CONDITIONED MEDIUMS IN REGENERATIVE MEDICINE

Shcherbak D., Lutsenko T.
National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", denys.y.shcherbak@gmail.com

Abstract

Conditioned medium (CM), a bioactive cell culture supernatant, has emerged as a promising cell-free therapeutic strategy in regenerative medicine. This review examines CM's complex composition, production methods, mechanisms of action, and applications in tissue repair and immunomodulation, particularly from MSCs. It also addresses delivery strategies, regulatory issues, and challenges in clinical translation.

Keywords: *mesenchymal stromal cells, conditioned medium, cellular pre-conditioning, regenerative medicine.*

Introduction. Conditioned medium (CM) stands out in regenerative medicine as it refers to the culture medium harvested after cells have secreted a repertoire of proteins, cytokines, growth factors, extracellular vesicles (EVs), and other soluble factors over a defined incubation period [1]. Unlike direct cell transplantation, CM offers a cell-free modality that mitigates risks such as immune rejection, tumorigenicity, and embolism while retaining the paracrine benefits of stem cell therapies [4]. MSC-derived CM (MSC-CM) has garnered particular attention due to its potent immunomodulatory, pro-angiogenic, anti-apoptotic, and antimicrobial properties, positioning it as a versatile candidate for diverse regenerative applications. The therapeutic efficacy of CM is primarily attributed to its secretome, which encompasses growth factors (e.g., VEGF, bfgf, HGF), cytokines (e.g., IL-6, Tgfβ), chemokines, lipids, non-coding RNAS, and EVS such as exosomes [1]. EVs serve as nanocarriers delivering functional proteins, lipids, and RNAs to target cells, modulating cellular behavior and gene expression. The secretome mediates tissue regeneration through multiple mechanisms: promoting angiogenesis, inhibiting apoptosis, modulating inflammation, and stimulating resident progenitor cells. For instance, CM enhances chondrocyte proliferation and matrix synthesis in osteochondral defects, underscoring its potential in cartilage repair [2]. Standard CM production involves culturing cells under serum-free or defined conditions, followed by medium collection and concentration. Preconditioning strategies – such as hypoxia, inflammatory cytokine priming, oxidative stress, or three-dimensional (3d) culture – have been employed to augment the therapeutic potency of CM [4, 10]. Hypoxic culture increases secretion of angiogenic factors, while oxidative preconditioning (e.g., H_2O_2 treatment) enhances antioxidant and cytoprotective components in MSC-CM [11]. Moreover, 3D spheroid culture can mimic the *in vivo* microenvironment, further enriching CM with regenerative cues. CM has demonstrated efficacy across various tissue models, showcasing its potential in diverse regenerative applications. MSC-CM promotes cartilage repair in osteochondral regeneration, reduces inflammation, and enhances subchondral bone remodelling [2]. For vascular grafts, MSC-CM-loaded microparticles improve acute patency and endothelialisation in silk-based constructs [5]. In liver fibrosis models, adipose MSC-CM attenuates collagen deposition, modulates cytokine profiles, and fosters matrix remodelling in cholestatic injury [9].

Additionally, CM exhibits antimicrobial activity, mediated partly by LL37 secretion, contributing to infection control in sepsis and pneumonia models [7]. Early clinical studies suggest CM's potential in dermatology and gastrointestinal repair. Gingiva-derived MSC-CM accelerates wound healing, enhances skin rejuvenation, and shows promise in alopecia treatment [3]. H_2O_2-pretreated MSC-CM promotes intestinal mucosal repair in inflammatory bowel disease by activating antioxidant pathways (Nrf2/Keap1/ARE) and reducing epithelial apoptosis [11]. Photobiomodulation combined with MSC-CM facilitates bone defect repair, indicating synergistic effects in critical-size models [6]. Effective delivery of CM and its components remains a focus. Encapsulation in microparticles or incorporation into hydrogels allows sustained release and localised retention of bioactive factors [5]. Exosome-enriched fractions of CM offer a cell-free vesicular therapy with enhanced stability and targeting capabilities [1]. Scaffold integration and topical formulations have been explored for cutaneous and orthopaedic applications, optimising bioavailability and therapeutic outcomes. CM-based therapies present advantages, including ease of manufacturing, sterilisation, storage, and off-the-shelf availability, circumventing the complexities of living cell logistics [1, 8].

Materials and methods. A systematic literature search was conducted in PubMed, Web of Science, and Scopus covering January 2000 to March 2025, using keywords "conditioned medium," "secretome," "mesenchymal stromal cells," and "regenerative medicine" [12]. Original research articles and reviews reporting CM production protocols, secretome characterisation, mechanistic studies, preclinical/clinical applications, and regulatory aspects were included. Data were extracted on cell sources (e.g., BM-MSC, WJ-MSC), culture conditions (serum-free duration 24–72 h), preconditioning stimuli (hypoxia, H_2O_2, cytokines, 3D spheroids), CM isolation (centrifugation, ultrafiltration), concentration (TFF, lyophilisation), and evaluation assays for bioactivity [13]

Results and discussion. MSC-CM is generated by culturing MSCs in serum-free medium for 48–72 h, followed by centrifugation (1,500 × g, 10 min), 0,2 μm filtration, and ultrafiltration (3 kDa cutoff) to concentrate bioactive factors. Proteomic and cytokine profiling consistently identify high VEGF, bFGF, HGF, IL-6, TGF-β, and extracellular vesicles carrying miRNAs, underscoring a rich regenerative secretome. The therapeutic effects of CM arise from paracrine signalling that promotes angiogenesis, inhibits apoptosis, and modulates immune responses in injured tissues. Hypoxic preconditioning further enhances CM potency by upregulating angiogenic and anti-inflammatory factors within EVs, boosting vessel formation and tissue repair. Advanced delivery formats – such as hydrogel encapsulation and PLGA microparticles – provide sustained release and localised retention of CM components, improving therapeutic outcomes in liver, cartilage, and vascular models. Key translational hurdles include batch-to-batch variability in secretome composition, lack of standardised potency assays, and the need for GMP-compliant manufacturing protocols to ensure safety and consistency [14, 15].

Conclusions. A conditioned medium leverages the MSC secretome as a cell-free therapy that promotes angiogenesis, immunomodulation, and tissue regeneration.

Preconditioning and advanced delivery systems enhance its efficacy. Future work should prioritise standardised production, clinical trials, and regulatory guidelines to integrate CM into regenerative medicine [16].

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