Stem Cell niches and overview of cell signaling

- ✓ **Adult stem cells (ASCs) are an attractive resource as they can remain quiescent for extended periods, proliferate to replenish stem cell pools or mature into related lineages.**

- ✓ **Whereas the biology of the stem cells and the microenvironment or niche, which they inhabit, are relatively well understood, the capacity to efficiently simulate a functional, artificial niche that can be used in developing stem cell-based medical treatments is still lacking.**

- ✓ **The aim of niche bioengineering is therefore to develop strategies, devices and novel techniques to mimic local environments for the controlled, ex vivo culture of pluripotent, omnipotent or multipotent cells.**

- ✓ **This would facilitate the manufacture of tissues for the replacement of injured or failing systems, or for further study, e.g. to understand why neoplastic transformation occurs when stem cells escape regulation by the niche.**

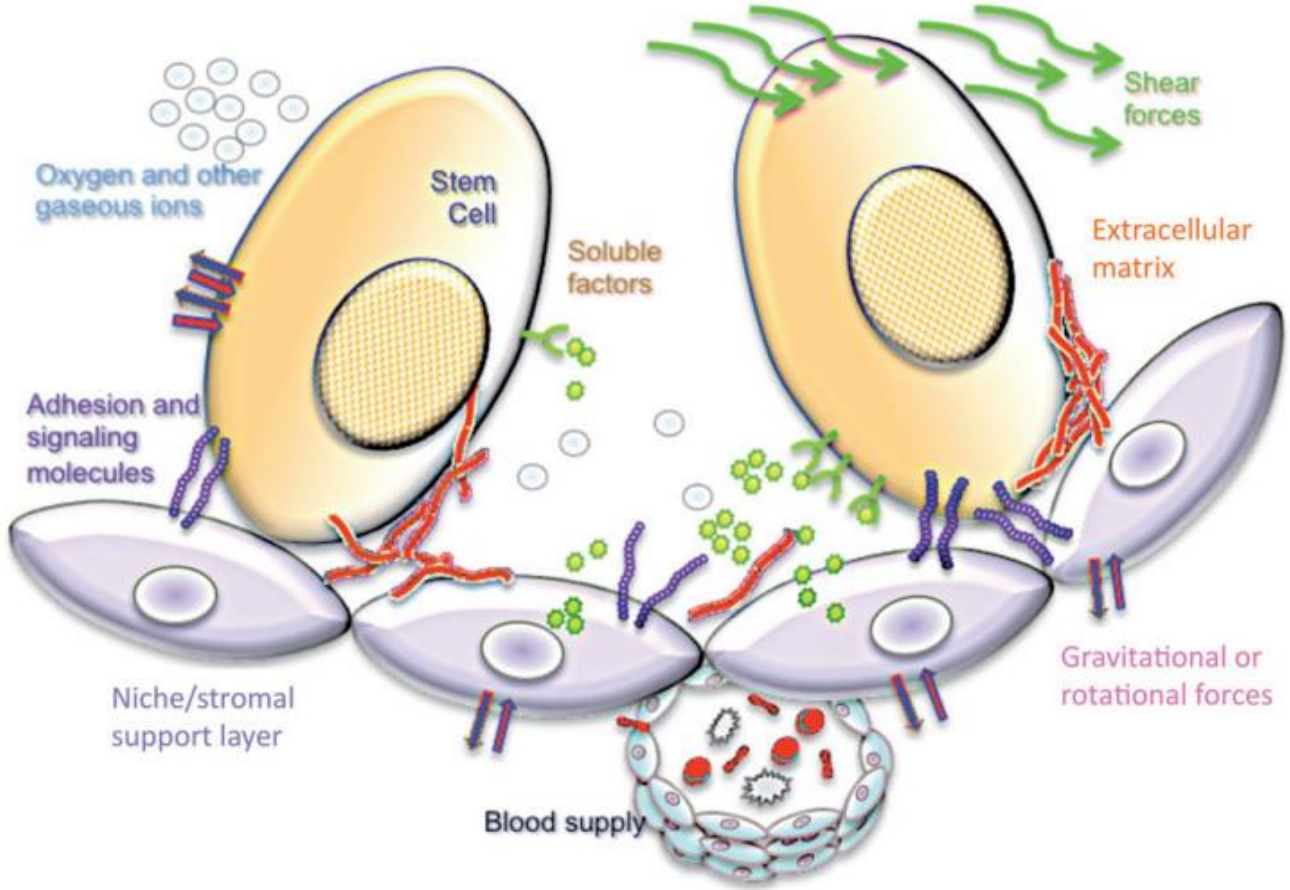
- ✓ **The three adult stem cells (ASC) niches discussed herewith are amongst the most active, and include those housing the hematopoietic, gastrointestinal and skin (including hair follicle) stem cells.**

- ✓ **However, the ASC niche is a highly complex structure due to the precisely coordinated appearance and interactions of numerous mechanical and biochemical factors.**

- ✓ **Without a functional niche, stem cells cannot properly regulate the fine balance between**
- ✓ **quiescence,**
- ✓ **active proliferation,**
- ✓ **differentiation and**
- ✓ **death**

- ✓ **The challenge to devise effective niche engineering strategies is further compounded by the inherent diversity of ASC niches, which often undergo endogenous remodeling to meet the specific homeostasis requirements of their host tissue**

- ✓ **The stem cell microenvironment supporting stem cell growth and differentiation**
- ✓ **Stem cells were originally observed to reside within an extremely specialized and precisely ordered microenvironment known as the ‘‘niche’’,**
- ✓ **In the hematopoietic system, studies indicated that the bone marrow (BM) possessed a greater capacity than the spleen to support hematopoietic stem cells (HSCs).**
- ✓ **Stem cell niches are now known to exist within both embryonic and somatic tissues, in vertebrates and invertebrates.**
- ✓ **By surviving within such a protected and restricted anatomical space, cells can interact with niche factors to sustain their stemness and regulate their differentiation in a spatiotemporally-dictated manner.**
- ✓ **Crucially, this process can be induced by biophysical elements including shear stress and oxygen tension, and by biochemical elements such as paracrine and autocrine signaling factors (Figure 1).**
- ✓ **Ex vivo culture of tissue (adult) stem cells has been improved by the development of niche engineering methods which replicate features of the endogenous niche.**
- ✓ **These artificial niches now include three-dimensional (3D) platforms incorporating elements such as ECM proteins, biochemical and biophysical factors (flow and shear), and pore size.**



General components of a stem cell niche. The individual elements of a stem cell niche that have been explored and exploited for niche creation are depicted in the schematic, and include both biochemical and cellular and biophysical factors.

Bioengineering the stem cell niche- BIOMATERIAL

- ✓ Artificial niches should include critical components such as
- ✓ mechanical factors (e.g. flexibility and tensile strength),
- ✓ gradients of oxygen tension,
- ✓ an optimal composition of biochemical and growth factors,
- ✓ as well as the appropriate mixture of extracellular matrices.

- ✓ Major technological breakthroughs in these areas have been driven by an increased understanding of niche biology and improvements in the biomaterials and culture systems available.

- ✓ Biomaterials evolved from a need to overcome the inadequacies of existing stem cell culture protocols.

- ✓ Each new biomaterial seeks to replace a niche factor, such as a support cell, glycosaminoglycan or protein.

- ✓ Ideally, the material has properties such as
- ✓ flexibility or tensile strength,
- ✓ can be molded into a multidimensional structure,
- ✓ is allogeneic or non-xenogenic and
- ✓ minimally cytotoxic, and
- ✓ can be easily customized for a particular niche.

- ✓ **Biomaterials can be natural, and thus inherently possess desirable properties such as adhesion, and include hyaluronic acid (HA) and fibrin, which are ingredients in the commonly used Matrigel.**

- ✓ **Synthetic biomaterials include polyacrylamide and polyethylene glycol (PEG), some of which have the advantage of being degradable in vivo.**

- ✓ **Of great interest to biologists is the capacity to infuse biomaterials with selected growth factors.**

- ✓ **For example, the chemokine Stromal Cell Derived Factor-1 (Sdf-1) was infused into chitosan-based nanoparticles, which allows its controlled release while simultaneously protecting it from heat and proteolysis, and this was demonstrated to support MSC proliferation and mobilization.**

- ✓ **Unfortunately the need to harvest the natural components for various commercially available biomaterials (e.g. Matrigel) means that these will inevitably be affected by undesirable batch-to-batch variation in functional efficacy and even contain undefined factors that preclude them from clinical applications.**

- ✓ **These issues justify the use of technologies such as designer peptides and biomimetic scaffolds, such as hydrogels which are made by polymerizing either synthetic (e.g. PEG) or natural (e.g. agarose, chitosan, collagen) hydrophilic macromolecules.**

- ✓ **More instructive approaches would integrate the effects of specific chemokines.**

- ✓ **Growth factor ligands can be brought into direct contact with their target stem cells through several means, e.g. by simply immobilizing them on a chosen inert surface, or by infusing them into biomaterials used to construct the scaffolds on which cells would be cultured.**

- ✓ **As hydrogels are elastic and can be infused with growth factors, they can be biofunctionalized for studying stem cells.**

- ✓ **To create specific niches, predefined concentrations of ECM ligands that encourage adhesion (e.g. integrins), or of regulatory proteins (laminin, fibrinogen, collagen, fibronectin) can be grafted into the biomaterial.**

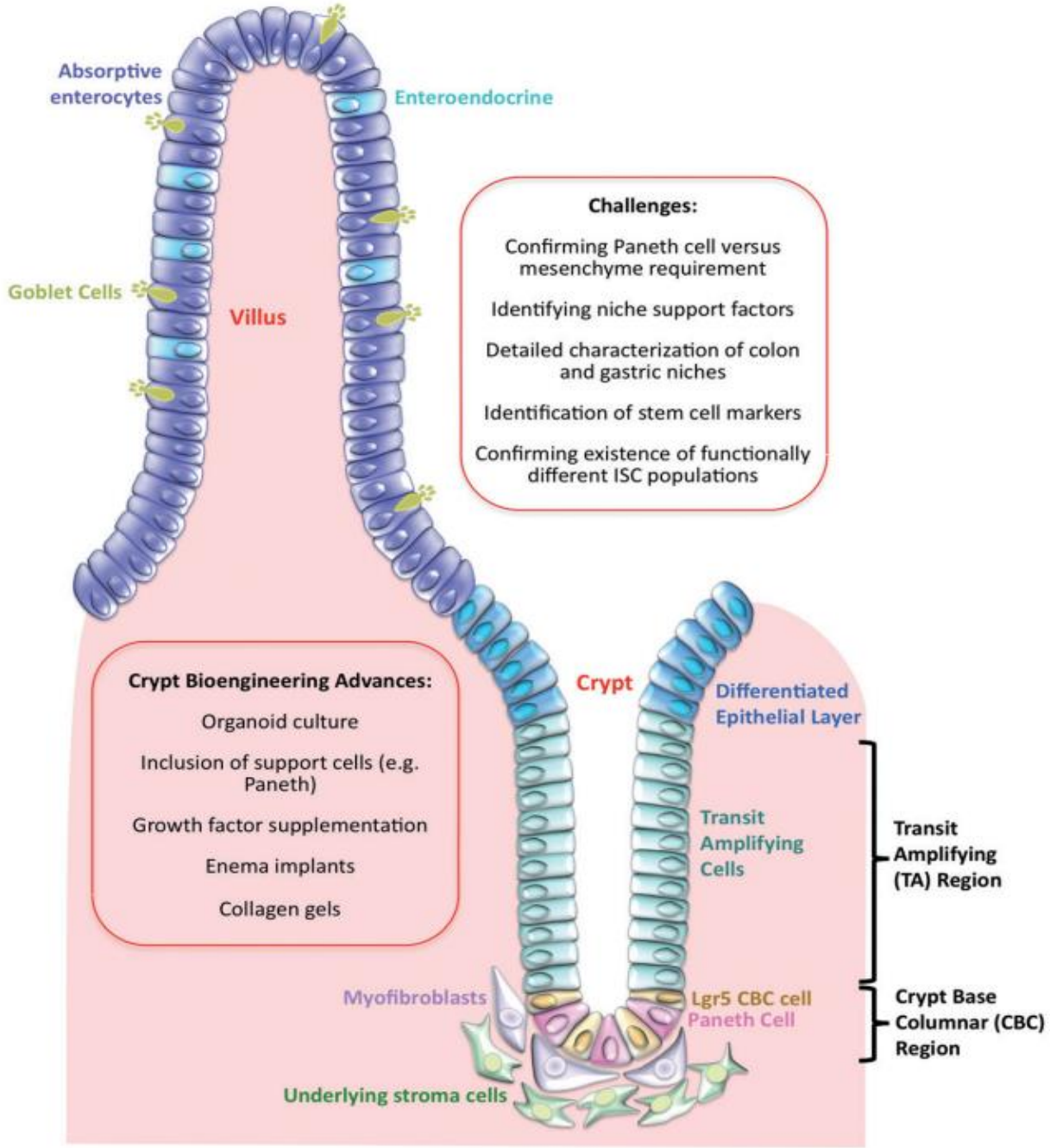
- ✓ **For example, muscle cells could be simply cultured in hydrogel-containing tissue culture plates, using standard myogenic (bFGF-containing) media.**

- ✓ **Transplantation of myogenic hydrogels has shown that they can effectively contribute to muscle development in vivo.**

- ✓ **In all examples, the overriding goal of stem cell niche engineering is to recreate the native environment of the stem cell and provide all the biochemical and physical elements required for their maintenance, proliferation and differentiation.**

Gastric gland/intestinal crypt engineering

- ✓ **Components of the gastric/intestinal stem cell niche**
- ✓ **The intestinal stem cell (ISC) niche or crypt comprises a complicated network of cells and signaling molecules within an intricately organized architecture.**
- ✓ **In mammals, ISCs occupy the base of multiple crypts within the intestinal epithelium.**
- ✓ **Two distinct ISCs are thought to be responsible for maintaining this organ.**
- ✓ **ISCs that express Lgr5 (leucine-rich repeat-containing G protein-coupled receptor 5, a target of the Wnt pathway) are rapidly proliferating and found at the crypt base, flanked by Paneth cells, while more quiescent, reserve ISCs are purportedly detected at the þ4 (or 4th cell) position, from the crypt base.**
- ✓ **While the former are considered to maintain the daily renewal of the intestinal lining, the latter appear to function only during injury or regeneration.**
- ✓ **The Lgr5 ISCs are believed to exist in a stem cell-permissive zone containing environment responsive factors (elements which react to changes in the stem cell's habitat, e.g. signaling molecules) produced by Paneth cells and underlying mesenchymal stromal components (e.g. fibroblasts, immune cells, neural cells) that collectively modulate local signaling pathways (Wnt, Notch and BMP in mammals) essential for regulating stemness, proliferation status and lineage specification.**



General components of the gastrointestinal stem cell niche. Engineering strategies used to create this niche have only recently been devised, and many technical and biological challenges must be resolved before a coherent, artificial ex vivo niche can be constructed.

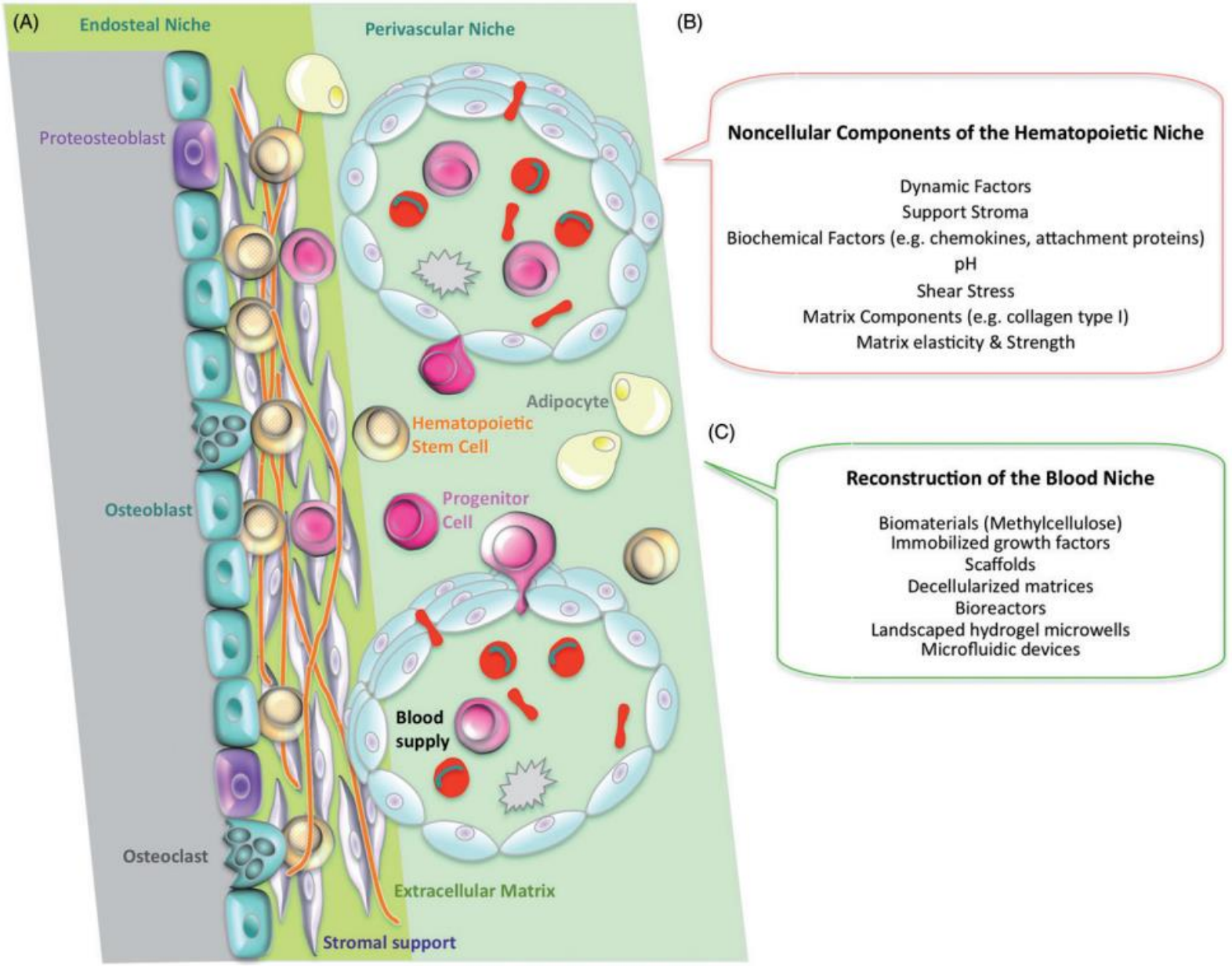
- ✓ **Blood**
- ✓ **Components of the blood stem cell niche**

- ✓ **Evidence for the blood microenvironment was derived from studies showing that unless HSCs reside within their niches, they fail to renew, and there exists only a limited number of sites in which HSC expansion occurs.**

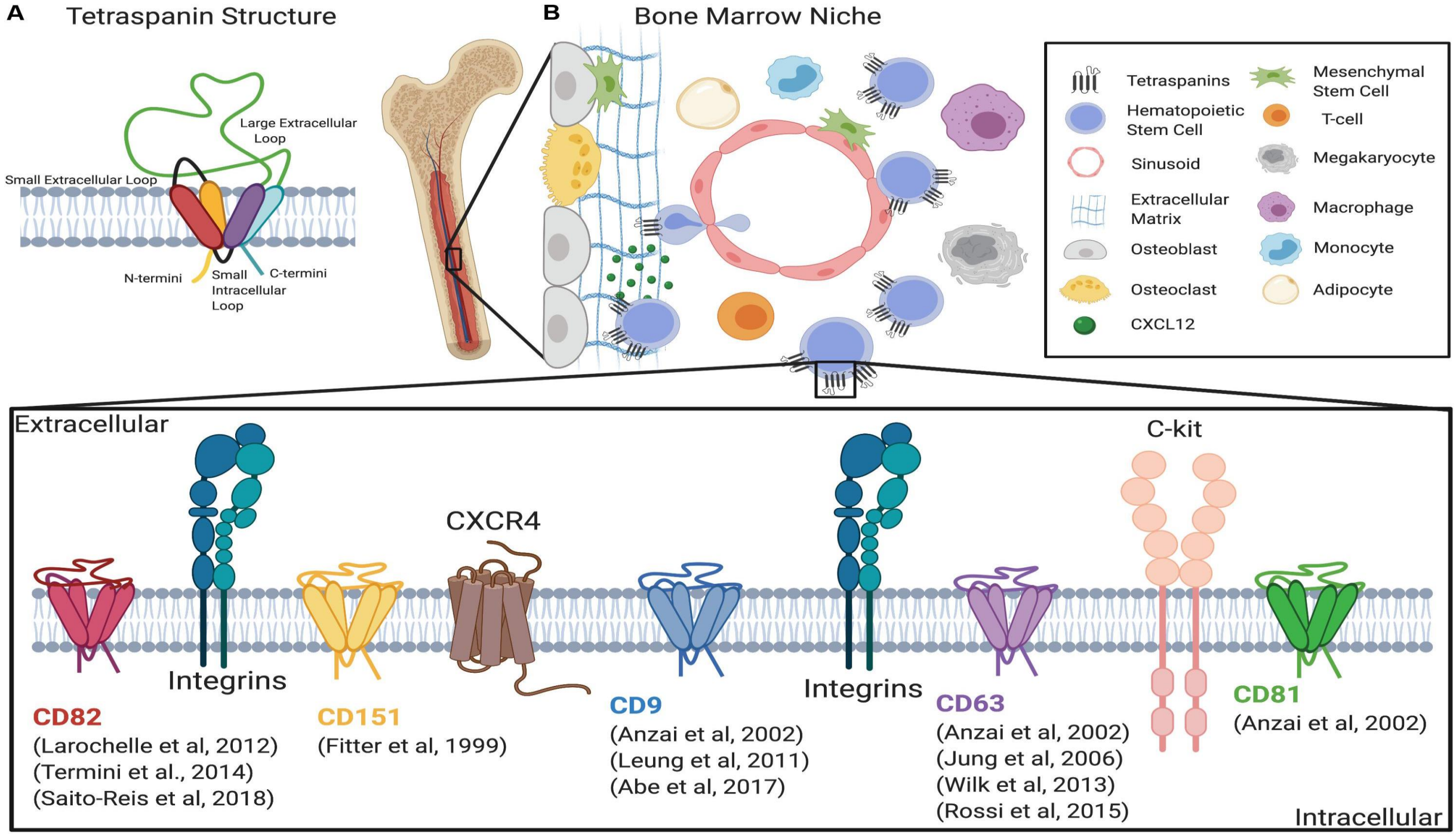
- ✓ **HSC niches in the bone marrow comprise the osteoblastic (or endosteal) and the vascular (or perivascular) niches.**

- ✓ **These locations are functionally distinct, with different cell signaling patterns, ligand compositions and physical interactions.**

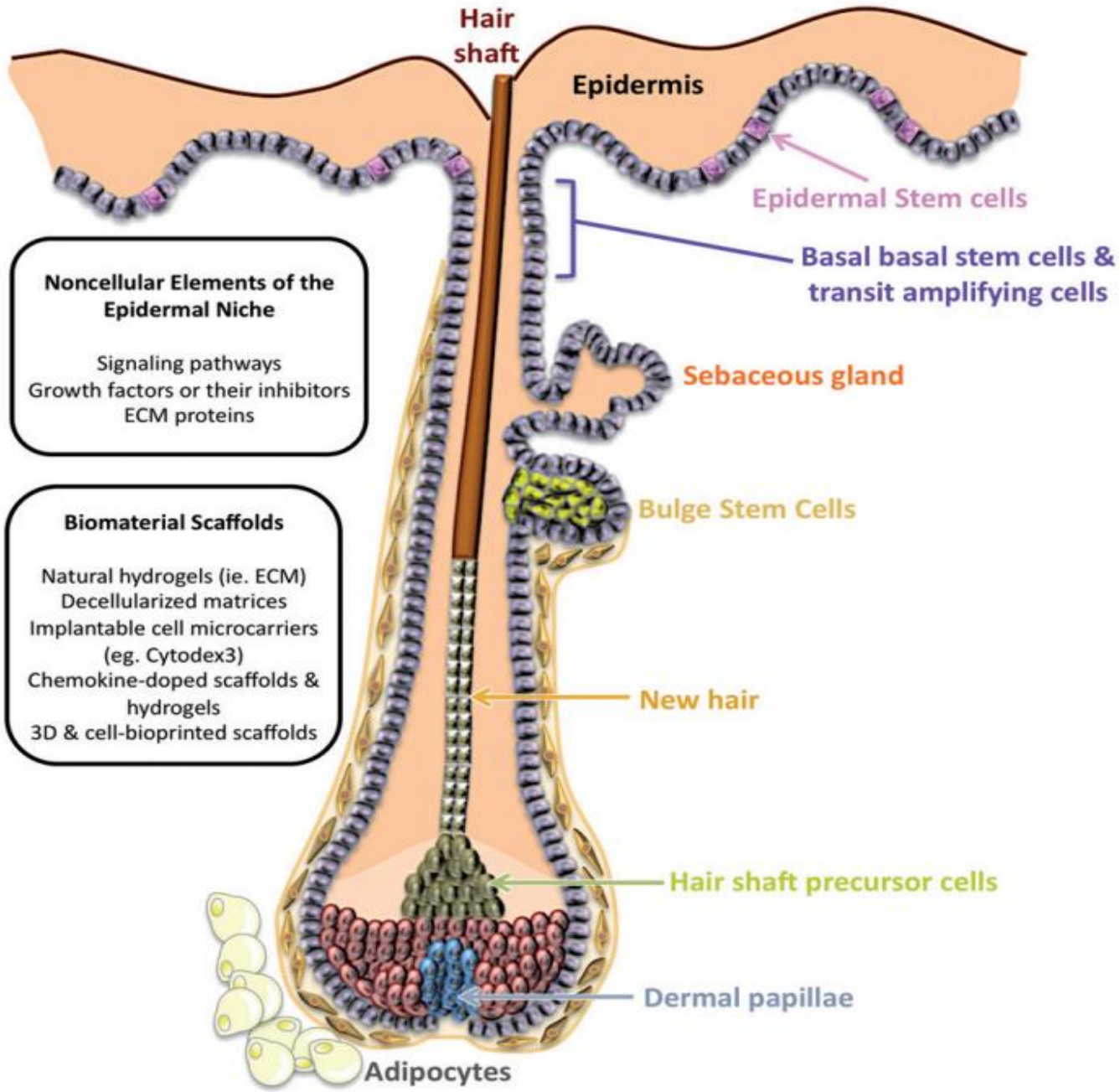
- ✓ **The niches also contain N-cadherins, a4b1-integrins and CD44, all of which serve the singular purpose of allowing HSCs to adhere to ECM components (e.g. collagen and hyaluronin).**



Engineering the hematopoietic niche. (A) Schematic illustration of the two blood-producing microenvironments in the bone marrow, the endosteal niche and the perivascular niche, and some of their constituent cells. Quiescent or less proliferative HSCs occupy the endosteal niche while progenitors tend to be located within the perivascular niche. (B) Individual factors that must be considered when attempting to replicate the hematopoietic niche. (C) Recent technologies used to reconstruct the blood-producing environment.


A
Tetraspanin Structure
Large Extracellular Loop
Small Extracellular Loop
N-termini
Small Intracellular Loop
C-termini
B
Bone Marrow Niche
Tetraspanins
Mesenchymal Stem Cell
Hematopoietic Stem Cell
T-cell
Sinusoid
Megakaryocyte
Extracellular Matrix
Macrophage
Osteoblast
Monocyte
Osteoclast
Adipocyte
CXCL12
Extracellular
CXCR4
C-kit
Integrins
Integrins
CD82
(Larochelle et al, 2012)
(Termini et al., 2014)
(Saito-Reis et al, 2018)
CD151
(Fitter et al, 1999)
CD9
(Anzai et al, 2002)
(Leung et al, 2011)
(Abe et al, 2017)
CD63
(Anzai et al, 2002)
(Jung et al, 2006)
(Wilk et al, 2013)
(Rossi et al, 2015)
CD81
(Anzai et al, 2002)
Intracellular

- ✓ **Skin**
- ✓ **Components of the skin stem cell niche**
- ✓ **As a major organ with abundant regenerative potential and ready accessibility, the mammalian skin is an ideal system in which to experiment with artificial niche engineering.**
- ✓ **This niche is characterized by an intricately organized cellular architecture, comprising an interfollicular epidermal (IFE) layer, hair follicles and sebaceous glands.**
- ✓ **All of these contain highly proliferative stem cells that undergo terminal differentiation to repair or regenerate the skin.**
- ✓ **For example, mitotically active cells in the IFE differentiate and migrate outwards to generate keratinocytes.**
- ✓ **Within the hair follicle, bulge regions also contain stem cells.**
- ✓ **These are characterized by their overlapping (either fully or partially) expression of CD34, keratin and Lgr5.**
- ✓ **Hair follicle stem cells that express Lgr5 are found in the hair germ and the bulge base, and actively divide to produce the entire follicle, including the infundibulum and the isthmus, whilst within the skin, Lgr6þ stem cells are responsible for epidermal generation.**
- ✓ **Stem cell activation within the bulge and mobilization to the germ region, where proliferation occurs, require signals and regulatory molecules such as the proliferation-inhibiting BMP, or the activating b-catenin.**
- ✓ **Extrinsic regulatory factors include adipocytes, muscle fibers and the dermal papilla (DP).**
- ✓ **The DP comprises mesenchymal cells, which provide signaling factors like fibroblast growth factor, Wnts and noggin, to facilitate stem cell activation.**
- ✓ **These are crucial factors that must be included when constructing this niche.**
- ✓ **In fact, the function of the epidermal stem cell (EpiSCs) may be entirely dependent on local niche factors, since whole hair-follicle and skin structures can regenerate even when EpiSCs are transplanted into a foreign environment.**

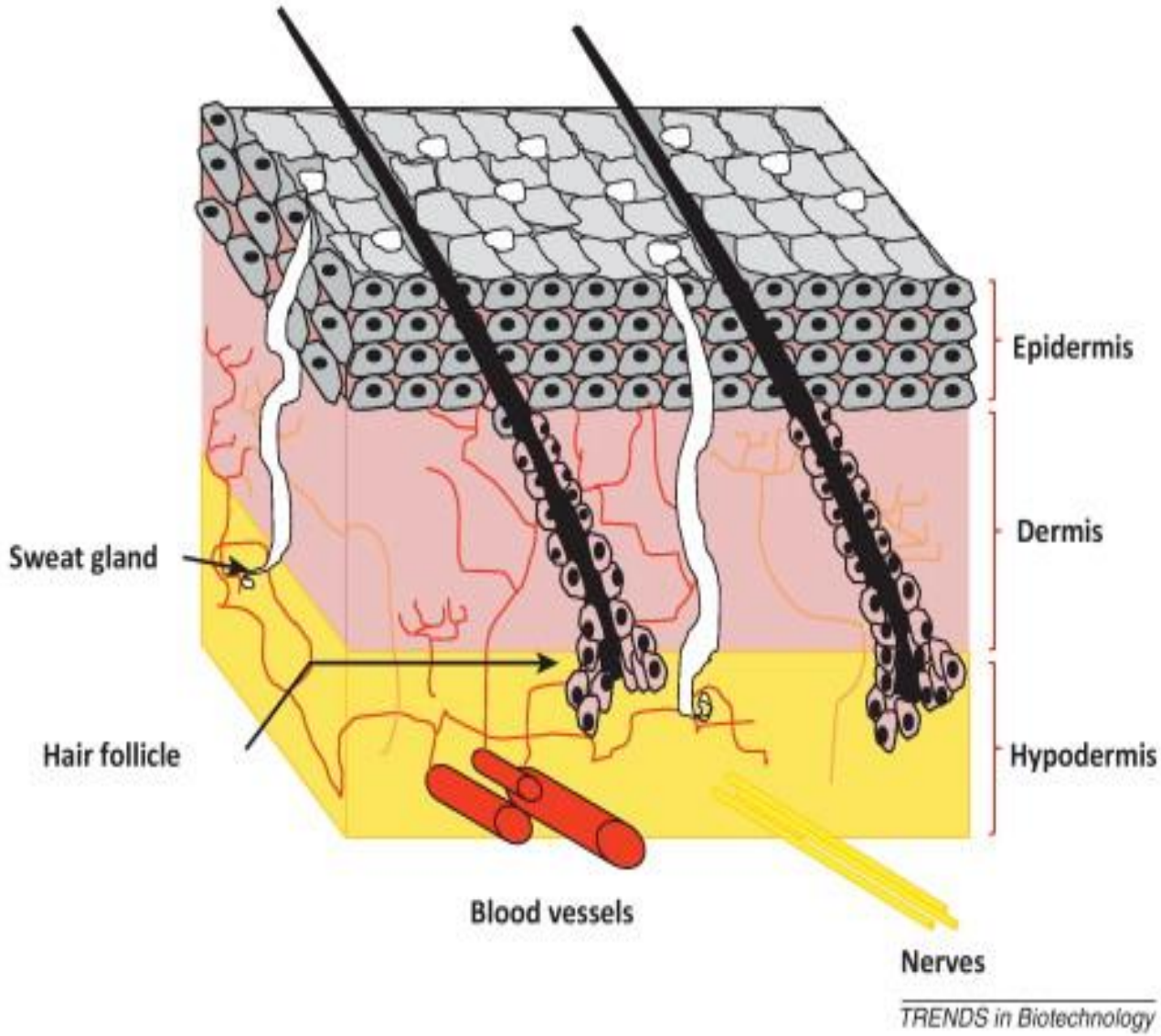


Engineering the epidermal (skin and hair follicle) stem cell niche.

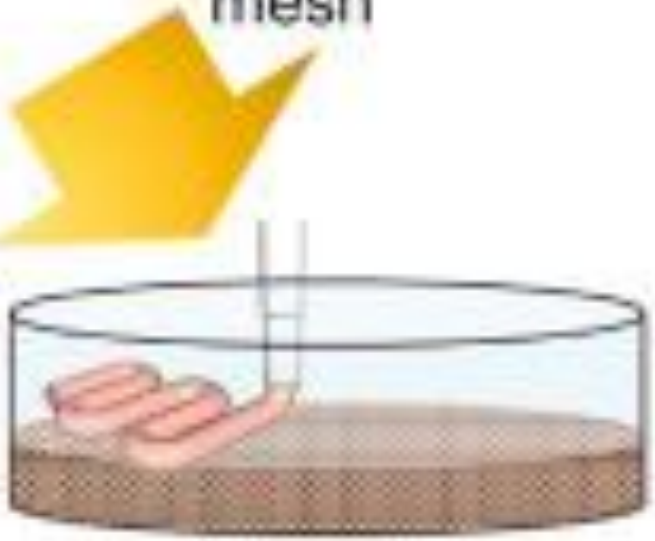
(A) Schematic of the cellular components of the niche.

(B) Biochemical and regulatory factors that modulate stem cells include signaling molecules, chemokines or inhibitors and extracellular matrix proteins.

(C) Advances in engineering the epidermal stem cell niche include the use of artificial and natural materials to create dermal scaffolds, which can be integrated into rotary or microgravity culture vessels.


Epidermis
Dermis
Hypodermis
Sweat gland
Hair follicle
Blood vessels
Nerves
TRENDS in Biotechnology

Preparation: Cells, Collagen hydrogel, microporous PCl mesh

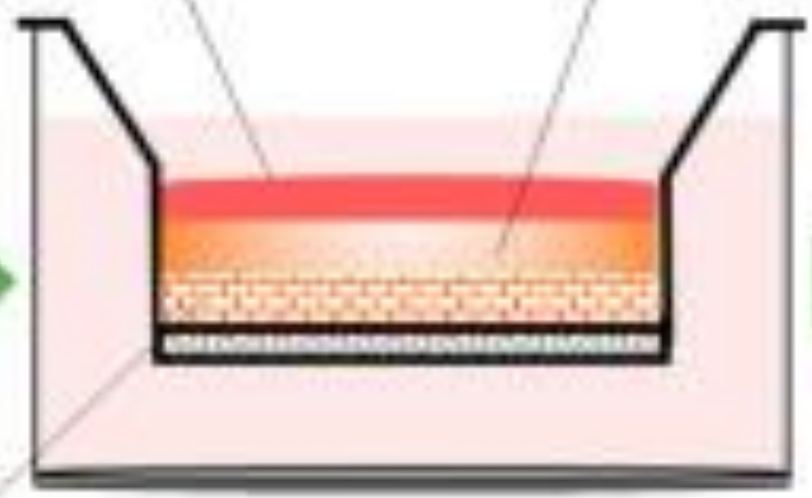


3D bioprinting

①

Keratinocyte cells

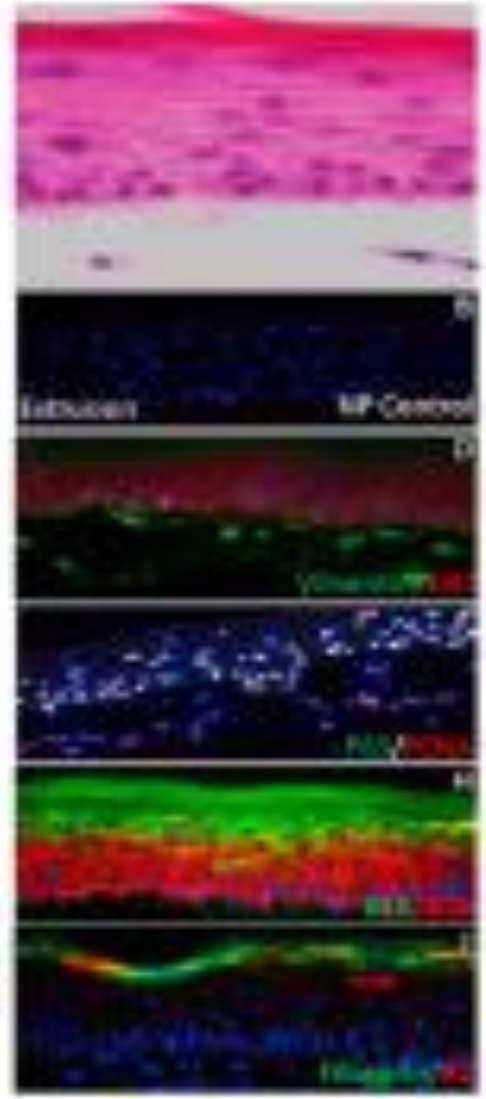
Fibroblast cells/collagen

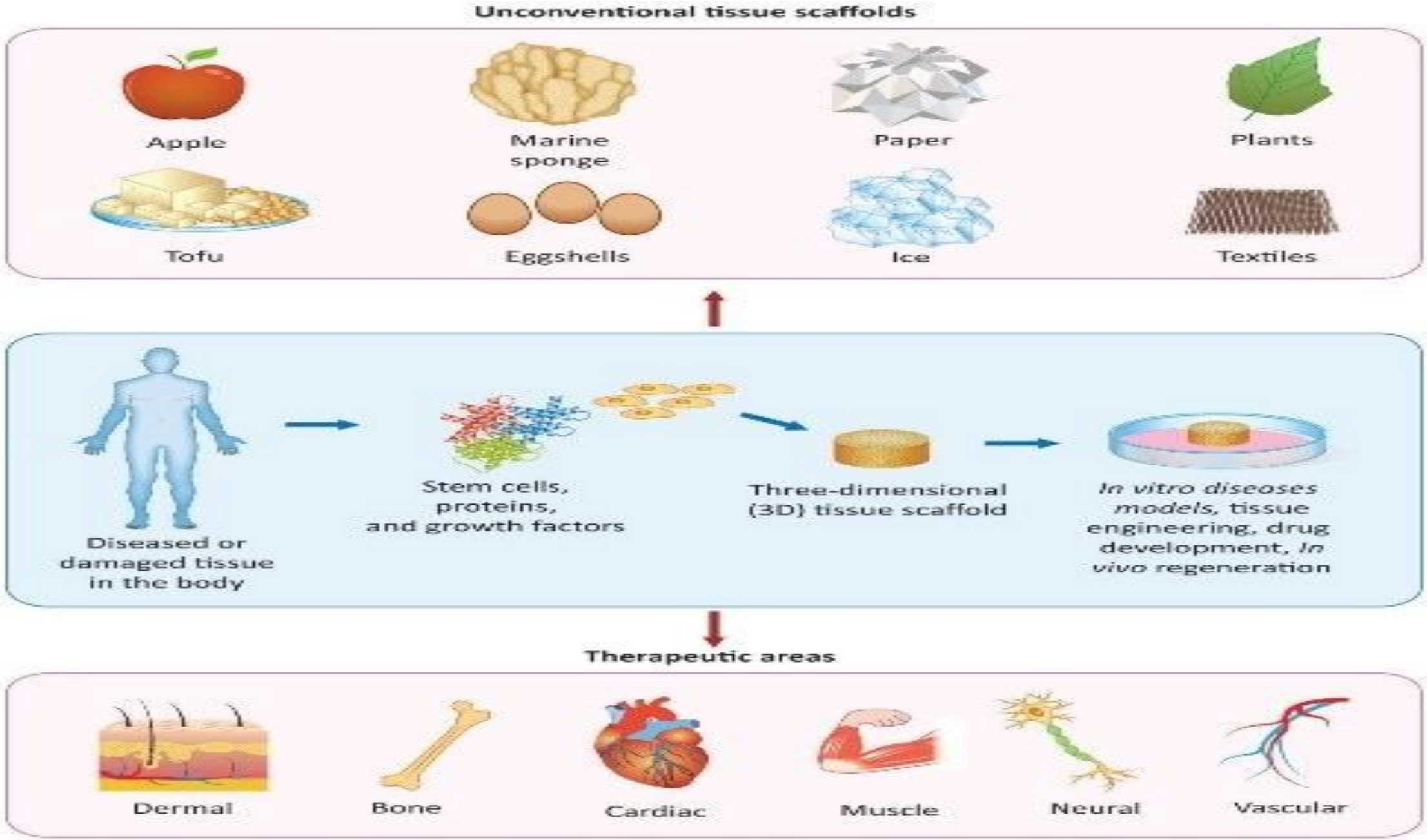


acellular layer

② **Air-Liquid interface lifting**

③ **Characterizations**




Unconventional tissue scaffolds
Apple
Marine sponge
Paper
Plants
Tofu
Eggshells
Ice
Textiles
Diseased or damaged tissue in the body
Stem cells, proteins, and growth factors
Three-dimensional (3D) tissue scaffold
In vitro diseases models, tissue engineering, drug development, In vivo regeneration
Therapeutic areas
Dermal
Bone
Cardiac
Muscle
Neural
Vascular

PROSPECTS AND CONCLUSIONS

- ✓ These platforms are difficult to create and face many technical problems associated with trying to recreate a multicellular structure that is constantly influenced by its constituents.
- ✓ The hurdles in fabricating the required engineering facilities or making platforms accessible to all biologists are not trivial.
- ✓ Many are expensive, not amenable to high-throughput scalability (or conversely, nano-scaling), or require extensive training and optimization.
- ✓ The ideal would be to establish a mass production protocol that identically reproduces each biological property of a stem cell in its entirety, including proliferation rate, which may be altered by ex vivo culture, since variations to any of these properties may negatively impact the integrity of the resulting cells and their eventual application in a clinical setting.
- ✓ Nevertheless, much progress and innovation has been made within the tissue-engineering field, with a corresponding increase in biological understanding.
- ✓ Technologies from other fields, such as the semiconductor industry have accelerated biomaterials development.
- ✓ Photolithography can be used to sculpt complicated cell architectures, or combined with microfluidics to fabricate microtissues.
- ✓ Microfluidic technologies facilitate the creation of niche features such as morphogen concentration gradients and would enable its more precise reproduction at the biochemical level.

- ✓ **Future niche design strategies could benefit from incorporating current technologies and methods successfully used in other tissues.**
- ✓ **For example, hydrogel landscaping could be applied to sculpting the vascular networks necessary for supporting more complex niche architectures.**
- ✓ **The transition to 3D systems, though technically challenging, must be realized, since the absence of multidimensional spatial organization introduces unacceptable physiological biases.**
- ✓ **In 3D, cells are afforded greater contact with soluble factors, such as cytokines or growth factors, and it will be interesting to see whether biofunctional 3D stem cell niches can be created from commonly used biodegradable synthetic polymers or ECM factors.**
- ✓ **Of greatest relevance to biologists is the capacity to customize niches and thus provide one of the most costeffective, versatile and instructive tools for pharmacological research.**
- ✓ **It is theoretically possible to exchange any of the abovementioned soluble or insoluble factors for an appropriate biomimetic surface or therapeutic compound.**
- ✓ **Thus, the niche can serve as a delivery vessel for drugs.**
- ✓ **The targeting of tumor-initiating cells residing in niches as a potential chemotherapeutic strategy is one such example.**

- ✓ **In the treatment of promyelocytic leukemia, an antagonist of CXCR4 (a chemokine receptor for the SDF-1 ligand), AMD3465, can be potentially engineered for timed-release into artificial bone marrow niches to enhance sensitivity towards the drug ADM3100.**

- ✓ **Clinical trials for niche components are now in process, such as one to determine whether decellularized matrix obtained from umbilical cord blood Wharton's jelly can be used as a scaffold for the growth and differentiation of mesenchymal cells.**

- ✓ **The fields of bioengineering and stem cell biology have converged to a point where it is now realistic to expect that maturation of scientific knowledge in one will lead to more sophisticated discoveries in the other.**

- ✓ **While progress from the biologist's bench to the patient's bedside has not been rapid, these advances indicate that the field is on track to realizing the full potential of stem cells in regenerative medicine.**

- ✓ **As bioengineering systems or biomaterials become more refined, responsive and accurate, so will our capacity to replicate cellular microenvironments, and it is hoped that this synergy will provide a precious and tantalizing resource for the future of medicine as well as basic biology.**