Review

The rise of mechanical metamaterials: Auxetic constructs for skin wound healing

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Abstract

Auxetic materials are known for their unique ability to expand/contract in multiple directions when stretched/compressed. In other words, they exhibit a negative Poisson's ratio, which is usually positive for most of materials. This behavior appears in some biological tissues such as human skin, where it promotes wound healing by providing an enhanced mechanical support and facilitating cell migration. Skin tissue engineering has been a growing research topic in recent years, largely thanks to the rapid development of 3D printing techniques and technologies. The combination of computational studies with rapid manufacturing and tailored designs presents a huge potential for the future of personalized medicine. Overall, this review article provides a comprehensive overview of the current state of research on auxetic constructs for skin healing applications, highlighting the potential of auxetics as a promising treatment option for skin wounds. The article also identifies gaps in the current knowledge and suggests areas for future research. In particular, we discuss the designs, materials, manufacturing techniques, and also the computational and experimental studies on this topic.

Keywords

Skin tissue engineering, auxetic materials, additive manufacturing

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Introduction

The importance of skin for human health is unquestionable, as it is the human body's first defense against physical and biological threats. Acting as a mechanical barrier to the outside environment, preventing the entry of pathogens and micro-organisms into the body,[1] thermoregulation and self-healing[2] are among its main functions.

This complex and large organ has two layers called epidermis and dermis, which are populated by different cell types and have different functional, mechanical, and biological characteristics.[3]

The epidermis is a thin, poorly vascularized layer located on the outermost part of the skin.[4] It is composed of keratinocytes that proliferate outwards and, as they differentiate, fill with keratin, creating a layer of dead cells that provides protection against external agents and prevents the loss of water and other substances. The dermis is the inner of the two layers of the skin. It is a thick, highly vascularized layer of connective tissue composed of fibroblasts and extracellular matrix (ECM) of collagen, elastin and glycosaminoglycans among other components.[5] This dermal ECM is the major contributor to the mechanical properties of the skin,[6] where the combination of stiff collagen fibers and flexible elastin fibers, as well as their cross-linking, results in an interesting mechanical behavior consisting of a characteristic "J-shaped" stress-strain curve[7] together with an auxetic behavior.[8,9] Skin mechanical properties may vary depending on several factors[10,11]

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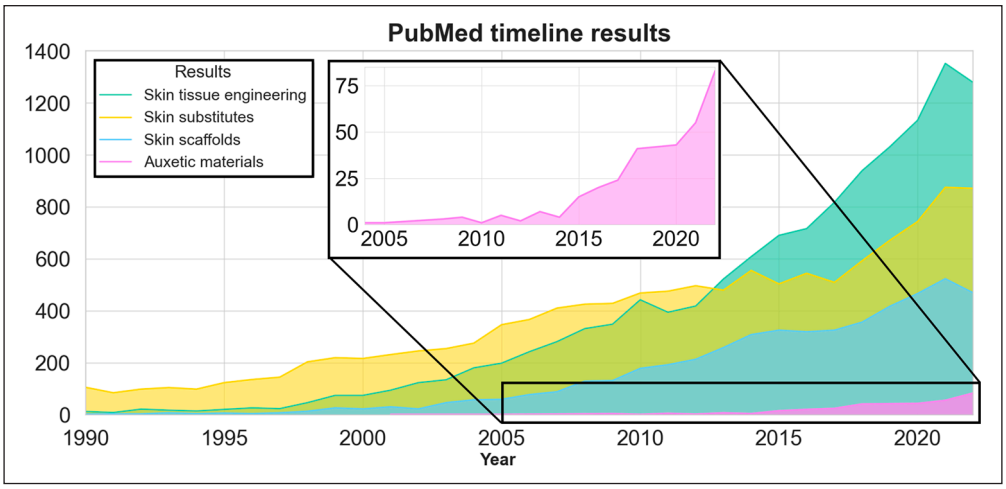

Figure 1. PubMed search results from skin tissue engineering related topics, showing the increasing interest and development in recent years.

that may be local, such as the particular location of the body being measured, its current care status or the direction of the sample fibers with respect to Langer's lines,[12] or global, such as the patient's age or gender.[13,14]

Mechanical alterations of the skin, such as those resulting from wound formation, give rise to processes in which physiological functions are also affected due to loss of tissue integrity and functionality, leading in extreme cases to disabilities or even death.[15] The importance of these wounds depends on their size, where small or superficial wounds quickly heal due to the high regenerative capacity of the skin,[16] while larger wounds (area >4 cm),[17] such as those caused by burn damage or acute trauma, may require more healing time and even additional surgical interventions, sometimes even requiring skin substitutes to achieve good repair and regeneration.[18]

In this context, many different skin substitutes have been developed in the last decades.[3,6,19–23] The most common and widely used solution is transplantation,[20,24] where the affected area is covered with tissue of the same or similar type as the missing tissue, taken from another part of the patient's body or from a donor, whether of the same species or not, known as autografts, allografts, and xenografts, respectively.

The graft transplantation method, although effective in healing the wound and giving rise to a tissue quite similar to the original one, requires a separate surgical intervention to remove the tissue to be transplanted, with all the risk that this entails, in addition to leaving a scar in another part of the body. Furthermore, it is also a procedure limited by the availability of tissue to extract,[21] so that in cases of exceptionally large area skin wounds, these solutions become unfeasible. In fact, besides immunoreactions,[25,26] there is another drawback to the use of human skin grafts that is the non-zero risk of disease transmission, as there has been at least one case of HIV transmission between donor and recipient.[27]

To overcome these problems, tissue engineering research has been focusing on the development of scaffolds or tissue substitutes that act as templates for cell infiltration and subsequent regeneration of damaged skin, culminating in a plethora of skin substitutes,[3,28,29] some of them showing promising techniques and results,[30] and even reaching the market and clinic.[31] As an example, searching on PubMed for related keywords such as "skin tissue engineering," "skin substitutes," and "skin scaffolds" from the last few decades yields a substantial number of results, indicating an increasing growth in this area during that time period (as shown in Figure 1). Similarly, a search for "auxetic materials" demonstrates an exponential rise in results from recent years, highlighting the increased interest in auxetic behavior research.

Moreover, the search of "auxetic materials" also reveals an exponential increase in the results from the last years, bringing to the board the rise of interest that auxetic behavior research has been experiencing.

Further research is still needed, and expected, as currently available materials present disadvantages such as high costs, issues of take and integration, and non-satisfactory esthetic outcomes.[32] Therefore, new strategies are needed in the design of novel and effective skin substitutes.

In this context, auxetic materials have gained attention for their potential use in skin wound healing due to their unique mechanical properties. Moreover, skin itself shows an auxetic behavior, as previously mentioned. As shown in Figure 2, auxetic structures can stretch/contract in multiple directions when they are subjected to external forces, allowing them to conform to the shape of the wound bed and provide good coverage and protection to the damaged tissue. In the context of stretching, auxetic structures exhibit mechanical behavior similar to that of skin. Both materials can withstand high levels of strain before experiencing stiffening at a certain point.

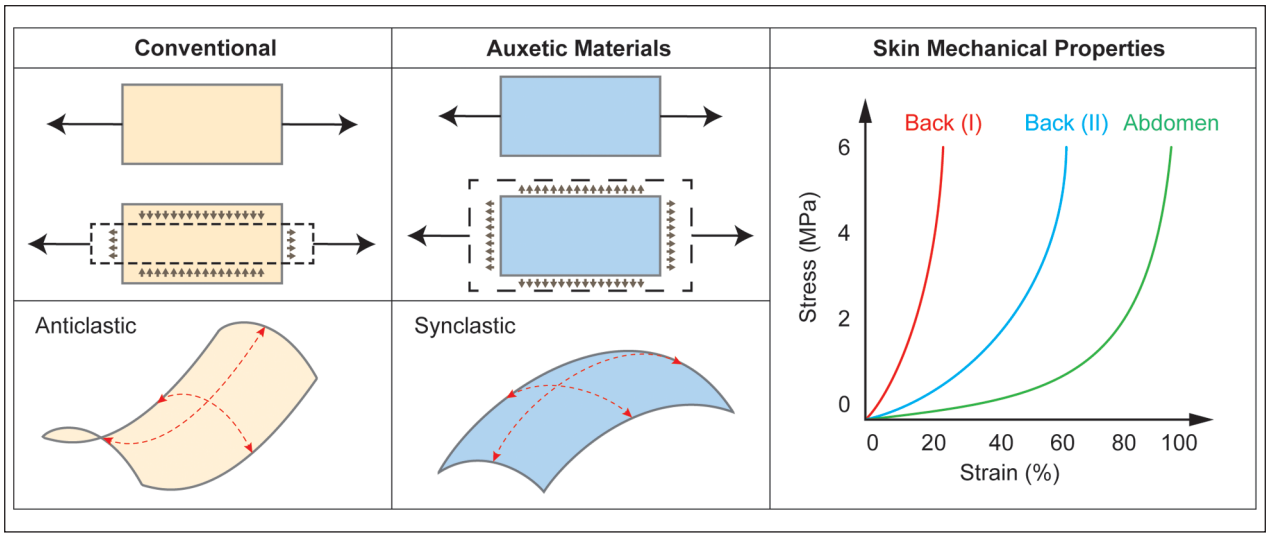

Figure 2. Left panels: schematic diagram comparing conventional and auxetic material behaviors. In addition to their negative Poisson's ratio, auxetic materials exhibit synclastic deformation which allows for more even distribution of pressure across the surface, potentially benefiting wound healing and reducing the risk of pressure sores in skin healing applications. Right panel: skin stress-strain J-shaped relationship for different parts of the body (Adapted from Jang et al.[34]).

Additionally, auxetic materials have exceptional out-of-plane bending behavior, allowing them to conform to synclastic surfaces and exert stable amounts of pressure along their shape when bent.[33]

This property may be very useful in wound healing applications, especially in areas such as joints, where the wound shape may be affected by the movement. The bending behavior of the scaffold may help maintain contact between the scaffold and the wound in these sites. This is an important function for skin repair, as it may help to prevent further damage and provide a suitable environment for cell proliferation and tissue remodeling. The porous structure also enables cell migration and infiltration,[35] which could enhance the formation of new tissue. Additionally, auxetic structures are known to have good mechanical strength and stability, which could help supporting the wound area during the healing process, preventing the scaffold from collapsing or deforming under the loads applied by the surrounding tissue. These properties, together with the developments of 3D printing technologies that allow both flexibility and a tight control of microgeometries, are rising auxetic scaffolds as a promising option for skin repair.

The aim of this review is to provide an overview of the current state of the art on research on auxetic constructs for skin tissue engineering. To this end, the review summarizes the auxetic designs that have been studied or proposed for skin wound healing, and outlines the manufacturing techniques applied to produce them, as well as the computational and experimental studies that have been conducted to evaluate their performance, from a critical point of view in order to understand their potential and limitations.

Auxetic materials

Auxetic properties and biological tissues

Auxeticity is a mechanical characteristic associated to materials exhibiting a negative Poisson's ratio, which means that they grow transversely when stretched longitudinally and vice versa, they shrink in the transverse direction when compressed longitudinally.[36] Materials exhibiting this behavior are included in a recently considered material group called "mechanical metamaterials," which englobes materials that have unusual mechanical properties, not commonly found in natural materials, due to their particular micro-scale structure rather than the properties of their constituents. These materials can be engineered to have particular properties such as negative stiffness,[37] negative Poisson's ratios, which are the auxetic materials, or negative mass density.[38]

However, despite this classification, auxetic properties have actually been found in natural materials for years. Actually, there are a lot of auxetic crystalline materials[39] and auxetic foams,[40] as their inner pore-based structure can develop an auxetic configuration. Even there are some biological tissues that have also been shown to develop auxetic behavior, as it has been reported in cow teat,[8] cat,[7] pig,[9,41] and salamander[42] skins, arteries,[43,44] tendons,[45] cancellous bone,[46] embryonic epithelia,[47] cornea,[48] and more recently, in marine sponges.[49]

These biological tissues develop the auxetic behavior mainly because of the structural organization and cross-linking of their ECM fibers, demonstrating the importance of ECM structure in the mechanical behavior of the tissue, and also leading to the idea of using auxetic scaffolds to mimic the mechanical properties of the ECM in order to provide better support for the implanted cells.

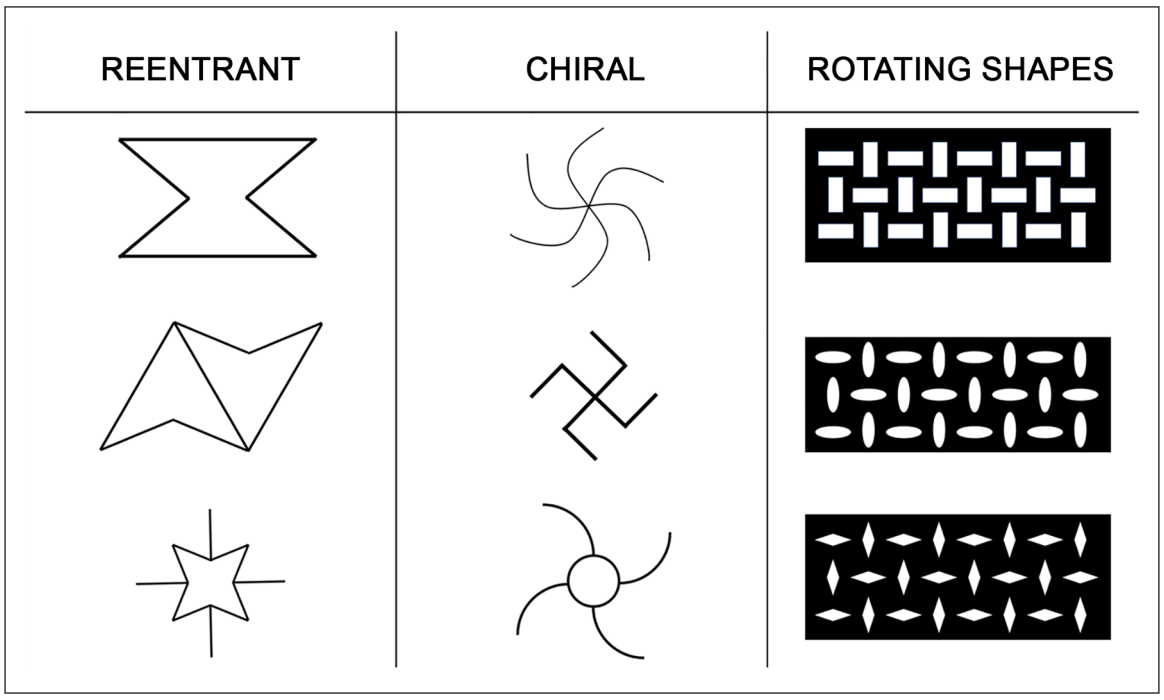

Figure 3. Examples of 2D geometry patterns with auxeticity development.

There are some reviews[50,51] on the topic of auxetic scaffolds for general tissue engineering, in which the collected studies show some encouraging results on cell migration, proliferation and differentiation enhancement due to auxetic scaffolds, in addition to studying the cyto-compatibility and the survival viability of different cell types when cultured in this kind of particular mechanical environments.

Nowadays, there are various paths to follow in order to achieve auxeticity in tissue engineering constructs. Apart from the auxetic foams already commented,[40] there are three main geometry design techniques based on repeatable basic patterns that are suitable for their application in manufacturing methods and could be useful to study the influence on cell behavior of this mechanical issue: reentrant, chiral, and rotating shape designs (Figure 3).

Reentrant designs consist of common polygons with an inversion in some of their angles, which gives the auxetic behavior to the structure. Controlling these angles and the size of the designs is crucial to set the structural auxetic behavior.

Chiral designs are similar fiber designs that are characterized by having a center, or axis, around which a radial fiber distribution coil. Thus, the coiling degree and fiber amount would be the main parameters to control the auxetic development of the structure.

Rotating shape designs are based on a pattern of cuts made on a membrane that results on a connected-shapes distribution that, when stretched, makes the shapes rotate around each other expanding the total area occupied by the membrane. The shape, size and distribution of the cuts are the main parameters to set the auxetic behavior of these designs.

To obtain reentrant and chiral designs, it is common to 3D print a scaffold by controlling the spatial distribution and orientation of the fibers to draw the 2D or 3D auxetic design. A widely used application of these techniques is to develop fiber reinforced scaffolds, where these fibers, made by biopolymers, enhance the mechanical behavior of other materials, such as hydrogel, gelatin, bio-inks, or tighter and thinner fiber structures. Hence, this reinforcing structure would be added to give physical support to the cell culture, and also affect synergistically the mechanical behavior as reported in some studies.[52–55]

In the case of rotating shape designs, the most common technique is to first manufacture the membrane, which could be made by both stiff materials,[56] as electrospun biopolymers, or soft materials,[57] as hydrogels, and then apply the cut pattern with other techniques such as laser cut, which has high precision and control. When applying this technique, the whole membrane acts as cell support and the mechanical behavior is more influenced by the properties of the constituent material. The existence of the cuts implies that there would be void spaces inside the scaffold when the membrane is stretched, so it is definitely something to consider when designing the scaffold application. More details regarding the manufacturing of this kind of scaffolds are described later on in the *Section 3.2. Fabrication technologies*.

Table 1. Publications about auxetic scaffolds related to skin tissue engineering.

Publication	Material	Fabrication method	Auxetic design	Cell culture	Particular details	Main conclusions
Chansoria et al.[58]	Bilayer patch of GelMA and PEGDA	Digital light processing	Reentrant, chiral, and rotating shape 2D macro designs	3T3 Fibroblasts	Patch design oriented to cover organs and to protect the bottom cell layer	Obtained good cell proliferation, area coverage and adaptation to dynamic organ mechanics
Flamourakis et al.[59]	Photopolymer SZ2080	Multiphoton stereolithography	Reentrant 3D micro designs	NIH-3T3 Fibroblasts	Cell-sized pore auxetic scaffold to study auxetic influence at cell level	Good cell penetration, proliferation, directionality, and scaffold shape adaptation to cell requirements
Jin et al.[60]	PCL fibers	Melt electro-writing	Reentrant 2D multiscale design	Human umbilical vein endothelial cells (HUVECs) and bone marrow stem cells (BMSCs)	Auxetic thick fiber macro design under a thin fiber layer that provides cell support. Intricate pore size scaffold with versatile mechanical properties	Different cell proliferation depending on cell type, solves the problem of biocompatibility and mechanical strength simultaneously

Auxetic scaffolds in tissue engineering

Despite the existence of various different auxetic scaffolds in the literature, to the best of our knowledge, a specific application of these structures to skin repair has not been reported. Table 1 summarizes the main characteristics of the studies where a link between the auxetic scaffolds and a possible application for skin tissue engineering could be suggested, while Figure 4 shows details of the auxetic constructs developed.

Although these studies greatly differ from each other, all of them show encouraging results about the use of auxetic scaffolds. For example, Chansoria et al.[58] developed and studied auxetic hydrogel patches designed for conforming to the complex mechanics of dynamic organs, such as heart, lungs, bladder, or skin. Their patches were composed of two layers obtained by photocuring simultaneously with 2D auxetic macro patterns, in the order of centimeters, both the non-fouling polyethylene glycol-diacrylate (PEGDA) top layer and the gelatin methacryloyl (GelMA) bottom layer, which allowed cell adhesion and proliferation. They applied different reentrant, chiral and rotating shapes designs to their patches and studied and tested them both numerically and experimentally, which they did by performing a parametric study that allowed them to relate the mechanical properties of the patches and the organs, and then select the ones they wanted to test and study their behavior and possible application. In this context, they were able to relate some of these designs with the skin mechanical behavior, but they did not further explore its application.

Flamourakis et al.[59] laser-fabricated small auxetic scaffolds with SZ2080, which is a hybrid organic-inorganic photoresist.[61] The high precision of the two-photon-polymerization technique, which they used to manufacture the scaffolds, allowed them to obtain 3D reentrant auxetic structures in the order of units of microns, which were about the same size of the cells, in order to better study the auxetic influence in cell response. After culturing mouse NIH-3T3 fibroblasts, they found that cells were able to penetrate and proliferate through the scaffold, and they also observed that cells were aligned along the scaffold structure and also adapting the scaffold shape to suit their requirements.

Jin et al.[60] went into the multiscale design of a fibrous poly(caprolactone) (PCL) scaffold composed by an 2D reentrant auxetic macro-structure, with fiber diameters of 400 microns, and a 2D web-like micro-structure that was composed of 10-micron diameter fibers. These multiscale scaffolds were designed not only to have an auxetic mechanical behavior due to their macro-scale design, but also to give physical support to the cells at the micro-scale. They seeded human umbilical vein endothelial cells (HUVECs) and bone marrow stem cells (BMSCs) which showed different behaviors, where HUVECs proliferated along the direction of the fibers, while BMSCs proliferated by filling the void spaces between the fibers. These scaffolds have an intricate pore size due to the micro-scale design, and their macro-scale design gives them a tunable mechanical behavior, thus it is a versatile way to develop auxetic scaffolds. However, they only studied the cell responses in static conditions, so dynamic testing of these structures would be interesting for observing the mechanobiological influence that auxetic behavior could have in the cells.

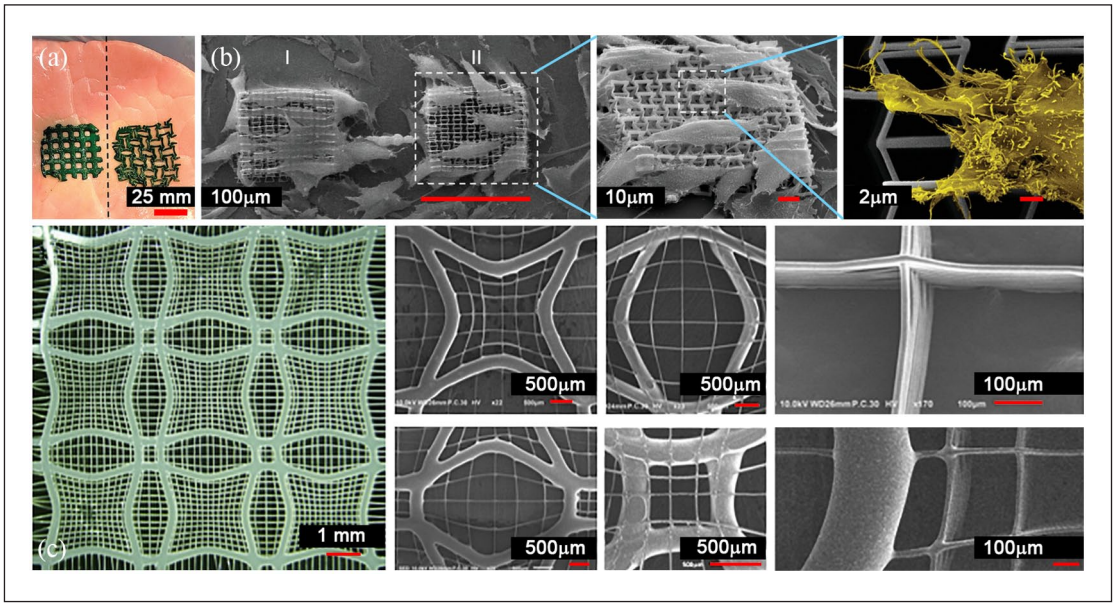

Figure 4. Details of the auxetic scaffold studies conducted. (a) Bilayer hydrogel non-auxetic and auxetic patches showing its in vivo application in pig lung. (b) Laser-made auxetic scaffolds showing NIH-3T3 fibroblast culture with cell scaffold directionality and attachment. (c) Multiscale auxetic scaffold where both macro and micro designs and fiber sizes are visible. Illustrations adapted with permission from Chansoria et al.,[58] Flamourakis et al.,[59] and Jin et al.[60] respectively.

The potential applications for skin tissue engineering of these publications diverge due to differences in sizes, scales, materials, and study subjects, although they could be adapted. For example, the hydrogel patches[58] could be manufactured in a reduced scale to decrease void spaces within the hydrogel, thus enhancing the coverage for large area wounds. In the same way, the 3D laser-manufactured[59] and the multiscale[60] fiber scaffolds could be an option to improve the effectiveness and mechanical and biological properties of fiber reinforced hydrogel scaffolds that could also be applied in large wounds.

Computational studies of auxetic materials

Simulations have several advantages over experimental studies among which we can highlight the cost and time efficiency and the ability to perform broad sensitivity analysis at different scales (from micro to macro) which can provide a more complete understanding of the behavior of studied systems. Computational studies on auxetic structures and designs have gained attention in recent years and have been used to predict mechanical behaviors and to optimize the material properties and geometries of the constructs.

For example, Jang et al.[34] did a numerical analysis of auxetic 2D chiral patterns designed for an application as a structural reinforcement for skin-mounted electro-physiological sensors. They ran a parametric study of the geometry and its different combinations, they fabricated some design samples with polymethylmethacrylate (PMMA) by photolithography and tested them experimentally, validating the numerical results. The mechanical properties measured were analogous to those of collagen and elastin in biological tissues and exhibited three phases of tensile loading: the first phase was characterized by bending-dominated deformation, followed by a second phase where fibers rotated, twisted, and aligned with the direction of stress, and finally, a stretching-dominated deformation mode in the last phase. During the final phase of mechanical loading, the mechanical modulus was observed to increase by several orders of magnitude compared to the initial phase. Additionally, when the auxetic designs were subjected to low strains, they exhibited a negative Poisson's ratio effect in that region. However, this behavior disappeared as the shapes were fully extended. They proposed their constructs to be used as sensors, but both the numerical analysis and the auxetic design could be scaled and the materials made biocompatible to fabricate fiber reinforced scaffolds for skin implantation.

A similar study was conducted by Liu and Zhang[62] where auxetic 2D chiral patterns but with straighter fibers were analyzed numerically. In this case, the parametric study was related to cat skin behavior, and the application was to develop architected cylindrical shells with shape memory effects. Results showed that the chiral patterns had the potential to exhibit isotropic Poisson's ratios ranging from −1 to 1, even over large strains. Furthermore, the developed design methods could identify appropriate geometries to achieve specific Poisson's ratios while also matching the mechanical properties of cat skin. Once

again, the analysis and designs could be adapted to the necessary scale and materials to also be applied in wound healing applications.

Lastly, there are two publications[63,64] where auxetic 2D patterns combining reentrant and rotating shapes designs as cuts in bilayer membranes with different properties simulating the epidermis and dermis layers of the skin were analyzed with a parametric study in order to understand the effect of the auxetic patterns on split thickness skin graft expansion. The results of this study showed that all auxetic graft designs confirmed the negative Poisson's effect. Moreover, when models were subjected to uniaxial strain, the meshing ratios of auxetic grafts exceeded 30, which was significantly higher than traditional grafts with ratios around 3. However, the study also identified some limitations, such as the assumption of skin as an isotropic and elastic material model, or the constant thickness assumption. Despite these limitations, further research and experimental investigations could enhance our understanding and contribute to developing a large skin graft area using a small size donor skin. This is particularly significant in skin transplantation and burn surgery.

In summary, computational studies provide a cost-effective and efficient way to analyze and optimize auxetic structures and designs, and to predict their behavior under different conditions, which can greatly assist experimental studies and the overall development of auxetic materials for skin tissue engineering applications. Further research is needed to develop constructs that can be easily integrated with other wound healing therapies and techniques.

Fabrication of auxetic scaffolds

Materials

Scaffolds are obviously a critical component in tissue engineering, as they provide the necessary support and microenvironment for cells to grow and differentiate into functional tissue. The materials used for manufacturing scaffolds must have both a combination of mechanical properties that makes them suitable for the corresponding manufacturing technology and also biological properties to not cause an adverse reaction in the body when it comes into contact with living tissue. The ideal scaffold material should be biocompatible, biodegradable, and should support cell attachment, proliferation, and differentiation.

A wide range of materials have been used to fabricate scaffolds for tissue engineering, including natural, synthetic, and composite materials.[65–67] Natural materials, such as collagen, gelatin, and chitosan,[68–73] are biocompatible and biodegradable, but they can be difficult to process and may lack the mechanical strength for some applications. Synthetic materials, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and PCL,[74,75] are easy to process and have well-defined mechanical properties, but they may be less biocompatible. Composite materials, such as those made from a combination of natural and synthetic materials, can offer the best of both worlds, providing the necessary mechanical properties while also being biocompatible and biodegradable.

Examples of new materials that have been used in scaffold fabrication are hydrogels, which have gained attention for their ability to mimic the mechanical properties of the native ECM.[76–78] Hydrogels can be made from natural or synthetic polymers and can be tailored to mimic the mechanical properties of the tissue it is supposed to replace, which can greatly improve the tissue regeneration process.

The auxetic constructs developed in the studies summarized in Table 1 showcase a diverse range of biomaterials, each with distinct properties and potential applications in skin tissue engineering. In order to provide a better comprehensive understanding of these materials, Table 2 outlines their main properties and composition, among other critical factors that influence their performance in tissue engineering applications.

In conclusion, the choice of materials for scaffold fabrication is a critical aspect in tissue engineering, as it affects the scaffold's ability to be adapted to the particular mechanical properties and microarchitecture of the tissue to replace.

Fabrication technologies

3D printing techniques are currently among the most promising and viable approach to manufacture functional and custom-fit scaffolds capable of promoting tissue regeneration. Such techniques can help in the challenge of accurately controlling the spatial distribution of pores and structures within the scaffold, which is critical for a proper cell development. Thus, depending on some factors such as the tissue to be replaced, the material utilized or the type of scaffold, there exist multiple ways to manufacture these tissue-engineered constructs.[87]

As the constructs may need specific requirements in their microstructures, not every 3D printing technique is suitable to be used in tissue-engineered scaffolds manufacturing. Thus, Figure 5 gathers details of the different microstructures achieved by some of the most used 3D printing techniques for the fabrication of tissue engineering scaffolds,[88–95] which can be grouped as electro-printing techniques, digital light processing (DLP) techniques, and 3D-bioprinting. Note that the geometries depicted are non-auxetic, as the figure focuses on the achieved scale and fiber organization.

The electro-printing category includes widely used and interesting techniques, such as electrospinning (ES) and melt electro-writing (MEW), which have been extensively studied for scaffold fabrication.[56,89,98,99]

ES, widely described in publications since the end of the last century,[100–102] is a fiber deposition process where an electrical charge is used to extrude a melt polymer solution into thin fibers, which are collected onto a grounded

Table 2. Descriptive list of biomaterials suitable for auxetic constructs for skin tissue engineering applications.

Biomaterial		Description	Main Properties
Poly-ε-caprolactone (PCL)[75,79]		Synthetic polymer: a polyester synthesized by ring-opening polymerization of ε-caprolactone using different catalysts or by 2-methylene-1-3-dioxepane.	• Biocompatibility Biodegradability • Hydrophobicity Semi-crystallinity Low meting point (∼*60°C*) Elasticity FDA approved
SZ2080[61,80–83]		Resin: a hybrid organic/inorganic photoresist made of two components: methacryloxypropil trimethoxysylane and zirconium propoxide.	• Biocompatibility • Long-term stability • Chemical and electrochemical inertia • Transparency • Photopolymerizable • Ultra-low shrinkage during polymerization
Gelatin methacryloyl (GelMA) / Polyethylene glycol diacrylate (PEGDA)	GelMA[84,85]	Modified natural hydrogel produced by the reaction of gelatin with methacrylic anhydride, whereby the amino groups on the side chains of gelatin are replaced by methacryloyl groups, thus forming modified gelatin.	• Biocompatibility • Biodegradability • Photopolymerizable • Thermostability • Tunable physicochemical properties
	PEGDA[83,85,86]	Synthetic hydrogel that is a PEG derivative fabricated through substituting terminal hydroxyl groups of PEG with acrylates.	• Biocompatibility • Biodegradability • Hydrophilicity • Photopolymerizable • Elasticity • Tunable physicochemical properties

collector in a spun way, resulting in a scaffold composed of a dense network of interconnected fibers. This technique allows for the production of fibers with diameters in the nanometer and micrometer range, which is compatible with the natural ECM fiber size range[103] and could promote cell attachment and proliferation. It also allows for the production of scaffolds with a high surface area, which can increase the interactions between cells and the scaffold. However, ES presents some limitations, as it can be challenging to achieve a uniform fiber diameter and distribution, and the fibers may have a smaller size than those produced by other techniques, which could result in weaker structures.[104] Furthermore, ES may not be suitable for certain types of polymers, as they may not be able to be stable at the high voltages required.

On the other hand, MEW, which has been under extensive development since 2011,[105] is a similar process that allows fabricating the polymer fibers with a higher resolution and precision where both position and orientation of the deposited fiber can be defined,[106] allowing a more precise control in the design of the object to be printed. It also involves using an electrical current to melt and extrude a polymer material through a fine nozzle, and then it is cooled and solidified as it is extruded, forming fibers and structures. In this case, the nozzle or the collector can be moved spatially to form the desired pattern or shape as the fiber is solidifying.

MEW offers some advantages over other 3D printing techniques, such as its ability to print at high resolutions with high precision, which allows for the creation of scaffolds with a high degree of control over the microarchitecture,[107] as the set up can be moved in the three dimensions to create complex shapes. It also can be used to create scaffolds with a wide range of pore sizes and architectures, which can be tailored to meet the specific requirements of the application. Nevertheless, MEW also has some limitations due to the specialized equipment required to develop this technology.

Both ES and MEW processes work by using, in addition to the application of the electric field, mechanical means to encourage the material deposition using a syringe pump that controls the polymer solution feed rate.[108] The ratio between the electrical and the mechanical parameters must be considered, as it defines the amount of polymer delivered to the jet that can be accepted for the flow rate provided by the electric field, seeking to avoid defects such as ribbon-like structures or spherical drops.[98,109,110]

ES and MEW techniques have been used to manufacture numerous types of biomedical devices applicable in various fields, such as multi-purpose biocompatible scaffolds,[55,111–115] cartilage,[116] and cardiac[117–119] tissue engineering scaffolds, constructs for stem cell therapy[120] or drug delivery devices.[121–124] Their application in skin tissue engineering scaffold manufacturing is also wide. For

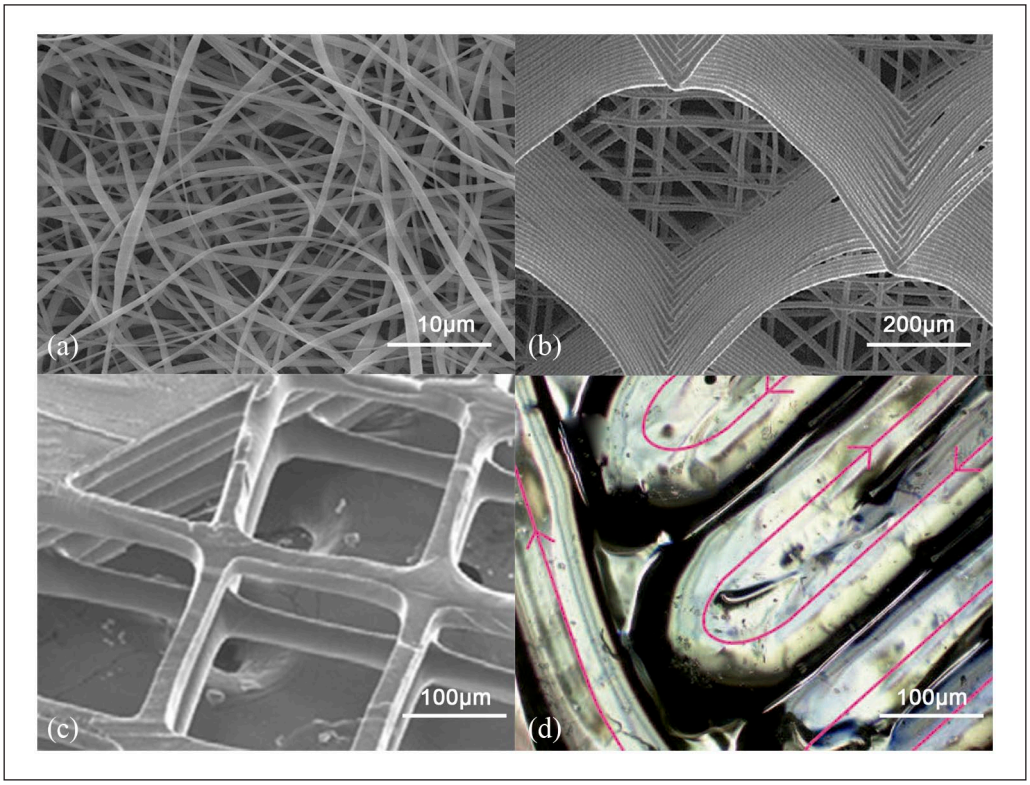

Figure 5. Different 3D printed structures achievable by: (a) Electrospinning. (b) Melt electro-writing. (c) Digital light processing. (d) 3D-bioprinting. Illustrations adapted with permission from Bhullar et al.,[56] Castilho et al.,[53] Soman et al.,[96] and Pourchet et al.,[97] respectively.

instance, it has been proposed the use of scaffolds with nanometric fiber size[125–128] or combined materials[129] for skin healing.

DLP techniques are an illumination method. Their application to additive manufacturing has been growing and becoming common in recent years due to its high performance in terms of resolution, printing speed, scalability, and material versatility, as it can be applied to soft materials like polymers and hydrogels, but also to metals and ceramics.[130–136]

In DLP applied to scaffold fabrication, a digital light projector is used to shine a pattern of light onto a photosensitive material solution, which contains a base-material, such as a hydrogel or a gelatin, and a photoinitiator, which reacts when exposed to the light, thus controlling the base-material solidification.[137] The light pattern is produced by a digital micromirror device (DMD) consisting of an array of micromirrors capable of changing their orientation via micro-actuators controlled by computer that allow them to reflect the light onto the material or onto an outer zone, where it is absorbed to prevent other surface reflections that could affect the precision of the fabrication process.[138] This precise control can be applied to obtain a wide range of shapes and geometries.

Besides the light pattern, other parameters, such as light intensity or wavelength, must also be controlled when using this process depending on the application, the photoinitiator, or the presence of cells, as they could be affected by the nature of the light applied.

The most commonly used polymers in DLP are acrylates, which are a class of monomers that can be polymerized to form polymers through photopolymerization. The use of these monomers is due to their excellent optical properties, low toxicity, and easy handling.

Photoinitiators are compounds that, upon exposure to light, generate free radicals or other reactive species that can initiate a chain reaction leading to the solidification of the material. When the photoinitiator is added to the acrylate, it serves as a catalyst that initiates the photopolymerization process when the acrylate is exposed to light. This process causes the acrylate molecules to form chemical bonds with one another, creating the solid scaffold. The photoinitiator is a crucial component of this process because it ensures that the polymerization reaction starts quickly and efficiently when the acrylate is exposed to light. Without it, the reaction would proceed at a much slower rate, making it difficult to form solid structures. Additionally, the properties of the photoinitiator can be tuned, such as its absorption wavelength, to match the light source and the rate of polymerization to control the polymerization kinetics of the acrylate.

DLP has several advantages over traditional scaffold fabrication methods, such as being able to create complex and highly porous structures with sub-millimeter resolution

and high precision, as well as include gradients of mechanical and chemical properties in the scaffold.

DLP applications in the medical field are vast. In fact, this process has been used for medical devices such as implants, scaffold manufacturing,[118,139,140] or to study biological mechanisms as drug delivery.[141] Furthermore, its application into skin tissue engineering is promising. For instance, Zhou et al. have developed a functional living skin combining this technique with a bioprinting bioink that shows a superior performance in promoting dermal regeneration and also mimicking the physiological structure of natural skin.[142]

Finally, 3D bioprinting techniques are novel and also promising fabrication technologies developed in recent years that has been applied in several biomedical applications, including some encouraging results in skin regeneration studies.[143–150]

3D bioprinting involves a variety of techniques to control the printing process. Some of these techniques use extrusion-based methods, others rely on laser-assisted methods, and stereolithography is also a common option used for different applications.[151–153] Each technique offers a unique set of advantages and disadvantages, allowing researchers to choose the most appropriate approach for their specific application depending on the media deposited, which should also be carefully selected. The media printed in 3D bioprinting are bioinks, which are complex solutions composed of living cells, biomaterials and biological substances that can enhance the viability of the cells in its environment.[154,155]

These techniques allow the creation of geometrically biomimetic constructs suitable for patient-specific applications due to the control over localization and composition of the deposited solution, which can be designed to be similar to native tissue.[97,156]

Among the advantages of these techniques are their speed and efficiency, scalability, high degree of control and precision over each deposition step and the high cell density obtainable. However, they have some disadvantages such as complexity of the factors that must be reproduced and the difficulty in achieving a really close resemblance to the native tissue, due to its high intricacy. Some of the most commonly used biomaterials for 3D bioprinting are gelatin, collagen, and alginate, as these biopolymers are appropriate for mimicking the ECM due to their prominent level of cross-linked fibers and they also have suitable mechanical properties that support their printability.[35,157,158]

In the future, bioprinting is expected to become an increasingly important tool for creating functional, three-dimensional tissue structures that could perceive and respond to their surroundings.[159] Advancements in materials, printing techniques, and bioprinting methods are expected to lead to the creation of more complex and anatomically accurate tissue structures. Furthermore, the integration of bioprinting with other technologies such as stem cell research and bio-fabrication, is expected to enable the creation of functional, living tissue structures that can be used for tissue repair and regeneration. Additionally, the integration of bioprinting with computational techniques, such as computer-aided design and simulation, is also expected to play a significant role, as it has the potential to change the way we think about tissue engineering and regenerative medicine.

Figure 6 presents a schematic diagram of the described fabrication techniques to summarize some of their main features to better understand their potential use for different skin tissue engineering applications.

Conclusions and future perspectives

This review article has provided a comprehensive overview of the current state of research on the use of auxetic constructs for skin healing applications. The unique mechanical properties of auxetic materials, such as negative Poisson's ratio and their ability to accommodate large deformations both uniaxially and biaxially, have been found to have a positive impact on cell infiltration and proliferation,[58–60] making them a promising treatment option for skin tissue engineering scaffolds.

This review has examined various types of auxetic materials that have been tested numerically and experimentally, where studies have shown that auxetic scaffolds could promote wound healing by enhancing the cell microenvironment and being more adjustable to the complex body geometries. Additionally, the review has identified different biomaterials and fabrication techniques available to develop auxetic micro and macro structures, each presenting different application possibilities.

Advanced fabrication technologies such as 3D printing techniques or electrospinning can contribute significantly to the development of auxetic scaffolds. For example, 3D printing enables the fabrication of complex structures with tailored properties, while electrospinning can be used to create nanofibrous scaffolds with high surface area-to-volume ratios.

Computational studies have proven to be an important tool in predicting the mechanical behavior of auxetic structures and designs, aiding in the optimization of their geometry designs and material properties for skin tissue engineering applications. The analysis of auxetic material designs through computational studies also contributes to obtain and adjust key parameters, such as strain rates or stiffness, to mimic the mechanical properties of the native ECM.

Nevertheless, several hurdles and challenges remain in the development and application of auxetic materials. These include the need to improve our understanding of their behavior and mechanical properties under different loading conditions, as well as a proper evaluation of the

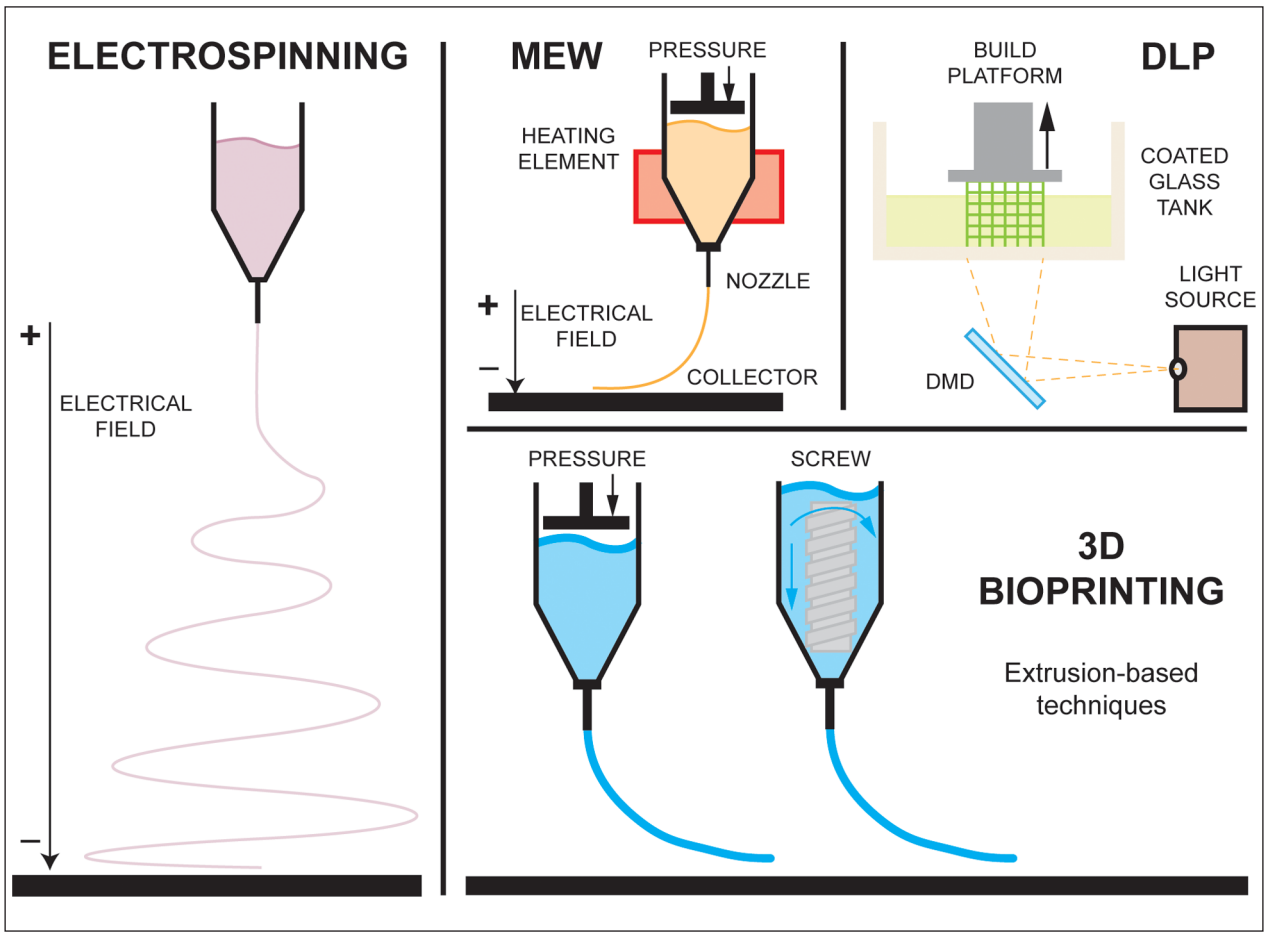

Figure 6. Main features of the advanced fabrication technologies described to manufacture auxetic skin tissue engineered constructs.

biocompatibility and long-term stability of these materials to ensure their safety and efficacy. To this end, relevant experiments would need to be performed, evaluating their quality and performance. These experiments should include tests to measure their tensile and compressive properties or their biocompatibility and degradation behavior. Furthermore, it is important to consider the practical implications of using these materials in a clinical setting, including regulatory compliance, cost-effectiveness, and scalability. This may require conducting extensive preclinical testing, such as in vitro and animal studies, in order to establish the safety of the materials. Also, while these materials have the potential to offer significant benefits over traditional skin grafts, they may also be more expensive to produce due to the use of advanced fabrication technologies or specialized biomaterials, which is intrinsically linked to the ability to manufacture these materials on a larger scale to meet the demands of the clinical market. To address these challenges, researchers and manufacturers may need to explore new approaches to manufacturing that can increase efficiency and reduce costs, for example using automation or high-throughput processing methods that may help to streamline the production process and reduce both time and costs involved.

In conclusion, the use of auxetic materials in skin tissue engineering holds great promise, and advanced fabrication technologies are rapidly advancing this field. Nonetheless, a more comprehensive understanding of their properties and behavior, coupled with well-designed experiments, is needed to overcome remaining hurdles and facilitate their successful translation into clinical applications.

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