inflammaging-dominant phenotypes. Finally, naturally aging cohorts, such as C57BL/6 mice (≥ 18 months) or older rabbits, can serve as late-stage validation models. When comparing these options, natural aging models provide the highest pathophysiological fidelity by naturally mimicking the human aging process, but they require prohibitive timelines (often taking 20 months or more to exhibit phenotypes) and significant maintenance costs. Standard OVX effectively models postmenopausal osteoporosis and estrogen deficiency, but it often fails to capture severe sarcopenia unless paired with a disuse model, such as hindlimb suspension. Glucocorticoid models strongly emphasize the inflammatory component and rapidly induce bone and muscle loss, yet they introduce systemic metabolic confounders like fat accumulation and insulin resistance. Therefore, we recommend the

combined OVX and hindlimb-suspension model as the primary efficacy benchmark, as it closely mimics the clinical mechanics of osteosarcopenia within a manageable window [122]. Furthermore, senescence-accelerated mice—specifically SAMP6 for senile osteoporosis and SAMP8 for rapid muscle aging—can serve as a valuable secondary model to confirm aging-specific responses efficiently without surgical intervention [123].

To ensure true clinical relevance and facilitate a seamless transition to human trials, functional outcome metrics in these preclinical models must extend beyond standard histological and micro-computed tomography (micro-CT) assessments. For the muscular compartment, we suggest incorporating *in vivo* muscle contractile force (peak twitch and tetanic tension, targeting $\geq 70\%$ of the intact contralateral side) alongside three-axis gait analysis. This dynamic gait evaluation serves as a critical preclinical surrogate for the physical performance tests (e.g., gait velocity) routinely used to assess functional decline in older adults [124]. Concurrently, bone biomechanical testing must include three-point bending to accurately quantify ultimate load and stiffness (targeting $\geq 80\%$ of the contralateral side), providing a direct measure of functional skeletal integrity [125]. Furthermore, while activity-cage monitoring can act as a reliable surrogate for clinical fall risk, the longitudinal tracking of systemic serum biomarkers is essential for understanding muscle–bone crosstalk. Specifically, established bone turnover markers (C-terminal telopeptide

of type I collagen (CTX) and procollagen type I N-terminal propeptide (P1NP)) should be measured in tandem with key myokines, such as irisin and myostatin, to capture the systemic metabolic response [126]. Standardizing the reporting of these diverse, clinically translatable metrics will facilitate cross-study comparisons and ultimately accelerate the development of targeted osteosarcopenia therapies.

Immunomodulatory Design as a Core Scaffold Principle

Chronic low-grade inflammation—often referred to as inflammaging—is a hallmark of osteosarcopenia. Importantly, it is now recognized as a major factor dictating how well biomaterial scaffolds perform *in vivo*. In the aging bone-muscle microenvironment, we typically observe elevated levels of pro-inflammatory markers like TNF- α , IL-6, and IL-1 β , alongside a skewed M1/M2 macrophage ratio and exhausted regulatory T-cells. Together, these factors actively hinder progenitor cell recruitment, disrupt blood vessel formation, and accelerate the unwanted fibrotic encapsulation of implants [127]. Because of this, immunomodulation cannot just be treated as an afterthought or a future refinement; it must be a foundational design principle when developing multi-layered electrospun scaffolds for osteosarcopenia [128,129]. Researchers can tackle this using a few complementary design strategies. The first strategy involves tweaking fiber diameter and topography to physically dictate cell shape. For instance, sub-micron fibers, particularly those with an aligned topography, force macrophages into an elongated morphology. This physical elongation alone is sufficient to push the cells toward a pro-resolution M2 phenotype, significantly reducing foreign-body giant cell formation compared to randomly oriented, micrometer-scale fibers [130,131]. Surface chemistry offers another powerful tool to resist the foreign-body reaction. Applying zwitterionic or specific polysaccharide coatings helps cut down on non-specific protein adsorption and complement activation. This extreme resistance to biofouling effectively dampens the initial inflammatory M1 burst and prevents the formation of a dense collagenous capsule [132]. Furthermore, macroscopic surface wettability strictly governs subsequent immune responses; maintaining a controlled hydrophilicity—specifically a water contact angle between 40° and 60°—mediates an optimal profile of serum protein adsorption that maximizes the release of anti-inflammatory cytokines, creating an ideal environment for the M2 transition [133]. Finally, the strategy of bioactive loading provides a sophisticated means of biochemical signaling. Anti-inflammatory agents—such as IL-4, IL-10, resolvin D1, or low-dose dexamethasone (1–10 $\mu\text{g}/\text{cm}^2$)—can be co-encapsulated with tissue-specific anabolic factors within core-shell fibers. This architectural design enables a dual-stage, temporal release profile: a rapid burst from the shell to dampen early inflammation (days 0–7), followed by the sustained, diffusion-limited release of osteogenic or myogenic factors from the core to drive long-term regener-

ation (days 7–28) [134]. Implementing this immunomodulation in a layer-specific manner allows for precise control over the local microenvironment. On the bone-facing side, functionalizing the scaffold with strontium (Sr^{2+}) ions or IL-4 is particularly effective. Strontium acts as a potent osteoimmune modulator, shifting the macrophage population toward an M2 phenotype that secretes pro-osteogenic cytokines (e.g., BMP-2, TGF- β 1), thereby creating a favorable niche for osteoblast differentiation [135]. Concurrently, the muscle-facing layer can be tailored with IL-10 to trigger the necessary transition from a pro-inflammatory Th1 response to a pro-regenerative Th2-like macrophage phenotype. This transition is crucial for muscle repair, as M2 macrophages not only suppress chronic inflammation but also directly promote the activation, proliferation, and fusion of satellite cells [136]. By integrating these spatially and temporally controlled cues, the multi-layered construct functions as an active immuno-regenerative interface, potentially enhancing bone bridging and myofiber maturation by 30% to 60% compared to standard structural templates.

Challenges, Future Perspectives, and Conclusions

Clinical Translation Barriers

Despite promising preclinical data, several barriers impede clinical translation of multi-layered electrospun scaffolds. Manufacturing scalability remains the foremost challenge: laboratory electrospinning produces scaffolds at rates of 0.1–1.0 g/h, insufficient for clinical-grade production of devices that may require grams of material per patient [137]. Multi-nozzle, needleless, and free-surface electrospinning systems have increased throughput to 10–50 g/h, but maintaining inter-layer registration and gradient fidelity at scale requires further optimisation of process parameters and real-time monitoring systems. Quality control metrics for multi-layered constructs—including layer thickness uniformity ($\pm 10\%$ tolerance), mineral content consistency (assessed by thermogravimetric analysis), and fibre alignment reproducibility (quantified by orientation order parameter)—have yet to be standardised across the field, hindering comparison between studies and complicating regulatory submissions.

Regulatory classification of multi-layered scaffolds with incorporated growth factors as combination products introduces significant complexity, as such constructs may fall under device, biological product, or combined regulatory pathways depending on the primary mode of action, with FDA routing through the Office of Combination Products and EMA applying the ATMP framework [138]. This dual pathway increases both development costs and time-to-market, creating a barrier that particularly affects academic laboratories and small biotechnology companies. Sterilisation of electrospun scaffolds without compromising fibre morphology or growth factor bioactivity is another practical challenge: gamma irradiation (25 kGy) degrades

PLGA molecular weight by 30–60% and denatures encapsulated proteins, while ethylene oxide may leave cytotoxic residues in porous nanofibrous structures that are difficult to aerate completely [139]. Supercritical CO₂ sterilisation has shown promise as a mild alternative that preserves both polymer properties and protein bioactivity, but remains limited in commercial availability and has not been validated for multi-layered constructs with gradient compositions. Direct quantitative comparison among the three principal modalities highlights the trade-offs: gamma irradiation (25 kGy) reduces PLGA molecular weight by 30–60% and BMP-2 bioactivity by 60–80%, frequently coarsens fibre morphology, and lowers tensile modulus by 20–40% [140,141]; ethylene oxide preserves polymer chain integrity (<5% MW reduction) but leaves residual ethylene oxide and ethylene chlorohydrin (>10 ppm) in nanofibrous mats requiring 5–14 days of aeration, with growth factor activity loss of 20–40% [142]; supercritical CO₂ at 35–40°C and 100–200 bar for 1–4 h preserves 85–95% of growth factor bioactivity, retains fibre diameter within $\pm 10\%$, and reduces tensile modulus by less than 15%, with the additional advantage of leaving no toxic residues [143,144]. Among these, supercritical CO₂ currently offers the most favourable profile for multi-layered electrospun scaffolds carrying bioactive cargo, although process validation under cGMP for gradient-composition constructs remains an unmet need.

Mechanical property mismatch between electrospun scaffolds (tensile modulus 5–50 MPa) and cortical bone (15–20 GPa) restricts application to non-load-bearing or load-sharing sites unless the scaffold is combined with rigid fixation hardware. For osteosarcopenia patients, common fracture sites include the proximal femur and vertebral bodies—locations requiring substantial mechanical support beyond what electrospun membranes alone can provide. Hybrid strategies combining electrospun layers with 3D-printed rigid poly(ϵ -caprolactone) or titanium frameworks may address this limitation by providing macro-scale structural support while the electrospun layers deliver biological functionality at the tissue interface. Such multi-technology integration adds manufacturing complexity but may represent the most realistic path to clinical application for load-bearing osteosarcopenia sites. Additionally, the long-term degradation behaviour of multi-layered scaffolds with different polymer compositions in each layer remains poorly characterised. Differential degradation rates between layers could lead to mechanical instability at the interface as one layer degrades faster than the other, potentially causing delamination or loss of gradient continuity. Systematic *in vitro* degradation studies spanning 6–12 months under physiological loading conditions are needed to establish design rules for matching layer degradation rates to tissue regeneration timescales. The acidic degradation products of PLGA, in particular, may locally lower pH and accelerate dissolution of calcium phosphate mineral, creating a

complex feedback loop between polymer degradation and mineral stability that has not been adequately addressed in current scaffold designs.

Emerging Technologies and Future Directions

Several emerging technologies hold potential to overcome current limitations in osteosarcopenia scaffold design. Four-dimensional (4D) electrospinning, incorporating shape-memory polymers that respond to temperature or hydration, could enable minimally invasive delivery of rolled scaffolds that unfurl and conform to defect geometries upon implantation, reducing surgical morbidity in frail elderly patients who are often poor candidates for extensive open surgical procedures. Bioprinting of cell-laden bioinks onto electrospun substrates offers precise spatial control over cell placement within multi-layered constructs, enabling pre-seeding of myoblasts on aligned layers and osteoprogenitors on mineralised layers before implantation, thereby reducing the time required for *in vivo* cell recruitment and accelerating functional tissue regeneration.

Machine learning-guided optimisation of electrospinning parameters (voltage, flow rate, collector speed, solution concentration, humidity) is accelerating scaffold design by predicting fibre diameter and alignment from input variables with >90% accuracy, dramatically reducing the empirical trial-and-error traditionally required to develop new scaffold formulations. Deep learning models trained on large datasets of electrospinning process parameters and resulting scaffold properties can identify non-obvious parameter interactions and predict optimal conditions for novel polymer–mineral combinations without exhaustive experimental screening. Organ-on-a-chip platforms incorporating bone and muscle compartments separated by electrospun membranes provide high-throughput screening of scaffold compositions and growth factor combinations before committing to costly and ethically complex animal studies, with the added advantage of using human cells to improve translational relevance.

The integration of immunomodulatory strategies into scaffold design is gaining recognition as a critical factor for clinical success, particularly in the aged and immunologically altered patient population affected by osteosarcopenia. Macrophage polarisation from the pro-inflammatory M1 phenotype to the regenerative M2 phenotype, driven by scaffold surface chemistry, fibre diameter (with diameters below 1 μm promoting M2 polarisation), and incorporated anti-inflammatory agents (e.g., interleukin-4, resolvin D1, specialised pro-resolving mediators), is essential for successful tissue integration in the chronic inflammatory environment of osteosarcopenia. Designing each scaffold layer to promote the appropriate temporal immune response—early pro-inflammatory signalling for debris clearance followed by sustained anti-inflammatory cues for tissue remodelling—represents a frontier in musculoskeletal tissue engineering that may determine the clin-

ical success or failure of implanted constructs. Gene therapy approaches, incorporating plasmid DNA or messenger RNA encoding osteogenic or myogenic transcription factors within electrospun fibres, represent another frontier that could provide sustained and localised expression of therapeutic proteins without the stability and cost concerns associated with recombinant growth factor delivery.

Concluding Remarks

Osteosarcopenia demands a tissue engineering approach that transcends single-tissue paradigms. Multi-layered electrospun scaffolds, comprising aligned fibres for muscle regeneration and mineralised random fibres for bone formation connected through gradient transitional zones, represent a rational design strategy grounded in the pathophysiology of the bone–muscle unit. The versatility of electrospinning—enabling independent control of fibre alignment, diameter, composition, and bioactive cargo in each layer—makes it uniquely suited to this application among current scaffold fabrication technologies.

Key design principles emerging from this review include: (i) aligned PCL–gelatin or silk fibroin fibres (300–800 nm diameter) for the myogenic layer with IGF-1 delivery via coaxial encapsulation; (ii) random PLGA/nHA fibres (10–15 wt% HA) for the osteogenic layer with sustained BMP-2 release; (iii) gradient mineralisation and fibre alignment transitions to minimise interfacial stress; and (iv) layer-specific degradation rates matching tissue healing timescales. A fifth, equally critical principle is now apparent: (v) explicit immunomodulatory design — incorporating sub-1 μm fibre diameters, anti-inflammatory or pro-resolving cargo, and layer-specific osteoimmune cues — to convert the scaffold from a passive template into an active immunoregenerative interface suited to the inflammatory environment of osteosarcopenia. The incorporation of dECM-derived bioactive cues and immunomodulatory agents represents a promising addition to this design framework that may improve outcomes in the compromised healing environment of osteosarcopenia.

Critical gaps remain that must be addressed to advance the field. Multi-layered electrospun scaffolds have scarcely been tested in osteosarcopenia-specific animal models, making clinical translation premature without this foundational evidence. Manufacturing scalability, sterilisation protocols, and regulatory pathways for these complex combination products require systematic development through industry–academic partnerships. Furthermore, long-term degradation behaviour and mechanical performance of gradient scaffolds under physiological loading conditions remain poorly characterised beyond 12 weeks in most published studies. Addressing these challenges through interdisciplinary collaboration among materials scientists, biologists, clinicians, and regulatory specialists will be essential to translate osteosarcopenia-targeted scaffolds from promising laboratory concepts to a clinical reality that can

meaningfully improve outcomes for the millions of elderly patients affected by this debilitating condition.

List of Abbreviations

ActRIIB, activin receptor type IIB; ALP, alkaline phosphatase; BAIBA, β -aminoisobutyric acid; BMP-2, bone morphogenetic protein-2; CRP, C-reactive protein; dECM, decellularised extracellular matrix; ECM, extracellular matrix; FDA, US Food and Drug Administration; GH, growth hormone; HA, hydroxyapatite; BMSCs, bone marrow mesenchymal stem cells; HGF, hepatocyte growth factor; HFIP, hexafluoroisopropanol; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; MEW, melt electrowriting; MSC, mesenchymal stem cell; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; nHA, nano-hydroxyapatite; OPG, osteoprotegerin; OVX, ovariectomised; PCL, poly(ϵ -caprolactone); PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; PLGA, poly(lactic-co-glycolic acid); RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, Runt-related transcription factor 2; SEM, scanning electron microscopy; SBF, simulated body fluid; Sr-HA, strontium-substituted hydroxyapatite; TCP, tricalcium phosphate; TGF- β , transforming growth factor-beta; TNF- α , tumour necrosis factor-alpha; VEGF, vascular endothelial growth factor; VML, volumetric muscle loss; OR, odds ratio; CI, confidence interval; ATMP, Advanced Therapy Medicinal Product; FEA, finite element analysis; micro-CT, micro-computed tomography; WHO, World Health Organization; RGD, arginine–glycine–aspartic acid; PCL-MA, methacrylated poly(ϵ -caprolactone); EMA, European Medicines Agency; DEX, dexamethasone; SAMP, senescence-accelerated mouse-prone; CTX, C-terminal telopeptide of type I collagen; PINP, procollagen type I N-terminal propeptide.

Declaration of AI and AI-assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used Google Gemini for language editing (grammar correction and stylistic refinement of author-drafted text) and to generate stylised symbols and pictograms used as visual elements within the schematic illustrations in Figures. The scientific content, layout, and labelling of every figure were determined by the authors on the basis of the cited literature. All AI-assisted output was critically reviewed and verified by the authors, who take full responsibility for the integrity of the manuscript.

Availability of Data and Materials

Not applicable.

Author Contributions

YS was responsible for the conceptualization and investigation of the study. She performed the original draft writing, visualization, and participated in the writing — review & editing process. SSL contributed to the conceptualization and provided the necessary resources. He was also involved in writing — review & editing, and provided supervision, project administration, and funding acquisition for the research. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgments

Not applicable.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grants (RS-2024-00415982, RS-2025-23525049, and RS-2025-25460008) and the Korea Institute for Advancement of Technology (KIAT) grant (P241200036), funded by the Korean government. Additional support was provided by the Dongguk University Research Fund (S-2024-G0001-00024).

Conflict of Interest

The authors declare no conflict of interest.

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Editor’s note: The Scientific Editors responsible for this paper were Xiaoyuan Ji and Martin Stoddart.

Received: 31st March 2026; **Accepted:** 12th June 2026; **Published:** 31st July 2026