Macrophage response in the immune microenvironment mediated by extracellular matrix: Recent progress

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Abstract

Regenerative medicine aims to repair tissue defects using biomaterials. Immune cells are the first line of response to implanted biomaterials. In particular, macrophages greatly affect cell behavior and the ultimate treatment outcome based on multiple cell phenotypes with various functions. The macrophage polarization status is considered as a general reflection of the characteristics of the immune microenvironment. Extracellular matrix (ECM) scaffold materials derived from native tissues are thought to be capable of inducing a pro-regenerative immune microenvironment. After reviewing previous literatures that explored the immune microenvironment mediated by ECM scaffolds, this paper describe the behaviors of immune cells, particularly macrophages. The mechanisms by which ECM scaffolds interact with macrophages are also discussed from the perspectives of the ECM ultrastructure along with the nucleic acid, protein, and proteoglycan compositions.

Impact statement

Macrophages show great heterogeneity in cell phenotype in response to biomaterial implantation. ECM scaffolds are thought to induce macrophage polarization toward pro-regenerative phenotypes. This review summarizes recent research on macrophage phenotypes mediated by ECM scaffolds conducted both in vitro and in vivo with traditional techniques and advanced technologies providing high resolution and throughput. The mechanisms by which ECM scaffolds interact with macrophages are also discussed, providing information for controlling the consistency of commercial

ECM products and applying them in clinical applications.

Main text

1 Introduction

Some tissue defects caused by trauma along with congenital or acquired diseases are difficult to repair through natural tissue regeneration or using autografts. Regenerative medicine aims to address these challenges by using biomaterials to recover the volume, structure, and function of organs and limbs. Generally, the aim of regenerative medicine can be summarized as replacement and regeneration. Replacement refers to imitating the missing portion of the human body, primarily in terms of appearance and function; replacement does not require the complete reproduction of the original structure. Regeneration refers to inducing the proliferation and differentiation of native cells along with forming tissue similar to the original tissue. In previous studies, the biomaterials fabricated for replacement (e.g., knee and hip implants, cardiac valve replacements, and oral implants) were designed to be passive and seldom interact with the host cells,[1] while the biomaterials designed for regeneration mainly target stem and progenitor cells.[2,3] However, it was recently revealed that immune cells respond firstly to biomaterial implants and direct the behavior of other cells via various signaling molecules.[4] The biomaterial-mediated local immune microenvironment has profound effects on the ultimate repair outcome. Instead of preparing biomaterials that hide from the immune system, optimizing immunomodulatory biomaterials has become the favored strategy. Extracellular

matrix (ECM) scaffolds derived from native tissues can be applied for both replacement (e.g., breast reconstruction) and regeneration (e.g., management of diabetic ulcers, burns, and other skin wounds). Multiple ECM biomaterials have been approved in different countries and are available on the market.[5] An example is Alloderm®, which is an acellular human dermal matrix graft for soft tissue repair. Alloderm® has been used for the reconstruction of abdominal wall, breast, palate, gingival, and defect after parotidectomy. Its medical application has been verified in clinical trials and related systematic reviews.[6-8] According to results observed both in vitro and in vivo, ECM is thought to be capable of inducing a pro-regenerative immune microenvironment and is thus recognized as an ideal referent for the design and fabrication of artificial synthetic biomaterials. However, it remains difficult to determine how this unique microenvironment is formed and to identify the components or structures in ECM that are responsible for the regenerative effect. The uncertain mechanism makes it challenging to ensure consistency in commercial products and achieve the wide clinical application of ECM scaffolds. An obvious problem is that commercial available ECM biomaterials could show different biological properties depending on tissue sources, namely the species of the donors, and processing methods by manufacturers. For example, four kinds of porcine dermal ECM biomaterials produced by different companies showed diverse influence on the proliferation, apoptosis, metabolism, and chemotaxis of host cells.[9] The lack of standardized criteria for the evaluation of ECM biomaterials is increasingly noticed in recent years, and prompts the exploration of the interaction between ECM

biomaterials and host immune system.[10] In this review, we describe the characteristics and behaviors of immune cells, especially macrophages, in the immune microenvironments mediated by ECM scaffolds. We also discuss the potential mechanisms by which ECM interacts with immune cells.

2 Key players in the typical ECM-mediated pro-regenerative microenvironment

The innate immune system is a nonspecific defense mechanism that detects ECM implantation and provides the first response. The innate immune system consists of polymorphonuclear cells, macrophages, and dendritic cells. Macrophages are the most important leukocytes and play a key role in the innate immune response and even the total immune microenvironment. Macrophages respond to stimuli based on the phagocytosis process and protein molecules expressed on the cellular surface. Beyond simply clearing the debris generated by cell apoptosis and senescence, macrophages pick up biological signals and initiate the downstream immune response. The metabolites and signaling molecules produced by macrophages can directly target stimuli (e.g., nitric oxide with microbicidal ability) or recruit other innate immune cells. When major histocompatibility complex class II (MHC II) is expressed on the cell surface, macrophages also affect the adaptive immune system via the antigen-presenting process to $CD4^+$ T helper (Th) cells. Therefore, macrophage status is considered to generally reflect the characteristics of the immune microenvironment. The multiple functions of macrophages are attributed to the great heterogeneity in cell phenotype. The mainstream theory adopts a dichotomous model of M1 and M2 phenotypes. The terms ‘M1’ and ‘M2’ were initially proposed to describe

macrophages expressing distinct arginine metabolic programs.[11] M1 cells transform arginine into NO, a diffusible gas that has a lethal effect on both microbe and host cells; M2 cells metabolize arginine to ornithine, a precursor necessary for collagen formation and cell proliferation. From these origins, the M1/M2 nomenclature system has been developed from diverse aspects, including activating stimuli, gene expression/transcriptome, metabolism, function, and surface markers.[12] However, numerous studies have indicated that the dichotomous model might be an oversimplification, with a continuum existing between the macrophage phenotypes M1 and M2. Therefore, we use the terms ‘M1-like’ and ‘M2-like’ in this review. Activated by bacterial endotoxins and IFN-γ, M1-like macrophages are characterized by a high expression of pro-inflammatory molecules and NO production. M1-like macrophages help initiate the inflammatory response in the early stage by recruiting immune cells. The persistent presence of M1-like macrophages can cause prolonged inflammation and damage the host tissue. M2-like macrophages are activated by IL-4 and IL-10 and promote stabilization and tissue maturation.[13] To differentiate between macrophage subtypes and evaluate the status of the entire microenvironment, markers of macrophage phenotypes in murine and human models have been identified (Table.1).

The dichotomous model of macrophages was applied to describe the microenvironments mediated by implanted biomaterials in the area of human regenerative medicine. M1-like macrophages are related to the degradation of scaffold materials and surrounding fibrous capsule formation. In contrast, M2-like

macrophages are recognized to be pro-regenerative and conducive to the proliferation and differentiation of progenitor cells. The immune microenvironments predominated by M1- and M2-like macrophages are described as fibrotic and regenerative microenvironments, respectively. However, as mentioned above, the M1/M2 nomenclature has been questioned because of the large discrepancies in surface markers, effector molecules, and gene expression between in vitro and in vivo models. It is difficult to identify the complex cellular phenotypes that coexist in vivo. In vitro experiments with pre-designed stimuli induce a relatively uniform macrophage population, while ignore the effect of communication between cells on macrophage polarization. Analyses of gene expression profiles suggest that the activated M1 and M2 phenotypes in vitro rarely overlap with the macrophages detected in vivo, which explains why the majority of markers identified in vitro fail to show equal potence in vivo.[32, 33]

In order to resolve the limitations of the classical M1/M2 system, Witherel et al. proposed a hybrid M1/M2 macrophage phenotype. This hybrid phenotype is prepared by exposing the macrophages to the M1- and M2-promoting stimuli simultaneously. Macrophages display both M1 and M2 markers in vitro, which is similar to the condition often observed in vivo. M1/M2 phenotype showed more favorable effects on the wound healing process than the M2-like phenotype alone.[34] However, this hybrid model cannot detect the information carried by each single cell. High-resolution techniques that recognize cells as individual units help reveal phenotypes that cannot be distinguished by analyzing hybrid cell populations.[35, 36] For

example, fluorescence-activated cell sorting (FACS) receives fluorescent signals from stained protein molecules. Based on the fluorescent signal, the characteristics of each single cell can be identified, and each cell subpopulation with common markers can be isolated. The staining and identification of up to 28 markers can be realized in one round, which is termed multicolor flow cytometry.[37] Single-cell quantitative polymerase chain reaction (qPCR), which is performed at the gene level, uses fluorescent mRNA-specific primers to detect the transcripts of each cell quantitatively. Commercial microfluidic approaches allow for the measurement of multiple primer pairs together in one assay.[38, 39] However, both FACS and single-cell qPCR rely on cell markers determined according to previous knowledge, which could introduce bias for cell sorting. Single-cell RNA sequencing (scRNseq) has emerged as an unbiased approach for establishing the transcriptional profiles of cells and revealing new phenotypes with similarity in gene expression.[40, 41] These high-throughput tests help understand the macrophage heterogeneity mediated by ECM by obtaining extensive information carried by thousands of cells. Thus, to achieve meaningful biological inference, clustering analysis and visualization algorithms are applied to handle the massive experimental data. Clustering analysis helps determine the relatedness between individuals (cells) based on the similarity they show. The relatedness can be then visualized as a two-dimensional scatter plot in which the scatters represent individuals (cells), and the distances between them are related to the calculated similarity.[42, 43] Cell subpopulations with common surface markers or transcriptional profiles can be identified. Frequently used tools for dimension reduction and

visualization include principal component analysis, t-stochastic neighbor embedding (t-SNE), and uniform manifold approximation and projection (UMAP).[44-46]

ECM derived from urinary bladder matrix (UBM) induced the response of multiple myeloid cells, including dendritic cells, neutrophils, monocytes, macrophages, and scaffold-associated macrophages (SAMs) in the murine volumetric muscle loss model. The cell phenotypes were determined using multicolor flow cytometry data visualized by t-SNE. SAMs were defined as the specific macrophage population with the surface profile $CD11b^{+}F4/80^{+}CD11c^{+/-}CD206^{hi}CD86^{+}MHCII^{+}$; they are likely related to the recognition of ECM fragment components. Considering the expression of both CD206 (an M1-like marker) and CD86 (an M2-like marker), the SAMs might suggest the existence of new subtypes between M1 and M2 phenotypes.[47]

Sommerfeld et al. constructed pro-regenerative and pro-fibrotic microenvironments by implanting UBM and polycaprolactone in to murine traumatic muscle defects. The UMAP plot for scRNAseq clustering analysis showed macrophage ($CD45^{+}CD64^{+}F4/80^{hi+}$) terminal clusters R1 and R2 (regenerative) and F1 and F2 (fibrotic). The surface marker combination of CD9, CD301b, and MHCII was also identified, thereby allowing the discrimination of these new phenotypes using t-SNE-visualized flow cytometry. Notably, the typical surface markers for M1- and M2-like macrophages (CD86 and CD206, respectively) presented considerable heterogeneity on a per-cell basis, and phenotypic subsets could not be distinguished.[21, 48] In summary, these findings challenge the relevancy of the classical M1/M2 system under physiological conditions mediated by biomaterials.

T and B lymphocytes can generate specific responses to the signals presented by the innate immune system. After ECM implantation, the amounts of both T and B cells increased significantly, and the ratio of $CD4^+$ to $CD8^+$ T cells increased.[49] $CD4^+$ Th cells adopt various subpopulations with distinct cytokine secretion profiles, which correspond to the macrophage phenotypes: T_H1 and T_H17 T cells with M1-like macrophages; and T_H2 T cells with M2-like macrophages. The specific macrophage phenotypes affect Th cell differentiation and shape diverse Th cell subsets.[50] In turn, Th cells promote the polarization of macrophages by secreting signaling molecules. As shown in a *Cd4*$^{-/-}$mouse model lacking $CD4^+$ Th cells, the expression of CD206, a commonly used M2-like macrophage marker, was impaired.[51] T_H2 T cells are necessary to shape the immune microenvironment mediated by ECM scaffold based on elevated IL-4 expression.[49] Taken together, these findings indicate that while adaptive immune cells are not responsible for initiating the immune response to ECM scaffold biomaterials, they help magnify the downstream signaling and ultimately produce the microenvironment together with the innate immune system (Fig. 1).

3 Mechanisms by which ECM scaffold materials interact with the immune system

3.1 Three-dimensional structure

The three-dimensional (3D) structure of ECM plays an important role in the immunomodulation process.[52] Macrophages exposed to homogenized acellular kidney matrix that had lost its ultrastructure showed a tendency toward M1-like polarization compared to those exposed to intact kidney-derived ECM, suggesting the importance

of preserving the inherent ECM ultrastructure.[53] Moreover, studies have indicated that the ability of ECM scaffold to encourage M2-like macrophages polarization might be lost when the 3D structure of the scaffold is altered by crosslinking. The crosslinking process for ECM biomaterials, which uses chemical agents such as carbodiimide and epoxy compounds, sometimes leads to unfavorable outcomes and even immune rejection.[54, 55] It is reasonable to infer that the unique capability of ECM to promote the pro-regenerative immune microenvironment largely depends on its 3D structure. A possible mechanism is as follows: immune cell migration is influenced by the orientation, porosity, and interconnectivity of matrix fibers. In two artificially assembled collagen hydrogel network models (dense and loose models) constructed as analogues to natural ECM, RAW 264.7 macrophages were virtually stationary when cultured alone in the densely connected network but migratory in the loosely connected network.[56] However, it remains difficult to identify the specific structure responsible for the effects of ECM. The decellularization process, which is conducted to remove the immunogenicity, has been shown to be affect the ECM ultrastructure.[57, 58] Currently, all commercially available biological scaffolds derived from xenogeneic or allogeneic ECM undergo the decellularization process to avoid the immune rejection caused by residual cellular and nuclear components.[59, 60] According to reviews of current decellularization protocols and agents, the decellularization process inevitably affects the native ECM ultrastructure, with the effect differing among the various methods.[61] This adds additional challenges in identifying the mechanisms related to the 3D ECM structure.

3.2 Residual cellular contents: nucleic acids

Nucleic acids remaining in the ECM scaffold may be derived from the residual nuclear contents after the decellularization process or from membrane vesicles secreted by cells into the extracellular space. The amount of double-strained DNA (dsDNA) is commonly measured to determine the thoroughness of decellularization in commercially available ECM materials, while the amount of nucleic acids in the form of single-stranded DNA and RNA has been neglected.[62, 63] Huleihel et al. measured the amount of nucleic acid in all forms in decellularized ECM materials and found that dsDNA only accounted for 25 to 40 percent of the total. Further investigation showed that exposure to nucleases cannot completely remove nucleic acids, especially small RNA molecules. These preserved nucleic acid molecules are packaged within lipid membrane vesicles, and protected from nucleases.[64] Extracellular vesicles (EVs) are nanosized vesicles released from cells for the purpose of intercellular communication. EVs transfer microRNA (miRNA), cytokines, chemokines, and other signaling molecules to regulate physiologic and pathologic processes.[65] Vesicles embedded within both laboratory-prepared and commercially available ECM materials with rounded structures and sizes similar to EVs have been identified using electron microscopy and osmium tetroxide staining; these vesicles are termed matrix-bound nanovesicles (MBVs) or ECM-derived extracellular vesicles.[64, 66] MBVs contains miRNA or protein cargos associated with the regulation of cell behavior, and can be separated and released after enzymatic digestion. Therefore, MBVs provide a reasonable mechanism for the interaction between ECM scaffolds

and immune cells. When exposing macrophages to labeled MBVs, the macrophages internalized MBVs rapidly within two hours and presented a gene expression profile, surface markers, and functions similar to those of macrophages treated with the parent ECM. MBVs can largely reproduce the influence of ECM on macrophage pro-regenerative polarization.[67] The specific miRNA cargos that function in MBVs require further identification. Among the enriched miRNAs in MBVs, three were selected according to the sequencing results: miR-125b-5p, miR-143-3p, and miR-145-5p. For all of these miRNAs, miRNA inhibition resulted in gene expression patterns opposite those of macrophages treated with MBVs

3.3 Proteins

The fibrotic network structure of ECM is mainly formed by proteins, including collagen, elastins, fibronectins, laminins, proteins connected to heteropolysaccharide chains, and other protein signaling molecules.[68] ECM macromolecules such as collagen improve the biocompatibility and tissue regeneration outcome.[69] Therefore, proteins offer a plausible explanation for the pro-regenerative effects of ECM. A metabolic signaling axis related to amino acid derived from ECM proteins has been demonstrated. Macrophages participate in the degradation of implanted ECM scaffolds via the phagocytosis process. The engulfed fragments, which consist mainly of proteins, are then digested in macrophage lysosomes and consequently generate amino acids and other nutrients. The accumulation of nutrients to a certain level results in the recruitment of nutrition sensors to the lysosomal surface. The mechanistic target of rapamycin complex 1 (mTORC1) in cytosol acts as a sensor for

signals of amino acid sufficiency. After the translocation of mTORC1, a complex formed by Ragulator proteins and v-ATPase on the lysosome membrane helps activate mTORC1 completely.[70, 71] Lamtor 1, one of the Ragulator proteins, has been shown to have an indispensable role in pro-regenerative macrophage polarization mediated by ECM scaffold materials in the presence of IL-4.[72, 73] He et al. established a knockdown macrophage cell line without intracellular Lamtor 1 for investigation.[74] Flow cytometry showed that the increase in the proportion of M2-like macrophage ($CD206^+$) among total macrophages induced by both ECM scaffold and collagen was reversed in the absence of Lamtor 1. In additional, the qRT-PCR analysis of the gene expression in $CD206^+$ macrophage indicated an almost complete loss of the expression of *Realmα* and *Arg1*, two signature genes identified as markers for M2-like polarization. The combination of intracellular amino acid sufficiency signal and the complex of relevant sensors and responders (Lamtor 1, v-ATPase, and mTORC1) with IL-4 forms a metabolic signaling axis for M2-like macrophage polarization.

The communication between macrophages and ECM depends on integrins. Integrins are transmembrane receptors expressed in almost all mammalian cells and can bind to different ECM proteins such as osteopontin and fibronectin.[75] Integrins that have been found to be associated with M2-like macrophage polarization include the following: integrin $\alpha_V\beta_5$ is directly targeted and induced by the nuclear receptor PPARγ (peroxisome proliferator–activated receptor γ) and drives M2 polarization[76]; integrin $\alpha_2\beta_1$ promotes M2 polarization by mediating the adhesion of human monocytic THP-1 cells to biomaterials[77]; and integrin $\alpha_V\beta_3$ enhances M2 polarization when activated by

the Arg–Gly–Asp tripeptide in ECM proteins.[78] However, the ligand of each kind of integrin needs to be identified in ECM scaffold materials.

ECM protein molecules also affect macrophage polarization by changing the behavior of immunomodulatory cells, such as mesenchymal stromal cells (MSCs). Fibrin and collagen regulate the paracrine process of MSCs and increase the releasing of cyclooxygenase-2 (COX-2) and TNF-α-stimulated gene 6 protein (TSG-6) from MSCs.[79] COX-2 is an enzymes for the synthesis of prostaglandin E2 (PGE2), which promote the pro-regenerative macrophage polarization.[80] TSG-6 acts on the CD44 receptor on macrophage membrane to inhibit TLR2-mediated NF-κB signaling, and prevent the pro-inflammatory macrophage polarization.[81]

Recently, several protein signaling molecules have attracted attention. IL-33 in its full-length protein form was recently detected in MBVs isolated from decellularized small intestine ECM and protected by the lipid membrane from proteolytic degradation. IL-33 is typically released after tissue injury to regulate immune cell behavior by combining to its receptor ST2.[82, 83] Western blot analysis confirmed the upregulation of Arginase1 expression in macrophages exposed to $IL33^+$ MBVs rather than $IL33^-$ MBVs, suggesting that IL-33 carried within MBVs is a potent mediator of M2-like macrophage polarization. In addition, the macrophages in *wt* and $st2^{-/-}$showed similar Arginase1 immunolabeling quantification in response to IL-33, indicating an uncharacterized, receptor-free transduction mechanism rather than the canonical ST2-dependent pathway.[84]

3.4 Proteoglycan and hyaluronan

Together with fibrous proteins, glycosaminoglycans (GAGs) and proteoglycans (PGs) are essential biomacromolecules for establishing the native ECM network structure. PGs are composed of a protein core and several (or a single) GAG side chains that are covalently attached to the core. The GAG chains are long linear heteropolysaccharides containing repeated disaccharides; they can also exist as free biopolymers or attach non-covalently to the PG core proteins.[85]

The immunomodulation effect of hyaluronan (HA), a GAG that consists of D-glucuronic acid and N-acetyl-D-glucosamine, has been recognized recently. In ECM scaffold materials, HA mainly exists as a large polymer and can be degraded in vivo either through the enzymolysis pathway by hyaluronidases or a nonspecific pathway by free radicals, resulting in small pieces with different molecular masses.[68] The locally depolymerized HA pieces are then partially drained by the lymphatic system and ultimately cleared through the lymph nodes or internalized by mononuclear phagocytes.[86] Consequently, the components of HA with various polymerization states influence the proliferation and polarization of macrophages and other innate immune cells dependent on combination with cell surface receptors such as CD44, toll-like receptors (TLR2 and TLR4), or the direct impact to intracellular pathways after being engulfed.[87] The roles of HA components have been investigated using solubilized urinary bladder ECM and primary rat bone marrow-derived macrophages (BMDMs). In BMDMs exposed to solubilized UBM, PGE2 production was upregulated, while no increase in TNFα or NO production was observed, indicating the absence of pro-inflammatory activity. However, when BMDMs were

treated with solubilized UBM digested with hyaluronidase, PGE2 production was downregulated, while NO secretion increased, suggesting an essential function of HA molecules in PGE2 production and NO inhibition.[88] The production of PGE2 dependent on the cyclooxygenase enzymes COX1 and COX2, and PGE2 plays an important role in pro-regenerative macrophage polarization mediated by ECM. The depletion of COX1 and COX2 was found to prevent the expression of M2-like macrophage marker CD206 in rodents.[80] In a 3D HA–mixed collagen matrix model, an HA-rich environment was confirmed to induce polarization toward M2-like monocytes/macrophages based on the elevated expressions of CD163, IL10, and CCL22.[89] Human THP-1 cells cultured in this model showed upregulated expression of CD44, a receptor for HA on the macrophage surface, along with the CD44-mediated activation of signal transducer and activator transcription-3 (STAT3), which is closely related to macrophage polarization.[90-92] These findings offer a potential explanation for the immunomodulation effects of HA. Notably, as these studies were conducted in vitro, the models used can hardly reproduce the components of depolymerized HA with diverse molecular weights found in vivo. In fact, qRT-PCR analyses of the mRNA levels of hyaluronidase and HA synthase indicated that the HA–mixed collagen model remains stable after being laden with THP-1 cells with no remarkable degradation.[89] However, the molecular weight of HA has been shown to be associated with immune response in previous investigations.[93, 94] Taken together, the in vivo interactions between immune cells and HA as well as other types of GAG warrant further consideration.

4. Conclusion

In the immune microenvironment mediated by ECM biomaterials, various macrophage phenotypes with different functions are induced. The possible mechanisms of interactions between ECM and macrophages are discussed in this review from the perspectives of 3D structure and composition (nucleic acid, protein, and PG) (Fig. 2). The classical M1/M2 macrophage dichotomous system showed limitations in previous studies. Recent advances in high-throughput and high-resolution techniques such as single-cell RNA sequencing and mass flow cytometry provide an opportunity to obtain insights into the in vivo behaviors of diverse cell phenotypes and the dynamic communications between them. The interpretation of the massive amounts of experimental data achieved by these techniques requires assistance from bioinformation techniques. Therefore, future exploration of the ECM-mediated immunomodulation process will require a multidisciplinary combination of biology, computer programming, and mathematical algorithms.

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Authorship confirmation statement

Wenlan Xiao，Chenyu Chu，Yi Man and Yili Qu conceived the review. Wenlan Xiao，Chenyu Chu wrote the manuscript. Sheng-An Rung plotted figure 1 and 2. All authors edited and approved of the final version of the manuscript.

Authors' disclosure statement

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Reference

1. Trindade R, Albrektsson T, Tengvall P, Wennerberg A. Foreign Body Reaction to Biomaterials: On Mechanisms for Buildup and Breakdown of Osseointegration. Clin Implant Dent Relat Res. 2016;18(1):192-203.
2. Nigro J, White JF, Ramshaw JA, Haylock DN, Nilsson SK, Werkmeister JA. The effect of bovine endosteum-derived particles on the proliferation of human mesenchymal stem cells. Biomaterials. 2010;31(21):5689-5699.
3. Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. Nature. 2009;462(7272):433-41.
4. Shook BA, Wasko RR, Rivera-Gonzalez GC, et al. Myofibroblast proliferation and

heterogeneity are supported by macrophages during skin repair. Science. 2018;362(6417):eaar2971.

5. Yao Q, Zheng YW, Lan QH, Kou L, Xu HL, Zhao YZ. Recent development and biomedical applications of decellularized extracellular matrix biomaterials. Mater Sci Eng C Mater Biol Appl. 2019;104:109942.

6. Zeng XT, Tang XJ, Wang XJ, et al. AlloDerm implants for prevention of Frey syndrome after parotidectomy: a systematic review and meta-analysis. Mol Med Rep. 2012;5(4):974-80.

7. Simpson A, Samargandi OA, Wong A, Graham ME, Bezuhly M. Repair of Primary Cleft Palate and Oronasal Fistula With Acellular Dermal Matrix: A Systematic Review and Surgeon Survey. Cleft Palate Craniofac J. 2019;56(2):187-195.

8. Ahmedbeyli C, Ipci SD, Cakar G, Yilmaz S. Laterally positioned flap along with acellular dermal matrix graft in the management of maxillary localized recessions. Clin Oral Investig. 2019;23(2):595-601.

9. Kulig KM, Luo X, Finkelstein EB, et al. Biologic properties of surgical scaffold materials derived from dermal ECM. Biomaterials. 2013;34(23):5776-84.

10. Choudhury D, Yee M, Sheng ZLJ, Amirul A, Naing MW. Decellularization systems and devices: State-of-the-art. Acta Biomater. 2020;115:51-59.

11. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol. 2000;164(12):6166-6173.

12. Jha AK, Huang SC-C, Sergushichev A, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity. 2015;42(3):419-430.

13. Spiller KL, Koh TJ. Macrophage-based therapeutic strategies in regenerative medicine. Adv Drug Deliv Rev. 2017;122:74-83.

14. Jaguin M, Houlbert N, Fardel O, Lecureur V. Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. Cell Immunol. 2013;281(1):51-61.

15. Ambarus CA, Krausz S, van Eijk M, et al. Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. J Immunol Methods. 2012;375(1-2):196-206.

16. Vogel DYS, Glim JE, Stavenuiter AWD, et al. Human macrophage polarization in vitro: maturation and activation methods compared. Immunobiology. 2014;219(9):695-703.

17. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, Ganesan H, Nino-Castro A, Mallmann MR, Labzin L, Theis H, Kraut M, Beyer M, Latz E, Freeman TC, Ulas T, Schultze JL. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity. 2014;40(2):274-88.

18. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM. Macrophage phenotype as a determinant of biologic scaffold remodeling. Tissue Eng Part A. 2008;14(11):1835-42.

19. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677-86.

20. Klopfleisch R. Macrophage reaction against biomaterials in the mouse model - Phenotypes, functions and markers. Acta Biomater. 2016;43:3-13.

21. Sommerfeld SD, Cherry C, Schwab RM, et al. Interleukin-36γ-producing macrophages drive IL-17-mediated fibrosis. Sci Immunol. 2019;4(40):eaax4783.

22. Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. Am J Respir Cell Mol Biol. 2015;53(5):676-688.

23. Huang C, Lewis C, Borg NA, et al.Proteomic Identification of Interferon-Induced Proteins with Tetratricopeptide Repeats as Markers of M1 Macrophage Polarization. J Proteome Res. 2018;17(4):1485-1499.

24. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2006;177(10):7303-11.

25. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 2014;6:13.

26. Chávez-Galán L, Olleros ML, Vesin D, Garcia I. Much More than M1 and M2 Macrophages, There are also CD169(+) and TCR(+) Macrophages. Front Immunol. 2015;6:263.

27. Lu G, Zhang R, Geng S, et al. Myeloid cell-derived inducible nitric oxide synthase suppresses M1 macrophage polarization. Nat Commun. 2015;6:6676.

28. Rőszer T. Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators Inflamm. 2015;2015:816460.

29. Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, Thomas B, Fabbri M, Crawshaw A, Ho LP, Ten Hacken NH, Cobos Jiménez V, Kootstra NA, Hamann J, Greaves DR, Locati M, Mantovani A, Gordon S. Genetic programs expressed in resting and IL-4

alternatively activated mouse and human macrophages: similarities and differences. Blood. 2013;121(9):e57-69.

30. De Paoli F, Eeckhoute J, Copin C, Vanhoutte J, Duhem C, Derudas B, Dubois-Chevalier J, Colin S, Zawadzki C, Jude B, Haulon S, Lefebvre P, Staels B, Chinetti-Gbaguidi G. The neuron-derived orphan receptor 1 (NOR1) is induced upon human alternative macrophage polarization and stimulates the expression of markers of the M2 phenotype. Atherosclerosis. 2015;241(1):18-26.

31. Raes G, Van den Bergh R, De Baetselier P, Ghassabeh GH, Scotton C, Locati M, Mantovani A, Sozzani S. Arginase-1 and Ym1 are markers for murine, but not human, alternatively activated myeloid cells. J Immunol. 2005;174(11):6561; author reply 6561-2.

32. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. Front Immunol. 2019;10:1084.

33. Huleihel L, Dziki JL, Bartolacci JG, et al.Macrophage phenotype in response to ECM bioscaffolds. Semin Immunol. 2017;29:2-13.

34. Witherel CE, Sao K, Brisson BK, Han B, Volk SW, Petrie RJ, Han L, Spiller KL. Regulation of extracellular matrix assembly and structure by hybrid M1/M2 macrophages. Biomaterials. 2021;269:120667.

35. Nestorowa S, Hamey FK, Pijuan Sala B, et al. A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. Blood. 2016 Aug 25;128(8):e20-31.

36. Hockley JRF, Taylor TS, Callejo G, et al.Single-cell RNAseq reveals seven classes of colonic sensory neuron. Gut. 2019;68(4):633-644.

37. Brummelman J, Haftmann C, Núñez NG, et al. Development, application and computational analysis of high-dimensional fluorescent antibody panels for single-cell flow cytometry. Nat Protoc. 2019;14(7):1946-1969.

38. Moignard V, Woodhouse S, Haghverdi L, et al. Decoding the regulatory network of early blood development from single-cell gene expression measurements. Nat Biotechnol. 2015;33(3):269-276.

39. Kellogg RA, Gómez-Sjöberg R, Leyrat AA, Tay S. High-throughput microfluidic single-cell analysis pipeline for studies of signaling dynamics. Nat Protoc. 2014;9(7):1713-26.

40. Giladi A, Paul F, Herzog Y, et al. Single-cell characterization of haematopoietic progenitors and their trajectories in homeostasis and perturbed haematopoiesis. Nat Cell Biol. 2018;20(7):836-846.

41. Papalexi E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol. 2018;18(1):35-45.

42. Petegrosso R, Li Z, Kuang R. Machine learning and statistical methods for clustering single-cell RNA-sequencing data. Brief Bioinform. 2020;21(4):1209-1223.

43. Ding J, Condon A, Shah SP. Interpretable dimensionality reduction of single cell transcriptome data with deep generative models. Nat Commun. 2018;9(1):2002.

44. Wu Y, Tamayo P, Zhang K. Visualizing and Interpreting Single-Cell Gene Expression Datasets with Similarity Weighted Nonnegative Embedding. Cell Syst. 2018;7(6):656-666.e4.

45. Becht E, McInnes L, Healy J, et al. Dimensionality reduction for visualizing single-cell data using UMAP. Nat Biotechnol. 2018

46. Tsuyuzaki K, Sato H, Sato K, Nikaido I. Benchmarking principal component analysis for

large-scale single-cell RNA-sequencing. Genome Biol. 2020;21(1):9.

47. Sadtler K, Wolf MT, Ganguly S, et al.Divergent immune responses to synthetic and biological scaffolds. Biomaterials. 2019;192:405-415.

48. Perciani CT, MacParland SA. Lifting the veil on macrophage diversity in tissue regeneration and fibrosis. Sci Immunol. 2019;4(40):eaaz0749.

49. Sadtler K, Estrellas K, Allen BW, et al.Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science. 2016;352(6283):366-70.

50. Arnold CE, Gordon P, Barker RN, Wilson HM. The activation status of human macrophages presenting antigen determines the efficiency of Th17 responses. Immunobiology. 2015;220(1):10-19.

51. Delgoffe GM, Pollizzi KN, Waickman AT, et al.The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol. 2011;12(4):295-303.

52. Hu C, Chu C, Liu LI, et al. Dissecting the microenvironment around biosynthetic scaffolds in murine skin wound healing, Sci Adv, 2020. In Press

53. Petrosyan A, Da Sacco S, Tripuraneni N, et al. A step towards clinical application of acellular matrix: A clue from macrophage polarization. Matrix Biol. 2017;57-58:334-346.

54. Elagin V, Kuznetsova D, Grebenik E, et al. Multiparametric Optical Bioimaging Reveals the Fate of Epoxy Crosslinked Biomeshes in the Mouse Subcutaneous Implantation Model. Front Bioeng Biotechnol. 2020;8:107.

55. Davidenko N, Schuster CF, Bax DV, et al. Evaluation of cell binding to collagen and gelatin: a study of the effect of 2D and 3D architecture and surface chemistry. J Mater Sci Mater Med.

2016;27(10):148.

56. Ford AJ, Orbach SM, Rajagopalan P. Fibroblasts stimulate macrophage migration in interconnected extracellular matrices through tunnel formation and fiber alignment. Biomaterials. 2019;209:88-102.

57. Hwang J, San BH, Turner NJ, et al. Molecular assessment of collagen denaturation in decellularized tissues using a collagen hybridizing peptide. Acta Biomater. 2017;53:268-278.

58. Ventura RD, Padalhin AR, Park CM, Lee BT. Enhanced decellularization technique of porcine dermal ECM for tissue engineering applications. Mater Sci Eng C Mater Biol Appl. 2019;104:109841.

59. Keane TJ, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. Biomaterials. 2012;33(6):1771-1781.

60. Dalgliesh AJ, Parvizi M, Lopera-Higuita M, Shklover J, Griffiths LG. Graft-specific immune tolerance is determined by residual antigenicity of xenogeneic extracellular matrix scaffolds. Acta Biomater. 2018;79:253-264.

61. Chakraborty J, Roy S, Ghosh S. Regulation of decellularized matrix mediated immune response. Biomater Sci. 2020;8(5):1194-1215.

62. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell. 2010;140(5):619-630.

63. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32(12):3233-3243.

64. Huleihel L, Hussey GS, Naranjo JD, et al.Matrix-bound nanovesicles within ECM bioscaffolds. Sci Adv. 2016;2(6):e1600502.

65. Rilla K, Mustonen AM, Arasu UT, Härkönen K, Matilainen J, Nieminen P. Extracellular vesicles are integral and functional components of the extracellular matrix. Matrix Biol. 2019;75-76:201-219.

66. An M, Kwon K, Park J, et al.Extracellular matrix-derived extracellular vesicles promote cardiomyocyte growth and electrical activity in engineered cardiac atria. Biomaterials. 2017;146:49-59.

67. Huleihel L, Bartolacci JG, Dziki JL, et al.Matrix-Bound Nanovesicles Recapitulate Extracellular Matrix Effects on Macrophage Phenotype. Tissue Eng Part A. 2017;23(21-22):1283-1294.

68. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. Adv Drug Deliv Rev. 2016;97:4-27.

69. Lee J, Kim G. Three-Dimensional Hierarchical Nanofibrous Collagen Scaffold Fabricated Using Fibrillated Collagen and Pluronic F-127 for Regenerating Bone Tissue. ACS Appl Mater Interfaces. 2018;10(42):35801-35811.

70. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010;141(2):290-303.

71. Yonehara R, Nada S, Nakai T, et al. Structural basis for the assembly of the Ragulator-Rag GTPase complex. Nat Commun. 2017;8(1):1625.

72. Kimura T, Nada S, Takegahara N, et al. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. Nat Commun. 2016;7:13130.

73. Hayama Y, Kimura T, Takeda Y, et al. Lysosomal Protein Lamtor1 Controls Innate Immune

Responses via Nuclear Translocation of Transcription Factor EB. J Immunol. 2018;200(11):3790-3800.

74. He C, Yang Z, Jin Y, Qi X, Chu J, Deng X. ADM Scaffolds Generate a Pro-regenerative Microenvironment During Full-Thickness Cutaneous Wound Healing Through M2 Macrophage Polarization via Lamtor1. Front Physiol. 2018;9:657.

75. Kobori T, Hamasaki S, Kitaura A, Yamazaki Y, Nishinaka T, Niwa A, et al. Interleukin-18 Amplifies Macrophage Polarization and Morphological Alteration, Leading to Excessive Angiogenesis. Front Immunol. 2018;9:334.

76. Yao Q, Liu J, Zhang Z, et al. Peroxisome proliferator-activated receptor γ (PPARγ) induces the gene expression of integrin $\alpha_V\beta_5$ to promote macrophage M2 polarization. J Biol Chem. 2018;293(43):16572-16582.

77. Cha BH, Shin SR, Leijten J, Li YC, Singh S, Liu JC, et al. Integrin-Mediated Interactions Control Macrophage Polarization in 3D Hydrogels. Adv Healthc Mater. 2017;6(21).

78. Wang H, Morales R-TT, Cui X, et al. A Photoresponsive Hyaluronan Hydrogel Nanocomposite for Dynamic Macrophage Immunomodulation. Adv Healthc Mater. 2019;8(4):e1801234.

79. Sun L, Li J, Gao W, Shi M, Tang F, Fu X, Chen X. Coaxial nanofibrous scaffolds mimicking the extracellular matrix transition in the wound healing process promoting skin regeneration through enhancing immunomodulation. J Mater Chem B. 2021;9(5):1395-1405.

80. Dearth CL, Slivka PF, Stewart SA, et al. Inhibition of COX1/2 alters the host response and reduces ECM scaffold mediated constructive tissue remodeling in a rodent model of skeletal muscle injury. Acta Biomater. 2016;31:50-60.

81 Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages. Blood. 2011;118(2):330-8.

82. He R, Yin H, Yuan B, et al. IL-33 improves wound healing through enhanced M2 macrophage polarization in diabetic mice. Mol Immunol. 2017;90:42-49.

83. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. Nat Rev Immunol. 2016;16(11):676-689.

84. Hussey GS, Dziki JL, Lee YC, et al. Matrix bound nanovesicle-associated IL-33 activates a pro-remodeling macrophage phenotype via a non-canonical, ST2-independent pathway. J Immunol Regen Med. 2019;3:26-35.

85. Garantziotis S, Savani RC. Hyaluronan biology: A complex balancing act of structure, function, location and context. Matrix Biol. 2019;78-79:1-10.

86. Vachon E, Martin R, Plumb J, et al. CD44 is a phagocytic receptor. Blood. 2006;107(10):4149-4158.

87. Johnson P, Arif AA, Lee-Sayer SSM, Dong Y. Hyaluronan and Its Interactions With Immune Cells in the Healthy and Inflamed Lung. Front Immunol. 2018;9:2787.

88. Meng FW, Slivka PF, Dearth CL, Badylak SF. Solubilized extracellular matrix from brain and urinary bladder elicits distinct functional and phenotypic responses in macrophages. Biomaterials. 2015;46:131-140.

89. Kim H , Cha J , Jang M , Kim P . Hyaluronic acid-based extracellular matrix triggers spontaneous M2-like polarity of monocyte/macrophage. Biomater Sci. 2019;7(6):2264-2271.

90. Kurdi M, Zgheib C, Booz GW. Recent Developments on the Crosstalk Between STAT3 and

Inflammation in Heart Function and Disease. Front Immunol. 2018;9:3029.

91. Niemand C, Nimmesgern A, Haan S, et al.Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. J Immunol. 2003;170(6):3263-3272.

92. Zhang G, Guo L, Yang C, et al. A novel role of breast cancer-derived hyaluronan on inducement of M2-like tumor-associated macrophages formation. Oncoimmunology. 2016;5(6):e1172154.

93. Shi Q, Zhao L, Xu C, Zhang L, Zhao H. High Molecular Weight Hyaluronan Suppresses Macrophage M1 Polarization and Enhances IL-10 Production in $PM_{2.5}$-Induced Lung Inflammation. Molecules. 2019;24(9):1766.

94. Rayahin JE, Buhrman JS, Zhang Y, Koh TJ, Gemeinhart RA. High and low molecular weight hyaluronic acid differentially influence macrophage activation. ACS Biomater Sci Eng. 2015;1(7):481-493.

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