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Understanding and utilizing textile-based electrostatic flocking for biomedical applications

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ABSTRACT

Electrostatic flocking immobilizes electrical charges to the surface of microfibers from a high voltage-connected electrode and utilizes Coulombic forces to propel microfibers toward an adhesive-coated substrate, leaving a forest of aligned fibers. This traditional textile engineering technique has been used to modify surfaces or to create standalone anisotropic structures. Notably, a small body of evidence validating the use of electrostatic flocking for biomedical applications has emerged over the past several years. Noting the growing interest in utilizing electrostatic flocking in biomedical research, we aim to provide an overview of electrostatic flocking, including the principle, setups, and general and biomedical considerations, and propose a variety of biomedical applications. We begin with an introduction to the development and general applications of electrostatic flocking. Additionally, we introduce and review some of the flocking physics and mathematical considerations. We then discuss how to select, synthesize, and tune the main components (flocking fibers, adhesives, substrates) of electrostatic flocking for biomedical applications. After reviewing the considerations necessary for applying flocking toward biomedical research, we introduce a variety of proposed use cases including bone and skin tissue engineering, wound healing and wound management, and specimen swabbing. Finally, we presented the industrial comments followed by conclusions and future directions. We hope this review article inspires a broad audience of biomedical, material, and physics researchers to apply electrostatic flocking technology to solve a variety of biomedical and materials science problems.

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NOMENCLATURE

a	Fiber acceleration
A_s	Surface area of substrate
C_0	Fiber drag coefficient
d_f	Diameter of fiber in m
E	Strength of electric field in V/m
g	Gravitational vector
K	Air drag coefficient
n_f	Number of flock fibers in field view
m	Fiber mass
q	Accepted charge
qE	Coulombic Driving Force
q_0	Theoretical max charge in Coulombs
t	Time (seconds)
v	Air flow field velocity
V_f	Total volume of all fibers in area of interest
V_s	Volume of nominal fiber
x_1	First x-coordinate of single fiber
x_2	Second x-coordinate of single fiber
y_1	First y-coordinate of single fiber
y_2	Second y-coordinate of single fiber
β	Fiber conductivity in S/m
γ_L	Kinematic viscosity of air
ε_0	Permittivity of free space
ε_r	Dielectric constant of fiber
ι	Length of fiber in m
ρ_f	Flock fiber density
ρ_L	Specific density of air
τ	Time constant of fiber charging
ϕ	Porosity
Ω	Resistance in Ohms

Abbreviations

AC	Alternating Current
Ag	Silver
AgNP	Silver nanoparticles
Au	Gold
BG	BioGlass
$CaCl_2$	Calcium Chloride
CHS	Chitosan
Cu	Copper
DC	Direct current
ECM	Extracellular matrix
EDC	(1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride)
FeCl3	Iron (III) chloride
GA	Gluteraldehyde
Gel	Gelatin
GPa	Gigapascals
HCl	Hydrochloric acid
hHSIL	Histological intraepithelial lesion
hMSC	Human mesenchymal stem cells
HTAB	Hexadecyltrimethylammonium bromide
MPa	Megapascals
MRSA	Methicillin-resistant Staphylococcus aureus
NaCl	Sodium Chloride
PA	Polyamide
PCL	Polycaprolactone
PEO	Polyethylene oxide
PGLA	Poly(glycolide-co-L-lactide)
PLA	Polylactide/Polylactic Acid
PLGA	Poly(lactic-co-glycolic-acid)
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PU	Polyurethane
PVP	Polyvinylpyrrolidone
RH	Relative Humidity
Saos-2	Sarcoma osteogenic cells
SARS-CoV-2	Sever acute respiratory syndrome coronavirus 2
SPIONs	Superparamagnetic iron oxide nanoparticles
TE	Tissue Engineering
US	United States
USP	US Pharmacopeia
UTS	Ultimate tensile strength
YM	Young's Modulus
Zn	Zinc
3D	Three-dimensional

I. INTRODUCTION

As a well-established textile finishing technique, modern electrostatic flocking (flocking) has been around for decades, with the first US patent awarded in 1864 and the modern flocking patent awarded in 1945.[1,2] Flocked products can be found in a variety of industries, including automotive, cosmetics, filtration, materials, fabrics, decorations, medical, and tissue engineering (Fig. 1).[1,3,4] Despite the length that flocking has been used in textile industries, little literature discussing the technical requirements to produce flocked materials exists. Flocking is a multi-component system that is comprised of flock fibers, fiber coatings, adhesives, substrates, electrostatic generators, and flocking boxes or charging electrodes.[5,6] As a general overview, flocking fibers are distributed atop an electrode or within a charged sieve box where an electrostatic generator produces an electric field, driving flock fibers (short individual fibers) toward a grounded electrode where an adhesive-coated substrate is affixed. After flocking, the voltage is reduced, the substrate is removed, and loose fibers are washed or brushed away and the adhesive is allowed to cure. Flocked surfaces are left with a coating of perpendicularly aligned fibers that give a velvety finish.

Given its flexibility as a coating platform, a wide range of fibers, adhesives, and substrates can be easily used for flocking.[7] Additionally, both top-to-bottom [Fig. 2(a)] and bottom-to-top[Fig. 2(b)] flocking setups are common, allowing for the coating of a variety of surfaces in a multitude of manufacturing methods.[1,5] Further, both alternating

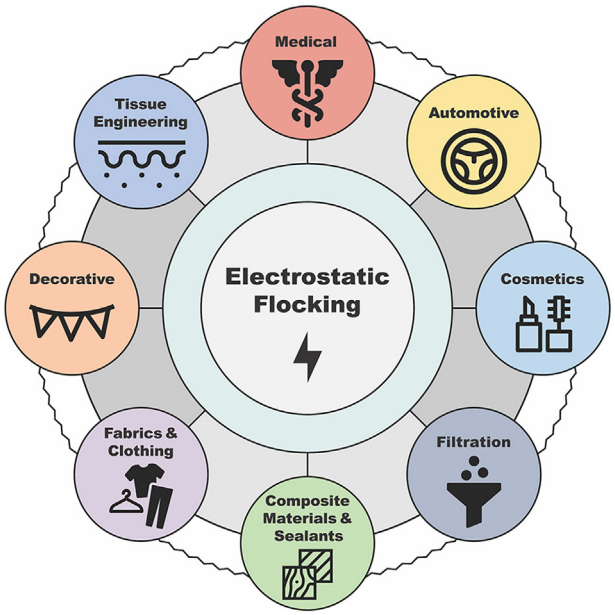

FIG. 1. Industries and applications utilizing electrostatic flocking.

current (AC) and direct current (DC) flocking setups are used although DC flocking setups are generally more common given their safety and ease of use. Applying flocking to biomedical sciences may prove a useful tool in creating 3D implantable structures or functionalizing the surfaces of existing structures. While the fundamental principles of flocking remain the same when applied for biomedical applications, there are additional design considerations that should be evaluated.[8] In this review, the theoretical framework, components, considerations, and applications of electrostatic flocking for biomedical applications will be examined.

Since the mid 1900s, electrostatic flocking has been utilized as a unique textile application to deposit a layer of vertically aligned fibers atop a designated substrate, generating a velvety surface of parallel fibers.[9–12] Generally, flock fibers are applied to a wetted, adhesive-coated surface via an electrostatic current. Flock fibers are generally considered small, synthetic, or natural short (microns to millimeters) fibers with electrically conductive surfaces or finishes. The flocking process can be broken down into distinct, sequential steps: pretreating the substrate, applying an adhesive to the substrate surface, flocking short fibers, curing, and cleaning the flocked material.[6] While there are several types of flocking methods, such as AC electrostatic, mechanical, and air-assisted techniques, this review will focus mainly on DC electrostatic flocking given its ease of use and broader application for biomedical-specific applications.[1,4] In order to best apply flocking to biomedical applications, it is best to first understand the theoretical framework and physics that underly the flocking process. Section II will outline the basic principles required to understand flocking.

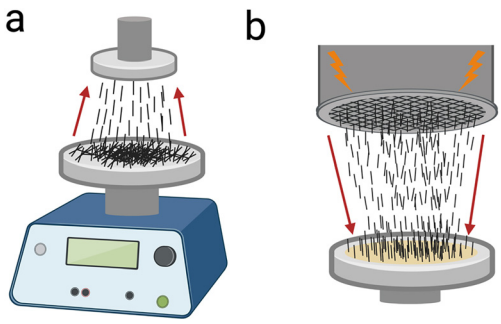

FIG. 2. Schematic illustration of electrostatic flocking set-ups with a (a) bottom-to-top configuration using a charging electrode and (b) a top-to-bottom configuration using a charged sieve box. Schematics inspired by excerpts and reproduced with permissions from Kim, Specialist yarn and fabric structures, in *Specialist yarn and fabric structures*, edited by R. H. Gong (pp. 287–317). Copyright 2011 Elsevier.[1]

II. PRINCIPLES OF ELECTROSTATIC FLOCKING

A. Flock fiber mechanics

Consider a DC electrostatic flocking setup using a two-electrode, bottom-to-top system, with flock fibers (fibers that have already been milled to a desired, uniform length) evenly covering a positively charging metal electrode.[1] As a high DC voltage is applied to the electrode, the surface of the fibers accumulate charges from the electrode. Positive charges move along the surface of the fibers, accumulating as time passes. The following equation lays out the mathematical framework for the direct charging of flocking fibers that remain in contact with a charged electrode:[13]

Fiber charging,

$$q = q_0[1 - e^{-t/\tau}]. \tag{2.1}$$

Time constant of fiber charging time,

$$\tau = \frac{\varepsilon_0 \varepsilon_r}{\beta}. \tag{2.2}$$

Theoretical maximum charge,

$$q_0 = \frac{\pi \cdot \varepsilon_0 \cdot l^2 \cdot E}{\left[\ln\left(\frac{4 \cdot l}{d}\right) - 1\right]}. \tag{2.3}$$

Here, q is the amount of charge a fiber accepts over t s in contact with the charging electrode, and q_0 denotes the theoretical maximum charge in Coulomb's. τ is the time constant of charging time of the fiber, which is further explained in Eq. (2.2), where ε_r is the dielectric constant of the fiber, ε_0 is the permittivity of free space $= 8.85 \times 10^{-12}$ F/m, and β is the fiber conductivity in S/m.[1] The maximum theoretical charge is described in Eq. (2.3), where E is the strength of the electrostatic field in V/m, l is the length of an individual fiber in m, and d is the cross-sectional diameter of a flock fiber.[1] As the equations demonstrate, the conductivity, geometry, and electrode contact time dramatically influence flock fiber flight in an electric field. That is to say, the longer a voltage is applied, the more charge accumulates on the fibers until they leave the charging electrode and move toward the grounded electrode. Additionally, the conductivity of a fiber dramatically impacts its ability to move and accumulate charges. For practicality, fibers should be conductive, insuring they accumulate a sufficient charge in a short amount of time. To highlight the importance of conductivity, consider a piece of untreated polylactic acid (PLA)

($l = 0.5 \times 10^{-2}$m, $d = 5.05 \times 10^{-5}$m, $\varepsilon_r = 3.25$, $\beta = 10^{-17.3}$S/m) at room temperature and in contact with a charged electrode ($E = 4 \times 10^5$V/m).

Here, the maximum theoretical charge, $q_o = 1.116 \times 10^{-8}$C, and charging time, $\tau = 5.739 \times 10^6$s, illustrate how low fiber conductivity quickly render certain polymers insufficient for flocking. Conversely, consider the same piece of PLA treated with a fiber finish composed of conductive materials that lowers the conductivity to $\beta = 10^{-6}$ S/m. The addition of a conductive treatment in this case would bring the charging time, τ, down to 2.876×10^{-5} s. This example demonstrates that perhaps the most important feature to consider for flocking is the conductivity of the fibers. Therefore, surface coating is often necessary to achieve flockability. Second, the time constant of charging is inversely proportional to the conductivity of the fiber or its finish. Finally, the time constant of charging is proportional to the dielectric constant of a fiber.[1] In summary, fibers with higher conductivity must charge for a shorter amount of time and are therefore more desirable for flocking applications.

While the goal of flocking is to orient fibers perpendicular to a substrate, the lines of electric fields are not typically completely perpendicular to the substrate. Nonetheless, as the electrostatic field is applied, the charge accumulation on the fibers creates a dipole that is oriented and lifted by a phenomenon known as Coulomb interactions—whereby repulsion or attraction between differentially charged particles occurs. The charged fibers travel along the lines of the electrostatic field until they enter the adhesive layer.[1]

When considering the charge shielding and electrostatic disturbances a large pile of flock fibers may have with flight patterns, it is more advisable to (1) uniformly distribute fibers atop the charging electrode to promote uniform charging and (2) slowly increase the voltage on the electrostatic generator. If voltage is suddenly applied via a switch rather than slowly with a dial, the fibers may rise as aggregates rather than as individual fibers.

A simple model for flock fiber motion during flight is described by Kleber and Marton in the following equation:[14]

Flock motion in flock zone,

$$ma = qE - Kv + mg. \tag{2.4}$$

Air drag coefficient,

$$K = \frac{C_0 \pi \rho_L (\gamma_L d_F)^{1/2} l_F}{2}. \tag{2.5}$$

Here, a is fiber acceleration, E is electric field, g is gravitational acceleration, v is air flow field, and K is air drag coefficient. Equation (2.5) describes air drag coefficient, where ρ_L is the specific density of air, γ_L is the kinematic viscosity of air, d_F is fiber diameter, l_F is fiber length, and C_0 is fiber drag coefficient (which must be determined experimentally).[14] During flocking, the Coulomb interaction (qE) greatly exceeds mg, and the fibers may move against gravity, aligning during flight.[15] In work from an unpublished thesis, Hou sought to investigate the velocity profiles of nylon flocking fibers and found that, due to external forces not incorporated into theoretical models, the actual velocity profile varied dramatically from the theoretical model.[16]

B. Charging fibers for flocking

As evident by Eq. (2.1), the transfer and accumulation of charges on the surface of a flock fiber is the single most important electrochemical aspect to a successful flock. Generally, three main methods are used for charging flock fibers: contact charging, corona discharging, and tribocharging.[1] Contact charging is simply conduction or the transfer of charges from one charged object to an uncharged object.[17] Here, the fibers act as conductors, receiving charges from the positively charged electrode [Fig. 3(a)]. Contact charging is arguably the easiest and most flexible approach, and Kim recommends using contact charging on conductive and semi-conductive fibers with surface resistivities ranging between 10^{-4} and 10^{-8} Ω.[1] Another technique, corona discharging, is a charging technique that relies on the ionization of air around an electrically charged conductor.[18] In the case of a top-to-bottom flocking technique where fibers are sifted through openings in a

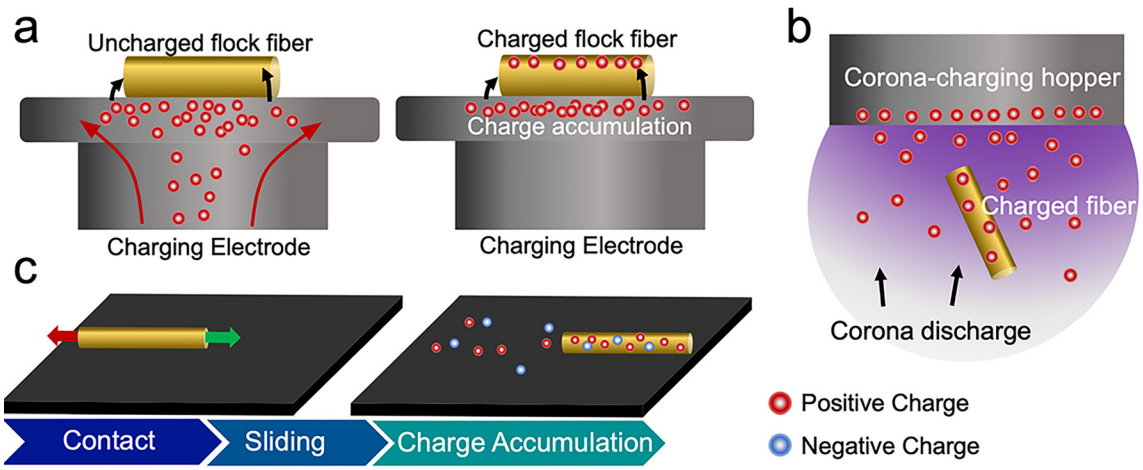

FIG. 3. Charging methods used to impart surface charges onto flock fibers. (a) Contact charging on a plate electrode showing movement of charges (left) and accumulation of charges (right), (b) corona discharge, and (c) tribocharging, where fibers make contact with and slide along a surface (left) to accumulate mix of positive and negative charges (right). Schematics inspired by excerpts and reproduced with permission from Kim, Specialist yarn and fabric structures, in *Specialist yarn and fabric structures*, edited by R. H. Gong (pp. 287–317). Copyright 2011 Elsevier.[1]

charged sieve, the corona discharge between meshes imparts a charge onto the fibers in passing, thus allowing them to rapidly accumulate charge before entering the electric field [Fig. 3(b)]. The corona charging method may be suitable for flocking from a top-to-bottom approach or on dielectric fibers with surface resistivities between 10^{-4} and 10^{-16} Ω.[19] Tribocharging is a form of conductive charging that uses the buildup of static electricity via friction between two materials with differences in surface electric potentials.[20] For example, fibers with lower concentrations of charges on their surface may be rubbed against a material with a high concentration of charges, resulting in a physical transfer of charges [Fig. 3(c)]. Kim cites this method as being most effective for charging electrically insulating fibers with surface resistivities between 10^{8} and 10^{18} Ω. Tribocharging does not require any voltage supply and may be broadly applicable to a host of biocompatible fibers [such as polycaprolactone (PCL) or polylactic acid (PLA)], given their electrical insulative nature.[15,21] Combining tribocharging and contact charging may enable fibers with very low conductivities to be flocked. Here, fibers would have to be mechanically tribocharged and contact charged by rapidly successive steps.

C. Flock density & porosity

Flock density, or simply the number of fibers in a given area, can be controlled by several factors during electrostatic flock assembly. Bershev and Lovova used a differential equation to relate flock density to flock time and fiber dosing.[22] Simply put, flock fiber density increases positively with the amount of time an electric field is present and with the mass of fibers loaded onto the charged surface. It is useful to note, then, that flock density can be controlled by adjustive the electric field intensity, or by changing the mass of loaded flock fibers. This concept was recently demonstrated by flocking 1 mm or 3 mm long polyamide fibers at 60 kV for 5 s or 15 s.[23] Densities for 1 mm fibers flocked for 5 s and 15 s were 72 ± 11.2 fibers/mm^2 and 104.5 ± 10.7 fibers/mm^2, respectively. For fibers 3 mm in length flocked for 5 s and 15 s, densities were 11.4 ± 1.8 fibers/mm^2 and 21 ± 3.3 fibers/mm^2, respectively [Fig. 4(a)–4(d)].[23] Desired flock densities vary dramatically based on applications, especially in biomedical applications.[24–27] For example, a flocked swab should have a high flock density in order to achieve a high surface-to-volume ratio. Conversely, if a porous anisotropic biological material is to be mimicked, lower flock density may be preferred as to better allow cells to infiltrate into and proliferate throughout flock fibers. A very simple equation can be used to express flock density:

Flock fiber density,[28]

$$\rho_f = \frac{n_f}{A_s}, \tag{2.6}$$

where ρ_f is the flock fiber density, n_f is the number of fibers in field view, and A_s is the surface area in field view.[28] Flock fiber density can be easily observed using simple microscopic imaging and counting. Flock fiber density also relates to porosity, with porosity negatively corresponding to flock fiber density. Walther proposed a very simple mathematical equation to estimate the porosity of a flocked scaffold using the following equation:[23]

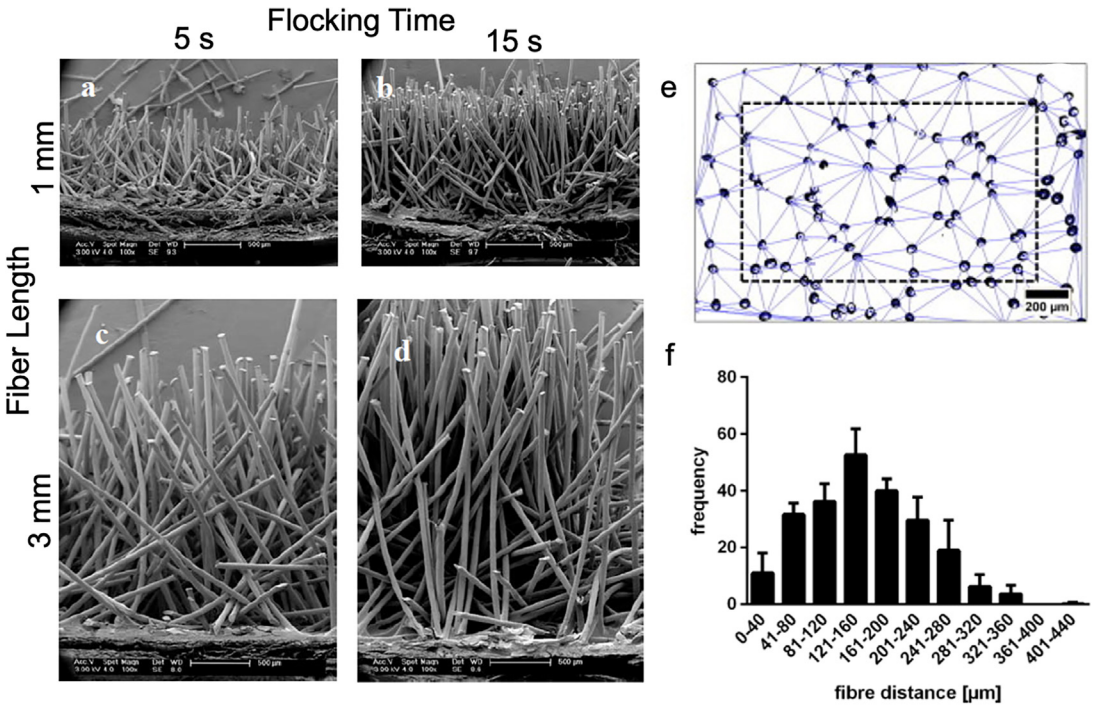

FIG. 4. Characterization of porosity and fiber density of flocked scaffolds. (a–d) SEM images of flocked scaffolds using 1 mm and 3 mm fibers for 5 s and 15 s illustrating differences in fiber density. (e) Delaunay–Voronoi triangulation used to calculate the Euclidian distance between adjacent chitosan fibers. Reproduced with permission from Walther *et al.*, Materials. **5**, 540 (2012). Copyright 2021 MDPI.[23] (f) Frequency distribution plot of the Euclidian distance between adjacent chitosan fibers. Reproduced with permission from Gosssla *et al.*, Acta Biomater. **44**, 267 (2016). Copyright 2016 Elsevier.[27]

Porosity,

$$\phi = \frac{V_s V_f}{V_s}, \tag{2.7}$$

where V s is the approximate volume of a geometrically equivalent shape, given by average fiber length and diameter of the flocked substrate, and V_f is the volume of all fiber flocked on the scaffold, which can be estimated by finding the volume of a single fiber and multiplying by the area covered by fibers. Walther additionally pointed out that the length of the fibers contributed to both density and porosity. Fibers that were 3 mm in length had lower densities and higher porosities than fibers with 1 mm.

One simple method to calculate fiber density and the average distance between fibers (fiber packing) can be accomplished by taking a cross-sectional image of the flocked surface with a scanning electron or standard microscope and calculating the Euclidian distance between fibers.[28] To accomplish this, four coordinates of each fiber can be taken using the Delaunay–Voronoi plugin on the widely used and publicly available ImageJ. Analyzing the Delaunay Triangulation using the plugin outputs the total number of selected fibers and their coordinates [Fig. 4(e)]. From here, the following equation can be used to calculate the distance between neighboring fibers, which is easily expressed as a distribution frequency [Fig. 4(f)]:

$$\text{Euclidian distance (d)} = \sqrt{(x_2 - x_1)^2 (y_2 - y_1)^2}. \tag{2.8}$$

Understanding the working theories behind electrostatic flocking, such as charging, fiber flight, flock time, DC electrostatic finishing, flock fiber density, and porosity, will enable researchers unfamiliar with textile engineering principles to explore electrostatic flocking for biomedical applications. To date, few research articles using electrostatic flocking for biomedical applications have been published, perhaps due to the limited information surrounding electrostatic flocking. Nonetheless, many biomedical applications using electrostatic flocking exist and necessitate not only the basic considerations for traditional electrostatic flocking but must fulfill a variety of other requirements for use *in vitro* and *in vivo*.

III. TUNING FLOCKING FOR BIOMEDICAL APPLICATIONS

The basic principles of electrostatic flocking are relatively simple; however, adjusting flocking for use in biomedical applications [specifically related to tissue engineering (TE) and regenerative medicine] adds a layer of complexity. Biocompatibility, mechanical stability, toxicity, and biodegradation are all essential considerations in selecting materials for flocking.[29] Considering biological requirements for flocking, the fibers, substrates, and adhesives must be resorbable, compatible with target tissues, immunologically inert, and should match native tissue architectures.[27,29] One specific biomedical application that may benefit from electrostatic flocking is TE. In TE, materials are designed to regenerate, heal, or substitute for damaged or diseased tissues.[30] Since flocking can create 3D structures while maintaining extremely high porosities, they may serve as ideal tissue scaffolds or tissue constructs.[31] To date, Gelinksy and colleagues have improvised biological substituents for each component of a flocked scaffold using pure chitosan.[27,28] That is, the fibers, adhesive, and substrates are comprised or formed from chitosan only. Additionally, Boccaccini and colleagues used bioactive glass materials as flock substrates.[26] Most recently, McCarthy *et al.* flocked poly(ε-caprolactone) (PCL) fibers onto electrospun nanofibrous membrane substrates, 3D-printed scaffolds, and self-folding conduits.[32] Other possible substrates suitable for biomedical flocking include collagen or fibrin-based membranes and native bone or skin samples. Biocompatible adhesives based on gelatin, chitosan, and a blend of both chitosan and gelatin solutions have been previously reported.[26,27,32] However, many adhesives have been reported for biological applications, such as tannic acid and polyethylene glycol solutions, fibrin glues, cyanoacrylate derivatives, and polydopamine-based ceramics.[33–36] Adhesives with tunable biodegradation can be considered and applied based on intended use of the flocked scaffolds. Finally, biocompatible fibers are arguably the most important aspect of flocked scaffolds to consider. Other than being biocompatible and biodegradable, fibers should have excellent mechanical properties that offer structural support in damaged regions of tissue. Fibers should have excellent wettability to allow cellular adhesion. Eventually, porous, coaxial, or yarn-based fibers may be applied as flock fibers that can deliver drugs or bioactive scaffolds. Finding suitable flock components for various biomedical applications will make flock technology a widely applicable biological therapy. Sections III A–III D will outline considerations and approaches for engineering flocked devices for biomedical applications.

A. Generating flock fibers for biomedical applications

Since the bulk of the mechanical properties of a flocked material is determined by the fiber morphology, fiber preparation is perhaps one of the most important considerations for making flocked tissue scaffolds.[23] Many methods exist for creating fibers from a variety of materials, and industrially produced flocking fibers are readily available for purchase. While fibers can simply be purchased from flock fiber suppliers and have even been investigated as fibers for flocked TE scaffolds (flock scaffolds), they offer little clinical translation as they are not degradable, biocompatible, and contain coatings with unknown ingredients.[3,8] Therefore, designing a feasible system to produce a large amount of biodegradable, biocompatible, and cuttable fibers is a major hurdle in creating flock scaffolds.

1. Selecting fiber materials

In order to be a suitable flocking material for TE, a variety of characteristics must be met. Narrowing down the scope of usable materials can be accelerated by applying exclusion criteria from known biopolymers. First, the fiber material should meet all the requirements needed for TE scaffolds: biodegradable, biocompatible, immunologically inert, and mechanically stable under physiological conditions. Second, fibers should meet criteria required for electrostatic flocking: high melting point as to not deform during cutting, capable of accumulating charge with surface resistivity ranging from 10^6 Ω to 10^8 Ω, and hydrophilic to retain moisture and enable wetting with adhesives and fiber finishing.[1] Synthetic fibers such as rayon, polypropylene (PP), and polyamide (PA) are suitable for flocking, but do not degrade *in vivo*, whereas polymers such as polylactide and PCL are biodegradable; they are not inherently suitable polymers for flocking as they have surface resistivities far exceeding the flockable range.[21] Investigating known biopolymers with relatively high melting points should be the primary criteria, and considering the ability of the

polymer to be modified with conductive fillings or coatings should be the second exclusionary consideration. To date, only Gelinsky and colleagues and McCarthy *et al.* have reported the flocking of degradable fibers using wet spun chitosan and PCL, respectively, although they do not report or directly measure the conductivity of such fibers.[27,28,32]

2. Producing continuous fibers

Using textile techniques for TE is not a new concept. In fact, many different methods of forming yarns, fibers, woven and nonwoven textiles, and fabric-based TE scaffolds have been reported.[37–41] To this end, several methods for creating continuous fibers exist that offer the means to produce fibers suitable for flocking. Both melt spinning and wet spinning offer versatile platforms for producing continuous monofilament fibers from a variety of polymers.[42,43] Wet spinning consists of a polymer-solvent solution that is loaded into a syringe and extruded directly into a coagulation bath where phase separation rapidly solidifies the polymer for take-up [Fig. 5(a)]. Wet spinning allows for both hydrophilic and hydrophobic fibers to be produced, but utilizes solvent systems that may induce cytotoxicity.[42] Gossla *et al.* and McCarthy *et al.* both utilized wet spinning to create chitosan and PCL fiber tows, respectively.[27,32] In melt spinning, a heating reservoir is loaded with polymer pellets and is heated past the melting point of the polymer. Next, an external force (typically pressure) extrudes the molten polymer out of a spinneret, where it is subsequently cooled, stretched, and taken up on a roller [Fig. 5(d)].[44] Melt spinning allows for a simple setup with minimal variable parameters although its fiber production is often much slower and it may not be ideal for polymers with high melting points.[45] In addition to monofilament fibers, nanofiber-based yarns may also serve as suitable flocking fibers that offer different properties due to their multifilament composition. In dry-phase yarn formation, an electrospinning setup with a rotating funnel-shaped collector forms a cone-shaped web of nanofibers that can be anchored to a take-up roller and collected, or fibers are simply allowed to self-assemble into yarns and are directly taken up with a collector [Fig. 5(b)].[31,37] In wet-phase yarn formation, a polymer solution is loaded into a syringe with a positively charged needle tip suspended above a coagulation bath with a submerged grounding electrode. As the polymer solution is sprayed toward the electrode, a web forms on top of the coagulation bath, and a take-up roller winds nanofiber yarns off the surface of the bath [Fig. 5(c)].[46]

Many methods used may be employed to form continuous fibers for flocking and the fabrication method should be selected based on the desired size and function of the fibers. For TE, fiber diameters play a significant role in the material property and cell response. For example, fiber diameters seem to play an important role in cell orientation and mobility. For fibers with larger diameters, cell orientations may be increasingly random, while migration along a desired direction is slowed.[47] Additionally, the stiffness and elasticity of fibers should be considered in the design process as certain tissues are subject to different stresses and fibers may need to be flexible or brittle, depending on their intended use.[48,49]

3. Preparing flock fibers from continuous fibers

After collecting fibers on a take-up roller, a tow of fiber must be produced for cutting. Tows are best described as untwisted bundles of continuous fiber filaments and are made by removing fibers from

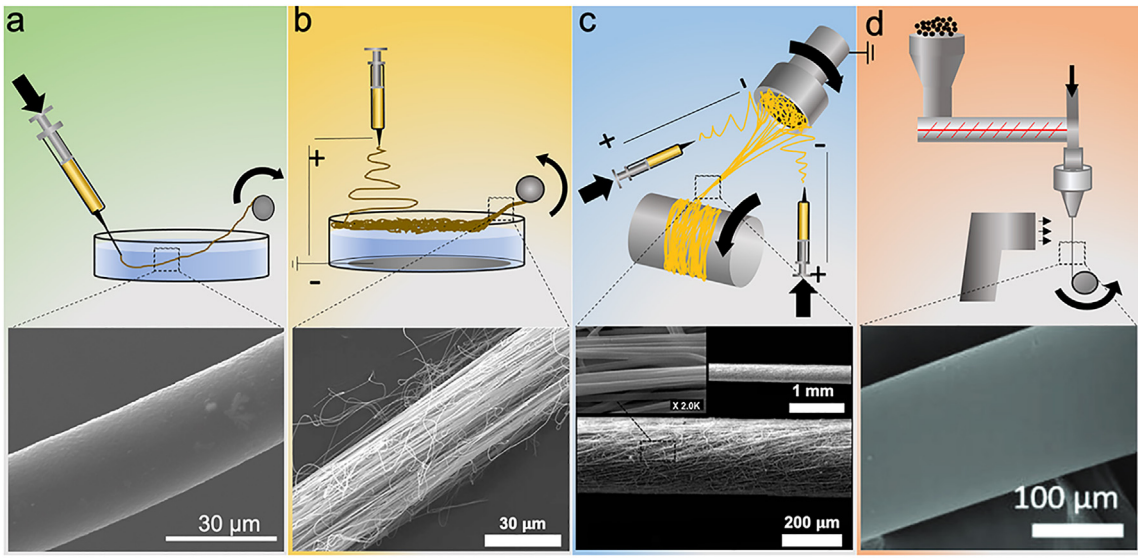

FIG. 5. Methods used to create microfibers suitable for electrostatic flocking and their accompanying SEM micrographs. Schematics inspired and adapted from Ali *et al.*, Electrospinning of continuous nanofiber bundles and twisted nanofiber yarns, in *Nanofibers – Production, Properties and Functional Applications*, edited by T. Lin (pp. 154–166). Copyright 2011 IntechOpen.[170] (a) Wet spinning. Reproduced with permission from McCarthy *et al.*, Adv. Healthc. Mater. **10**, 2100766 (2021). Copyright 2021 Wiley.[32] (b) Wet electrospinning. Reproduced with permission from Smit *et al.*, Polymer **8**, 2419 (2005). Copyright 2005 Elsevier.[46] (c) Yarn electrospinning. Reproduced with permission from Wu *et al.*, Acta Biomater. **62**, 102 (2017). Copyright 2017 Elsevier.[40] (d) Melt spinning. Reproduced with permission Zhao *et al.*, Adv. Funct. Mater. **28** (2018). Copyright 2018, Wiley.[44]

take-up rollers and mechanically stretching.[50] Subsequently, fiber tows are fed into cutting devices to be chopped into flock fibers. In order to cut fibers uniformly, either vertical or rotary cutting machines can be used.[1] In vertical cutting devices, such as the Pierret P26 [Fig. 6(a) i], fibers are fed directly into the path of a blade which moves up and down at high speeds [Fig. 6(a) ii].[51] Vertical cutting allows for adjustments in blade angle and is capable of shorter cut lengths. Furthermore, blades can be finely adjusted during the run which helps to prolong blade life. With really fine deniers or extreme prolonged blade life, fusion might take place which could require an aggressive agitation of the fibers to break up any fusion. Rotary cutters, such as the Van der Mast Fiber Chopper Chopcot® T6, use circular blades to slice through tow [Fig. 6(b) i-ii].[52] Although rotary cutters are used regularly in industry settings, vertical cutting is more cost-effective and a better-suited candidate for cutting biomedical-grade flock given its ease of use and maintenance. Resultant flock fibers prepared using industry standard methods are typically uniform in length (±15%–30%) and allow for precision cutting to generate uniform fibers of a variety of polymer materials [Figs. 6(c) and 6(d)]. Despite the high precision enabled by utilizing industry fiber cutters, they require a large amount of polymer to utilize and may not be suitable for lab use. To this end, a unique method that may be suitable in laboratory settings is reported by Cole (2016) and consists of freezing fibers which are highly aligned and cutting them on a cryostatic device [Fig. 6(e) i-iii].[53] Freeze drying or filtering with water allowed for a high yield.[53] Cole's method may be suitable for thermoplastic polymers as cryostatic cutting occurs under low temperatures. After cutting, resulting fibers should be collected and stored in a humidity-controlled room (RH = 50%–65%) to retain moisture, thus ensuring a conductive, wettable surface for DC electrostatic finishing to be applied.[54]

Complications with cutting brittle fibers may be uneven breaks along the cut line. Complications with cutting elastic or thermoplastic fibers may be tip fusion (sintering) or dog-boning (flattening of fiber tips). During cutting, blades can become very hot from friction and begin melting fiber tips. When this is the case, a post-cutting step may be necessary to separate fibers that may have sintered and fused along the cut line. Fibers may be separated by sonication, shaking, vigorous bubbling, or mechanical sieving.[55,56] McCarthy *et al.* describes a method to produce PCL flock fibers which includes a separation step utilizing probe tip ultrasonication and air bubbling.[32]

4. Flock fiber finishing

Perhaps the most important, and underreported, aspect of electrostatic flocking is DC electrostatic coating. For fibers to flock well, they must exhibit bulk semi-3 conductivity or surface conductivity. Historically, electrostatic finishes are applied to fibers after they are cut. Precise fiber finishing chemistry is important to not only enable fiber flight but prevent fibers from sticking to each other. Inorganic salts, ions, electrolytic solutions, or other coatings that may enhance conductivity tune a fiber's surface conductivity to an acceptable level for flocking.[1] Using throw spraying, mixing, or dip-coating, DC electrostatic finishes can be applied to batches of fibers to ensure uniformity. Ingamells *et al.* examined three different kinds of flock finishes and compared the quality of the finish.[54] Here, three finishes—cationic, anionic, and nonionic—were applied. Nonionic (paraffin wax) finishes demonstrated the highest flock activity (~0.15/2.0 g fibers lifted), followed by anionic (fatty alkyl sulphate) and cationic (fatty acid condensate) (~0.05/2 g fibers lifted and 0.03/2 g fibers lifted), respectively [Fig. 7(a)]. Another key aspect that Ingamells *et al.*

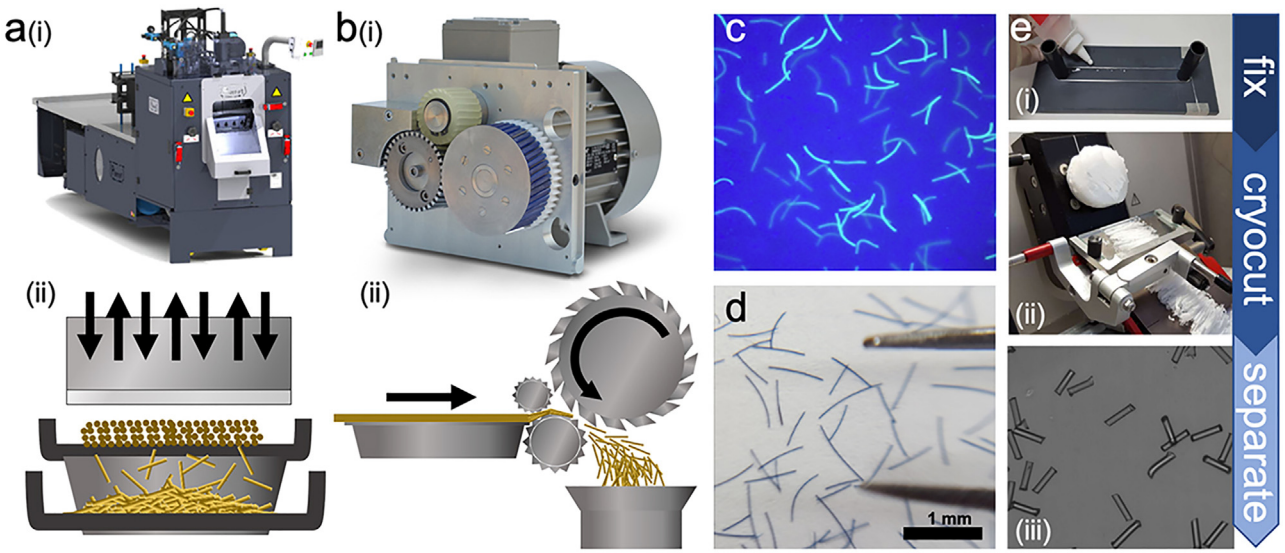

FIG. 6. Machines and representative schematics for preparing flock fibers from continuous fiber tow: (a) (i) Pierret P26 vertical precision short cut fiber cutting machine and (ii) cutting mechanism employed by vertical cutting. Photograph supplied and reproduced with permission from Pierret International. (b) A Van der Mast Fiber Chopper Chopcot® T6 rotary short fiber cutter and (ii) rotary cutting mechanism of action. Photograph supplied and reproduced with permission from Van der Mast. (c) An example of precision short-cut fluorescent flock fibers and (d) longer standard flock fibers prepared by Spectro Coating Corp. Photographs supplied and printed with permission from Spectro Coating Corp. (e) A novel, lab-appropriate technique to prepare thermoplastic flock fibers that uses (i) aligned fiber freezing, (ii) cryocutting, (iii) and water separation. Reproduced with permission from M. Cole, Sci. Rep. **6**, 34519 (2016). Copyright 2016 Nature Publishing Group.[53]

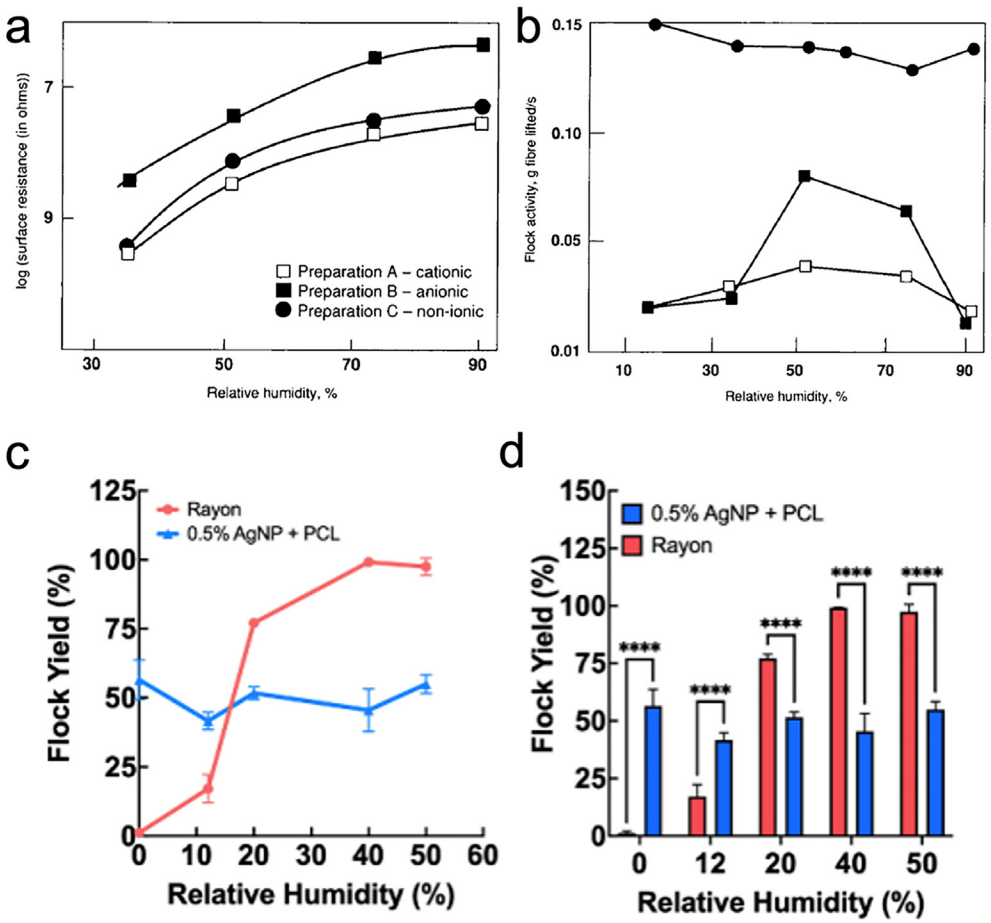

FIG. 7. Mechanisms of flocking. (a) Surface resistance as a function of relative humidity (RH) using different fiber finishes. Reproduced with permission from Ramadan and Ingamells, J. Soc. Dye. Colour **108**, 270 (1992). Copyright 1992 Wiley.[54] (b) The flock activity of finished fibers at different RH. (c–d) Rayon and AgNP/PCL fibers flocked at different RH showing different responses to the same RH conditions. Reproduced with permission from McCarthy *et al.*, Adv. Healthc. Mater. **10**, 2100766 (2021). Copyright 2021 Wiley.[32]

demonstrated is the importance of relative humidity (RH). Maintaining high RH (50–90%) is extremely important as moisture deposition on the surface of fibers increases the solubility of conducting salts as well as enhances the critical conductivity. Not surprisingly, increased RH leads to an increase in surface moisture content of the fibers, which in turn, results in increases in flock activity [Fig. 7(b)]. For biomedical applications, though, anionic or cationic finishes common in industry may induce cytotoxicity when used in biomedical applications.

It is reasonable to assume that optimizing electrostatic finishes for biomedical use may open the possibilities for using a wider range of polymers with inherently poor surface conductivities. Considering the low conductivity of polymers used in TE, a simple step can be employed to increase surface conductivity of hydrophobic polymers (such as PCL). Modifying such fibers with oxygen plasma treatment will increase hydrophilicity, which can, in turn, allow for higher moisture retention on the fiber surface.[57,58] Due to the ease of use and processing, oxygen plasma treatment may be a good starting point to improve the flockability of a certain polymer.

Another approach, adding conductive fillers during fiber fabrication, may prove a suitable method by itself or in combination with oxygen plasma treatment. In this case, conductive particles are directly incorporated into the fibers during fiber production. Simply mixing conductive nanoparticles like Ag, Au, Zn, or Cu with a polymer solution would allow for direct wet spinning, electrospinning, and melt spinning of inherently conductive fibers.[32,59–61] Directly incorporating conducting nanoparticles in an insulative polymer can yield an overall semiconductive material through a phenomenon explained by the percolation theory. The percolation theory dictates that conductive particles create a conductive matrix that charges can pass within and accumulate on.[62,63] By directly incorporating conductive agents in the fibers during production, any post-cutting finishes can be avoided altogether. To demonstrate how the percolation theory may be used as a separate, RH-independent mechanism for flocking, McCarthy *et al.* flocked AgNP/PCL fibers and Rayon fibers with proprietary finishes at a range of relative humidities and found that Rayon fibers flocked in a RH-dependent manner, much in agreement with Ingamells *et al.*, but the AgNP/PCL fibers had relatively uniform flock yields, regardless of

RH [Figs. 7(c) and 7(d)].[32,54] Further, McCarthy *et al.* provided a preliminary framework for flocking insulative biopolymers by filling fibers with conductive particles.[32] Three polymer types [PCL, PLA, and Poly(lactic-co-glycolic-acid) (PLGA)] were wet spun with silver nanoparticles (AgNPs), Iron (III) chloride ($FeCl_3$), hexadecyltrimethylammonium bromide (HTAB), sodium chloride (NaCl), superparamagnetic iron oxide nanoparticles (SPIONs), and zinc powder, and demonstrated significantly higher flock yields compared to untreated fibers. While some electrolytic surface treatments require higher humidity content for flocking, incorporation of highly conductive fillers appears to retain flockability at low RHs, which may be a consideration for environments that are usually dry. Additionally, some conductive nanoparticles have relevant therapeutics effects. Zinc nanoparticles, for example, have been shown to enhance dermal wound healing, while Ag nanoparticles have well-documented antimicrobial effects.[64,65] McCarthy *et al.* showed increased antimicrobial efficacy in an AgNP dose-dependent manner released from flock fibers against Methicillin-resistant Staphylococcus aureus (MRSA).[32]

Finally, another approach to improve the electrostatic properties of flocking fibers is direct deposition. Here, conductive metals are deposited onto the fibers' surfaces via sputter-coating, dip-coating, and direct chemical deposition. While sputter coating allows for a range of metals to be deposited on the fibers' surfaces and occurs at low temperatures, deposition occurs only on exposed surfaces, making uniform coating difficult.[66] Direct deposition of conductive metals by chemical reduction may be a suitable method to induce surface conductivity as fibers can be functionalized in batches easily.[67] Nonetheless, there are multiple options available to tune the electrostatic properties of fibers. Using DC electrostatic finishing with inorganic or organic salts, nanoparticle doping, plasma treatments, sputter coatings, and direct chemical deposition can enhance the flocking of a wide range of fibers. Modifying the conductive treatments to the intended biomedical application may increase efficacy and further tune flocked scaffolds.

5. Direct synthesis of conductive fibers

While applying a conductive finish may be a suitable option in some circumstances, directly creating conductive flocking fibers could help to reduce time and material costs during fiber production. For the most part, three options exist by which conductive fibers may be directly produced. First, simply selecting materials with suitable conductivities, such as chitosan or polypyrrole, may be directly spun into microfibers without changing the bulk material properties. However, most polymers used in TE are degradable thermoplastics (PCL, PLA, PLGA), and have extremely high resistivities. In order to reach satisfactory conductive properties, conductive particle doping and/or conductive particle surface deposition may be employed.

Small conductive nanoparticles, such as silver nanoparticles (AgNPs), are highly conductive. Mixing conductive nanoparticles into a polymer solution before microfiber synthesis can incorporate and evenly distribute conductive nanoparticles throughout the bulk of the microfibers, creating a composite material with semi-conductive properties. The mechanism by which doping insulative polymers with conductive particles creates semi-conductive composite materials is explained by the percolation theory. Determining the percolation threshold, or the material transition from insulative to conductive, from a set of equations gives an estimation for the fraction of conductive particles needed to achieve a flockable conductivity. Directly doping conductive nanoparticles into the polymer solutions is easy, fast, but may not be as efficient as depositing conductive particles onto the surface of fibers. In previous studies using pure chitosan, no surface treatments or fillers were reported although McCarthy *et al.* reported the use of several conductive fillers for flocking PCL, PLA, and PLGA.[27,28,32]

B. Adhesive selection for biomedical applications

As one of the three materials composing flocked scaffolds, adhesives play an important role in a flocked scaffold's mechanical stability and fiber bonding. In fact, according to Kim (2011), the performance of a finished flock is determined primarily by the adhesive.[1] Resistance to abrasion and rate of degradation are largely determined by the quality and hold of adhesives. In industrial applications, adhesives are water-based, solvent-based, plastisol, or thermosetting.[1] Although there are a variety of adhesives for industrial application, adhesives intended for biological use must be carefully selected to meet the biological requirements of the implant region. Many biomedical adhesives exist and range dramatically in composition, durability, and use. Weighing the biological and flocking requirements, suitable flocked scaffold adhesives can be selected.

1. Adhesive requirements

Adhesives for flocked scaffolds must meet a variety of requirements. First, they must have adequate wettability as to properly spread over the substrate and form around fibers.[1] The hydrophilicity of an adhesive may be tuned to match that of the fibers and substrates. In addition to proper wettability, adhesives should have sufficient elasticity to form bonds and retain mechanical flexibility after curing. An adhesive that cures to form a solid, brittle layer is not suitable for biological implantation as fracture can easily occur, compromising the structure of the scaffold. Additionally, adhesives should biodegrade in tandem with the scaffold. Perhaps the biggest factor necessitating development of new biocompatible flock adhesives is cytotoxicity during the life of the scaffold and after degradation. To date, only one type of flock adhesive has been tested *in vivo*, and its suitability for human use is not yet ascertained.[32] Finally, the adhesive must have enough electrostatic conductivity to meet the requirements of a normal flocking fibers. Generally, medical grade adhesives must fulfill the requirements of two standard tests: U.S. Pharmacopeia (USP) Class VI and ISO 10993.[68]

2. Synthetic adhesives

Many medical adhesives that fulfill the requirements set forth by UPS Class VI and ISO 10993 exist although no literature exists documenting their use as a flock adhesive. Epoxies and silicones comprise a major class of medical adhesives. Typically, epoxies come in either one or two-parts and may require mixing. Epoxies have excellent chemical resistance and physical bonding and quickly cure at high temperatures or slowly cure at room temperatures. Epoxies may prove difficult to uniformly spread, however, given their high viscosities.[69,70] Silicones also offer excellent flexibility, cures at room temperature and require no mixing.[71] Other synthetic adhesives may include UV/LED curing

systems, epoxy-polyurethane blends, and cyanoacrylates.[69,72] Application methods for synthetic adhesives is based largely on the viscosity of the solution. Knife coating, roller coating, dip coating, and spray coating are all viable techniques to apply synthetic adhesives. Synthetic adhesives have proven safety and efficacy in a broad range of biomedical applications, and undoubtedly may be suitable adhesives for a variety of flocked scaffolds.

3. Biological adhesives

While synthetic adhesives may be suitable for some biomedical flocking applications, it may be more desirable to have adhesives derived from biomaterials as their degradation and biocompatibility is ensured. An early protocol was established by Walther *et al.* that employed gelatin adhesives in concentrations ranging from 5 wt. % to 50 wt. %.[8] The conductivity of the gelatin was adjusted by dissolving the gelatin powder in 0.5M NaCl solutions. To cure gel adhesives, a cross-linking step using 1% (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (EDC) in 80 vol. % ethanol was carried out over a 20-h period. In a similar work, Gossla *et al.* dissolved chitosan (CHS 95/500) in acetic acid at 5 wt. % and allowed total dissolution before knife coating substrates.[27] Subsequently, the adhesive was thermally cured at 120 °C for 15 min. Both the chitosan and gelatin adhesives have been used in multiple reports and have demonstrated biocompatibility and biodegradation. McCarthy *et al.* investigated the stability and degradation of chitosan and gel-based adhesive systems and determined that gel (crosslinked with EDC) demonstrated the slowest degradation, with 50/50 gel/chitosan [crosslinked with glutaraldehyde (GA)] had a median degradation, and pure thermally cured chitosan had the fastest degradation rates.[32] All flock-related TE reports have utilized either the chitosan or gelatin adhesives systems.[23,26–28,32,73]

Besides gelatin and chitosan, there exist a variety of other suitable adhesives. Alginate gels, hyaluronic acid gels, collagen gels, or combinations of other natural polymers exhibiting adhesive properties may all serve as suitable adhesives that can be chemically crosslinked via EDC or GA or thermally cured.[74–76] Kim *et al.* reported the development of an adhesive inspired by plant-based compounds.[77] Here, tannic acid and poly(ethylene glycol) are mixed to form a stick substance that adheres well to skin, induce rapid hemostasis, and are antimicrobial.[34] Another biomimicking adhesive is reported by Han *et al.*, where a polydopamine-clay-polyacrylamide hydrogel showed high skin adhesion, excellent biocompatibility, hemostatic capacity, and antimicrobial efficacy.[78] Hundreds of biological-based adhesives exist, and further studies examining their potential application in flocking are necessary to establish which natural adhesives are best suited for flocking.

Though not biological materials, some electrospun nanofiber adhesives have been synthesized recently. Specifically, poly(methyl methacrylate) (PMMA), N-octyl-2-cyanoacrylate, and blends of PCL/polyethylene oxide (PEO)/polyvinylpyrrolidone (PVP)/Eudragit® RS100 have been reported.[79–81] Electrospun adhesives may be an exciting avenue of further investigation in flocking. Electrospinning is an enabling technology with proven biomedical applications and is easily and reliably scaled up.[82,83] By creating a sticky nanofiber membrane, there may not be a need for a substrate, thus reducing production and resources that would require spray coating a substrate with an adhesive layer. Further, electrospun membranes can be aligned or random and exhibit control over cell migration while offering a versatile fabrication process, allowing for a variety of functionalities to be incorporated.[84]

C. Substrate selection for biomedical applications

Flocking substrates are broadly considered anything receiving flocked fibers. According to the American Flock Association, almost any surface can be flocked. In industrial textile applications, it is common for woven and non-woven textile surfaces, papers, polymers, and metals to receive flock coatings. There are nearly no requirements a substrate must meet for the flocking procedure—as long as an adhesive layer can be applied, the object can be flocked. However, there are many requirements a substrate must meet for flocked tissue scaffolds. The mechanical and morphological properties of substrates should match that of the region and function they are serving. Considering the bulk porosity, density, mechanical strength, geometry, and composition of a substrate should be considered when selecting the type of substrate to flock.

1. Synthetic substrates

Given a substrate has no requirement to be suitable for flocking, there are many useful materials that may serve as ideal flocking substrates for TE. Electrospinning, molding, 3D printing, microfabrication, and a variety of other techniques have been reported as methods to create bioactive and biocompatible materials.[40,85–87] Polymers, ceramics, and metals may all be suitable comprising materials. Considerations for selecting a synthetic substrate are largely based on the biological material they replicate, their degradation rates, and their bioactivity. Balasubramanian *et al.* first reported using a bioactive glass [45S5 BioGlass (BG)] as a flock substrate for osteochondral TE scaffolds.[26] In their study, they noted the BG substrate retained its porosity and held the flocked fibers to its surface well. Additionally, they reported the formation of cauliflower-shaped structures from hydroxyapatite formation—a mineralization process that has been shown to enhance bone healing. Utilizing woven nanofiber yarn mats, electrospun nanofiber membranes, 3D-printed meshes, or metallic implants as flock substrates are feasible by either directional or object flocking. McCarthy *et al.* demonstrated the versatility of electrostatic flocking by surface coating electrospun nanofiber membranes, 3D-printed PCL meshes, and self-folding tubes which displayed regionally distinct hierarchical structures (Fig. 8).[32] When a substrate can work synergistically with the surrounding tissues, the desired biological outcome can be achieved faster, and risks, such as infection, can be further mitigated.

2. Biological substrates

Biomaterial substrates are developed from biological systems and may be composed of collagen, hydroxyapatite, chitosan, cellulose, and many other biologically derived structures that can retain shapes. Several substrates derived from biological materials have been investigated for use in electrostatic flocking. Steck *et al.* synthesized mineralized membranes by dissolving collagen type I in hydrochloric acid (HCl) and rinsing with calcium chloride ($CaCl_2$), tris buffer, and phosphate buffer.[73] The resulting membrane was formed under vacuum

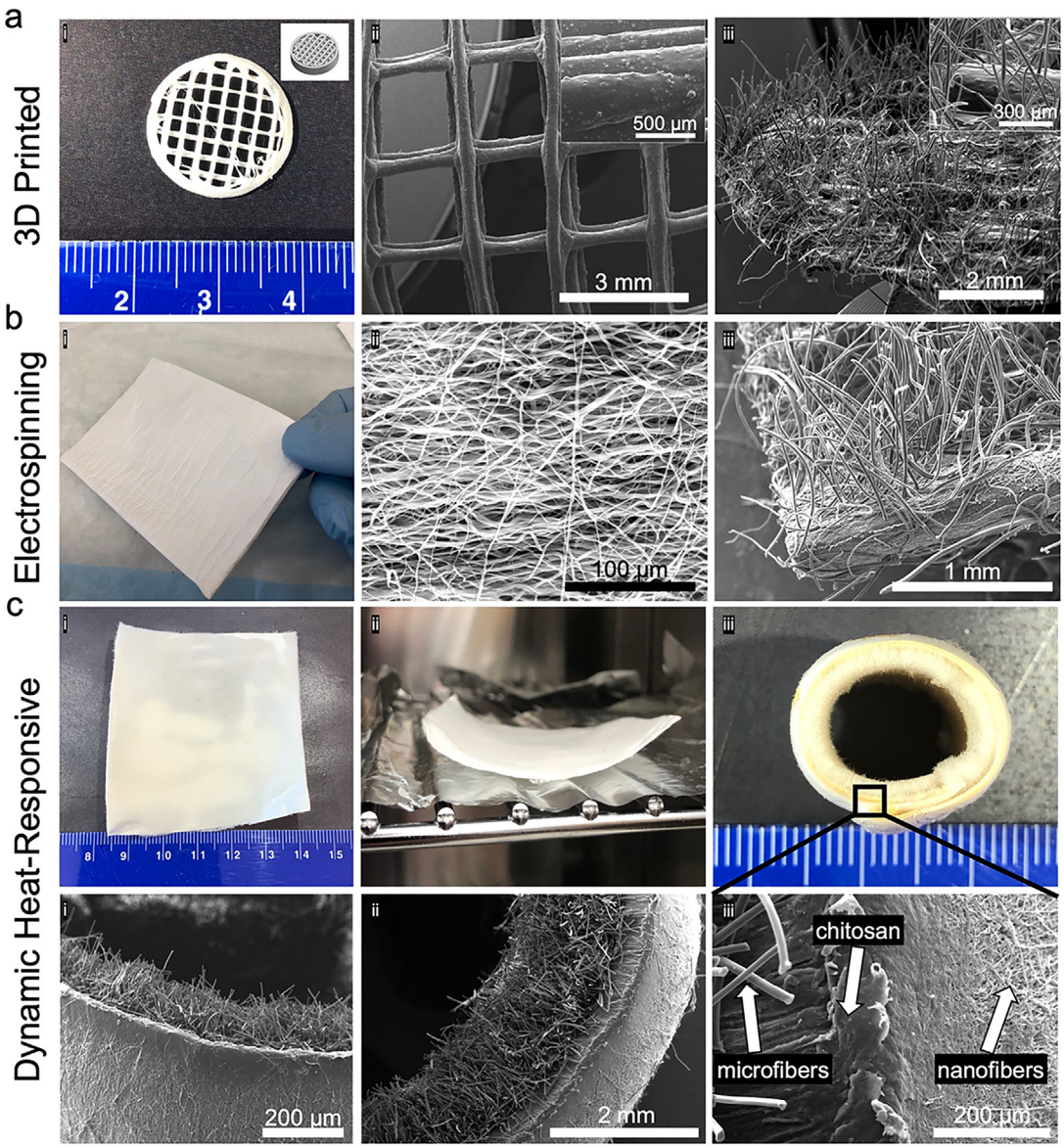

FIG. 8. Potential flocking substrates with preselected biomedical applications. (a) 3D printed multi-layered mesh, (b) electrospun nanofiber mat, and (c) self-folding hierarchical conduits. Reproduced with permission from McCarthy *et al.*, Adv. Healthc. Mater. **10**, 2100766 (2021). Copyright 2021 Wiley.[32]

filtration, crosslinked with EDC, and freeze-dried. Gossla *et al.* used a thick layer of chitosan (CHS 95/500) to act as both substrate and adhesive, noting that thermally cross-linking the chitosan formed a solid, flexible structure.[27] Other plant-based materials may be suitable to act as non-degrading substrates, such as cellulose-derived electrospun mats, which have been shown capable of releasing bioactive compounds.[88]

D. Post-flock finishing

After the final steps in the electrostatic flocking process (usually cross-linking or curing the adhesives after the application of flocking fibers), several steps must be taken to verify the quality of the flocked scaffold. Generally, loose fibers are vacuumed away prior to a series of cleanings and sterilizations. In order to assure the quality of the flocked surface, several mechanical tests are used to check the stability of the fibers, resistance to abrasion, and degradation.

1. Sterilization

Before using flocked scaffolds for *in vitro* or *in vivo* tests, proper cleaning and sterilization methods must be employed. First, any excess fibers not adhered to the substrates should be removed via vacuum and any residual fibers that persist may be blown off with compressed air.

A thorough rinsing of the scaffolds with water or phosphate-buffered saline (PBS) should be carried out at least three times before sterilization, based on previous reports.[23,28,73] Noting the influence sterilization may have on the mechanical properties of flocked scaffolds, Gossla *et al.* sought to explore how different sterilization techniques influenced the mechanical stability and cell viability of flocked scaffolds. Ethanol treatment, gamma-irradiation, supercritical carbon dioxide, steam autoclaving, and water-submerged autoclaving were treatments used to sterilize scaffolds.[27] First, the study compared the effects of each sterilization method on the ultimate tensile strength (UTS) and Young's Modulus (YM). Based on the results, each sterilization method decreased the YM by a significant amount, with water-submerged autoclaving dropping the YM from ~14 GPa to 6 GPa. Steam autoclaving and gamma-irradiation had the smallest decreases in YM, with both treatments dropping the YM to 12 GPa. The UTS was significantly reduced in all sterilization methods except for water-submerged autoclaving. UTS was reduced from 177 MPa (control) to 169 MPa 153 MPa, 150 MPa, 116 MPa, and 91 MPa for water-submerged autoclaving, steam autoclaving, gamma-irradiation, ethanol, and subcritical carbon dioxide, respectively. Cell viability was also assessed after each form of treatment in the form of cell count (cells/scaffold) and viability (live cells as % of control). After 24 h, ethanol-treated scaffolds had the most cells, while only subcritical carbon dioxide had a significant decrease in cell count. Cell viability across 96 h was observed, revealing mixed results with no trends at each treatment type. Although no pattern was observed, no treatment had a significant decrease in cell viability as compared to the control.[27] Other than the aforementioned methods, scaffolds could be prepared from sterile materials as to skip an additional sterilization step altogether. It is important to be mindful the kind of substrate and adhesive used during fabrication. For example, is a PCL nanofiber membrane was used as a substrate and the flocked scaffold was autoclaved, the PCL would likely melt due to its low melting point.

2. Mechanical testing

In the flocking industry, several mechanical tests are suggested to validate the stability of a flocked surface. The rub, pluck, and scuff tests are used to test the abrasion-resistance of a flocked surface.[1,32] Testing resistance to abrasion is critical, particularly in relation to TE scaffolds, as loose fibers may release from the adhesive and migrate into surrounding tissues, eliciting unwanted immune responses or localized inflammation. To date, no research has reported the abrasion resistance of flocked scaffolds; a critical oversight is needed to ensure the safety of implantable flocked scaffolds. To test abrasion, employing two simple tests—the rub and scuff test—is necessary. Here, a thumb, coin, crockmeter, or some other dry or wet rubbing device cycles back and forth over the surface of the flocked scaffold until failure is reached and fibers are removed. Scaffolds should be fixed and subject to adequate rubbing—both by force and cycle. In the pluck test, an MT 501 or some other caliper may be used to tear fibers out of the adhesive layer, thus testing the quality of the bond between adhesive and fibers.[1]

Additional testing to verify compressive strength, YM, and UTS should be performed to confirm the mechanical properties of the scaffold match that of the implanting region. Flocked fibers are particularly interesting due to their anisotropic material properties. With high compressive strength and high porosity, flocked scaffolds fulfill the mechanical requirements of tissue engineer scaffolds well. The mechanical requirements of flocked scaffolds depend largely on their intended use. For example, research investigating flock scaffolds for osteochondral engineering should consider the compressive and abrasive forces caused by joint movement, verifying that the scaffold integrity is sufficient under biologically relevant loads. On the other hand, flocked scaffolds intended for cranial defects may not need high compressive strengths since they are not subject to compressive loads. Luckily, mechanical properties of flocked scaffolds can be tuned by adjusting fiber type and geometries, fiber density, and adhesive type. In fact, both Walther *et al.* and Tonndorf *et al.* demonstrated that modifying the length of the fiber and the fiber density could be used to tune both porosity and compressive strength of scaffolds, with compressive strength increasing relative to decreases in fiber length.[23,28] McCarthy *et al.* also investigated the abrasion resistance of flocked scaffolds in gel/chitosan adhesives and found that nearly after 100 abrasive cycles, the mass of the flocked scaffold reduced by nearly half.[32] This may highlight the need to create better adhesives to prevent fiber loosening *in vivo*.

IV. POTENTIAL BIOMEDDICAL APPLICATIONS

Flocked scaffolds, though understudied, may serve as useful tools in regenerative medicine, TE, and several other biomedical applications considering its unique properties. Whether it is to mimic anisotropic parts of the body, induce high absorption using capillary action, cause hemostasis, or enhance the mechanical properties of tissues, flocking is a versatile technique that can be easily applied to many clinical applications. As TE constructs, flocked scaffolds offer improved porosity compared to other nanofibrous or hydrogel scaffolds. Additionally, flocked scaffolds exhibit bulk anisotropy and interconnectedness that is superior to other fiber and gel-based scaffolds. Not surprisingly, flocked scaffolds may have important roles to play in bone TE—specifically related to osteochondral, trabecular, and cortical bone engineering. Further, flocked scaffolds may serve an important role as wound healing constructs as they may promote the formation of granulation tissue along a given area by using the flock fibers as anchors in the wound bed. Advances in flocking will undoubtedly lead to innovations in textile-based TE and biomedical applications.

A. Tissue regeneration

Although TE has existed as an area of research for decades, there are a few TE constructs that are currently being implemented in clinical settings. For a TE scaffold to be clinically relevant, it should mimic the extra cellular matrix (ECM) microenvironment, enhance tissue formation and cell infiltration, match the porosity of the host tissue, facilitate neovascularization, and be mechanically supportive under natural stresses.[89] To date, all reports investigating flocking in biomedical applications have focused on TE using the flocked scaffolds as TE scaffolds. Preliminary studies have warranted further investigation as scaffolds were shown to have suitable biocompatibility with seeded cells, not alter gene expression, support cell differentiation, provide adequate mechanical strengths, and biodegrade over time.[8,23,26,27,73] Understanding how flock scaffolds offer improvements and shortcomings in TE is vital in progressing the technology toward clinically relevant therapies.

1. General requirements

Porosity has been a significant hurdle in designing successful TE scaffolds given the paradox of achieving high porosity while maintaining favorable mechanical properties continues to remain a challenge. Generally, a scaffold should have an interconnected porous network that can facilitate the movement of cells, nutrients, and growth factors.[90,91] Both pore size and interconnectivity are significant design factors to consider for TE scaffolds as they directly facilitate or inhibit the migration and infiltration of cells. Karageorgiou *et al.* points out that a minimum pore size of 100 μm is necessary for cell migration and nutrition transport although a pore size of 300 μm or greater was recommended as they could enhance formation of tissues and capillaries.[92] Here, the size of the pores also determines the formation of bone, where large pore sizes (>300 μm) lead to direct osteogenesis, while small pores induced osteochondral formation prior to osteogenesis. In many conventional biomaterial fabrication methods, such as electrospinning or hydrogel formation, tuning bulk porosity, pore size, and interconnectivity may be difficult. As Howard *et al.* summarizes, different cell types respond preferentially to a variety of pore sizes, suggesting that a method where these features can be tuned by intended cell type could have a broader range of applications.[30]

Flocking as a technique allows for easy tuning of porosity and interconnectivity. In flocked scaffolds, it is difficult to assign porosity since flock can largely be considered as surface decoration. However, if a flocked scaffold is considered as 3D construct rather than a surface-decorate substrate, porosity values often exceed 90%, following a flock porosity equation set forth by Walther *et al.*,[23]

$$\text{Porosity} = \frac{V_s - V_f}{V_s} \times 100\%. \tag{4.1}$$

Here, V s is the volume of the scaffold which is calculated as

$$V_s = l_f \times A_s. \tag{4.2}$$

Or it is simply the average fiber length (l_f) multiplied by the area of the substrate (A_s). Additionally, V_f is taken as the total volume occupied by fibers, calculated as

$$V_f = V_{nf} \times \rho_f, \tag{4.3}$$

where V_{nf} is the volume of a nominal fiber and ρ_f is the fiber density. Based on this simple formula, the porosity of a flocked scaffold can be easily derived. As noted by Walther *et al.*, achieving high porosity values (94.9 ± 0.8 to 97.6 ± 0.8) is directly correlated with fiber density, which is a function of several flocking parameters.[23] Applied voltage, loaded fiber count, distance between electrodes, and time applying voltage are all parameters that may be adjusted to fine-tune porosity of flocked scaffolds. Increasing applied voltage, loaded fiber count, and time applying voltage all increase fiber density while increasing distance between electrodes decreases fiber density. It is worth noting that both Walther *et al.* and Steck *et al.* demonstrate that increasing fiber length has little effect on porosity, but decreases compressive strength.[23,73] Nevertheless, flocked scaffolds offer a massive surface area-to-volume ratio that allows cells, oxygen, and nutrients to move and interconnect incredibly well since there is no connection between aligned fibers. In theory, cells can migrate throughout and along fibers with no hinderance. In both cases, Walther *et al.* and Steck *et al.* demonstrated that Saos-2 and human mesenchymal stem cells (hMSCs) could sustain proliferation on flocked scaffolds with varying fiber characteristics.[23,73] In the case of Steck *et al.*, both cell types saw significant, fiber-guided cell proliferation [Figs. 9(a) and 9(b)].[23] Over 14 days of incubation, Saos-2 cells saw an increase from 4000 cells/scaffold to nearly 225 000, while hMSCs saw an increase from 4000 cells/scaffold to nearly 800 000 cells/scaffold [Figs. 9(c) and 9(d)]. Similarly, McCarthy *et al.* demonstrated sustained cellular viability and proliferation along fiber lengths over a 28-day period, measuring the mean height of the cell tissue layer and determining that the cell migrated close to 550 μm in length [Figs. 9(e) and 9(f)].[32] Overall, flocked scaffolds owe their high porosity and interconnectivity to a specific material phenomenon called anisotropy—a highly desirable material characteristic for many TE scaffolds.[93–95]

In materials science, anisotropy is the phenomenon where material properties are directionally dependent. For example, an aligned bundle of straws would have a high compressive strength along the length of bundle and a much lower compressive strength along the cross-sectional area of the bundle. Anisotropy is also found throughout the body—in cartilage, bone, muscle, and in the ECM.[96–98] Mimicking the anisotropy found in different tissues is a continued avenue of research as designing anisotropic tissue scaffolds is challenging due to the precision needed to align micro- and nano-scale objects.[99] However, flocking, by nature, creates an anisotropic material that is oriented by the direction of aligned flock fibers. Mimicking anisotropy is not only morphologically important to replicate, but can direct cell migration into a defect, thus accelerating tissue healing or new tissue formation. In fact, anisotropic biomaterials have been shown to enhance wound healing, bone formation, and cartilage healing in several studies.[93,100,101] There is undoubtedly a need to recapitulate tissue anisotropy to best heal and regenerate a variety of tissues.

2. Bone repair

Applying flocked scaffolds to bone engineering is easily conceivable for both load-bearing and non-load-bearing tissue scaffolds. Since flocked scaffolds have tunable mechanical properties that can match the mechanical requirements of different bone regions while facilitating bone cell proliferation, flocked scaffolds may be well-suited as bone engineering scaffolds. Further, previous studies have demonstrated that fiber diameter can, on its own, impart some modulation of cell movement and proliferation by effecting the angle of alignment along the fiber.[102] To this end, flocking fibers of different diameters can contribute to the rate of cell infiltration, adding another layer of tunability in flocked tissue scaffolds. In fact, previous studies investigating flocked scaffolds as potential bone engineering scaffolds have shown promise by directing and differentiating cells toward bone-like cell lineages, supported tissue formation, and exhibited mechanical properties that outperformed scaffolds of similar design.[23] While there are many applications where flocked scaffolds may offer promise, bone TE may be an area of enhanced focus due to the demanding mechanical requirements and need for a high surface-area-to-volume ratio.[103,104] In the following, different bone-related applications for flocked tissue scaffolds are discussed. It should be noted, however, that there are yet to be any studies investigating the *in vivo* or clinical performance of flocked scaffold relating to bone TE.

Despite having seemingly simple appearances, bones are morphologically complex organs that exhibit a variety of mechanical

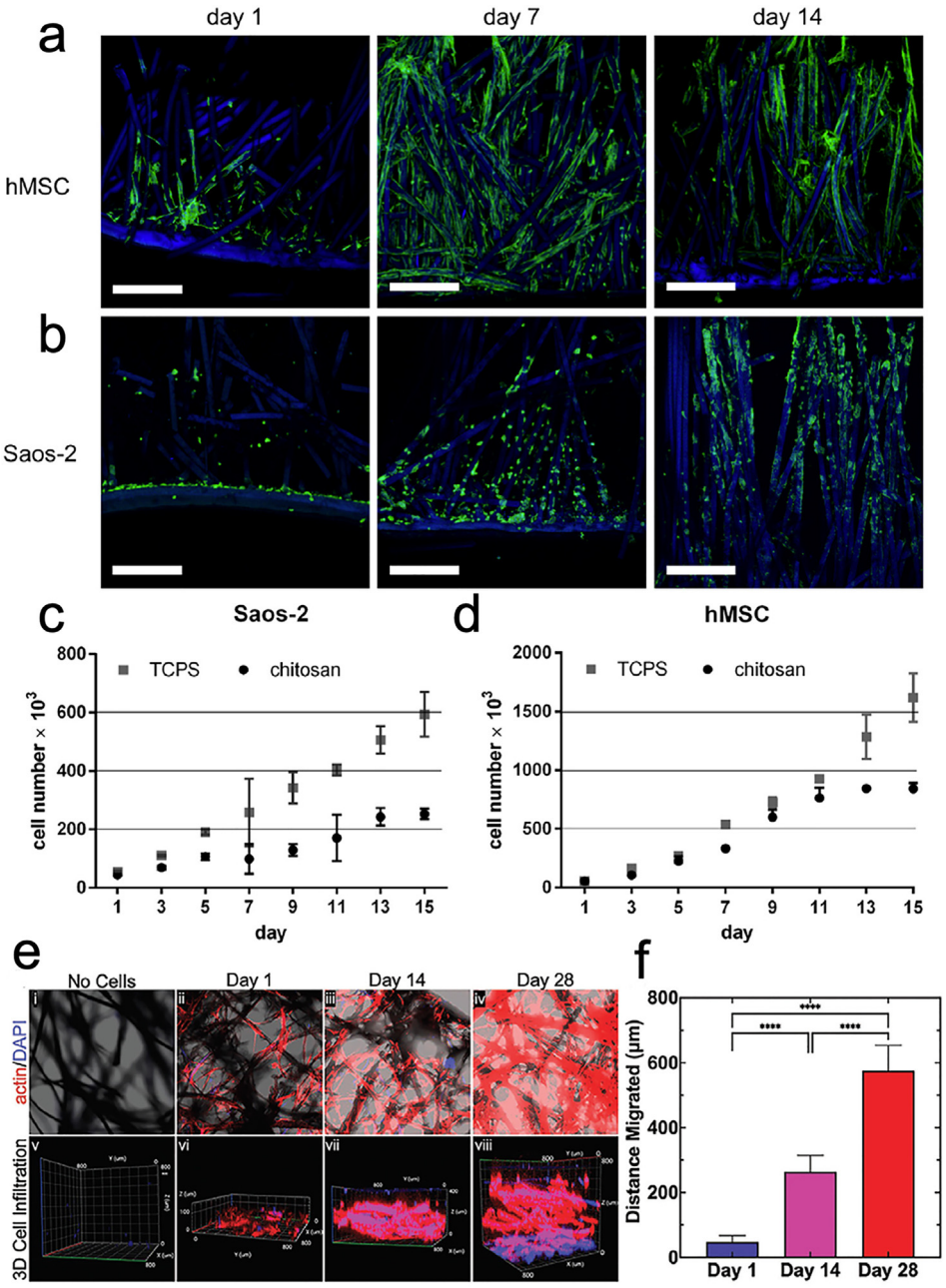

FIG. 9. Cellular response to flocked scaffolds. (a) hMSCs cultured on pure chitosan scaffodls for 14 days. (b) Saos-2 cells cultured on pure chitosan scaffolds over 14 days. Scale bar = 400 um. (c) Saos-2 and (d) hMSC proliferation cell counts after 14 days of culture. Reproduced with permission from Gosssla *et al.*, Acta Biomater. **44**, 267 (2016). Copyright 2016 Elsevier Ltd.[27] (e) Actin/DAPI stained hMSCs cultured on AgNP/PCL flocked scaffolds for 28 days. (f) Distance of leading cell edge migrated over a 28-day culture period. Reproduced with permission from McCarthy *et al.*, Adv. Healthc. Mater. **10**, 2100766 (2021). Copyright 2021 Wiley.[32]

properties varying by region. When considering how to best construct bone TE scaffolds, it is imperative to consider the different types of bone regions and their morphological and mechanical properties. The outermost layer of bone is called the periosteum and is broadly referred to as the bone covering. The periosteum has a dense, fibrous outer layer, composed mainly of collagen and fibroblasts, and an inner layer, the cambium, composed of mostly osteoblasts and chondrocytes.[105] While the periosteum does not exert tremendous mechanical strength to the bone, it serves to support blood vessels, and nerves, and assist in bone growth and repair.[106] Relative to flock scaffolds, it is likely that implanted scaffolds would be sutured under the periosteum. However, it would be conceivable to use the substrate of the flocked scaffold to form a continuum with the periosteum, so long as the substrate of the flocked scaffold meets the mechanical requirements of the periosteum.

Beneath the periosteum is another distinct layer called cortical bone. Like the periosteum, cortical bone is a dense covering that functions to protect the internal cavity of the bone. Unlike the periosteum,

cortical bone imparts the majority of the body's mechanical strength.[107] Cortical bone has high resistance to bending, torsion, and compression, and accounts for roughly 80% of the total bone mass in the body. With aging or other bone-degenerating diseases, cortical bone compartments become more porous, and thus weaker.[108] Many clinical approaches to treat osteoporosis of cortical bone have sought to protect or recover bone density with chemical and mechanical interventions, including bone tissue scaffolds.[109,110] As the most internal and porous part of the bone, trabecular bone plays a complex role in bone strength and repair. Unlike cortical compartments, only approximately 20% of the trabecular compartment is composed of bone, with the majority of the compartment composed of marrow.[111] While trabecular bone may not impart mechanical properties to the same magnitude as cortical bone, the trabecular compartment absorbed and transfers mechanical loads to the cortical bone. Trabecular bone has a much higher surface area-to-volume ratio and a much higher cell turnover rate.[111,112]

Given the mechanical and functional differences in different bone components, scaffolds must vary dramatically in design to best fit the region of defect they are intended to repair. For cortical bone, flocked scaffolds should have very high fiber densities such that the scaffold's resistance to compression matches that of the cortical bone. In practice, fibers that degrade slowly (to match the lower turnover rate of cells in the cortical bone) should be oriented parallel to the direction of compressive loads. Comparatively, should a defect occur in the trabecular compartment, a flocked scaffold with quickly degrading fibers and low fiber densities should be used. Previous studies have shown that hMSCs seeded on porous flock scaffolds differentiate and proliferate well.[23,72] Trabecular bone scaffolds should worry little about imparting significant mechanical properties and instead promote rapid proliferation of cells into a defect. By simply tuning fabrication parameters (such as flock time or applied voltage), dramatically different scaffolds can be used for different bone regions without changing their materials or methods of fabrication.

In the case of treating multiple layers of a bone defect with a single scaffold, it is possible to stack flocked substrates to create a composite or hybrid-type flocked scaffold that exhibits regionally different mechanical and material properties [Fig. 10(c)]. Such a scaffold would consist of having intermediate substrates with adhesives on both the top and the bottom, essentially gluing flock scaffolds on top of each other. Different fiber densities and fiber lengths could offer gradient or stepwise changes in material properties. While no instances of investigating sandwich-type composite flock scaffolds are reported, they may be an area of interest in the future.

One particular application for flocked scaffold is for the repair of cranial defects. Defects occurring in the calvarium typically arise from trauma or surgery.[113] Cranioplasty, which is the surgical repair of a cranial bone defect, is the most common procedure to correct cranial defects. Allografts, autografts, and xenografts of bone tissue have all shown improvements in regenerating cranial bone although complications following grafting are not uncommon.[114] Besides bone grafting, both polymeric and metallic tissue scaffolds have been implicated in improving cranioplasty outcomes.[115,116] However, these scaffolds often have low surface area-to volume ratios or have uniform material properties throughout the material.[116] Since cranial bone

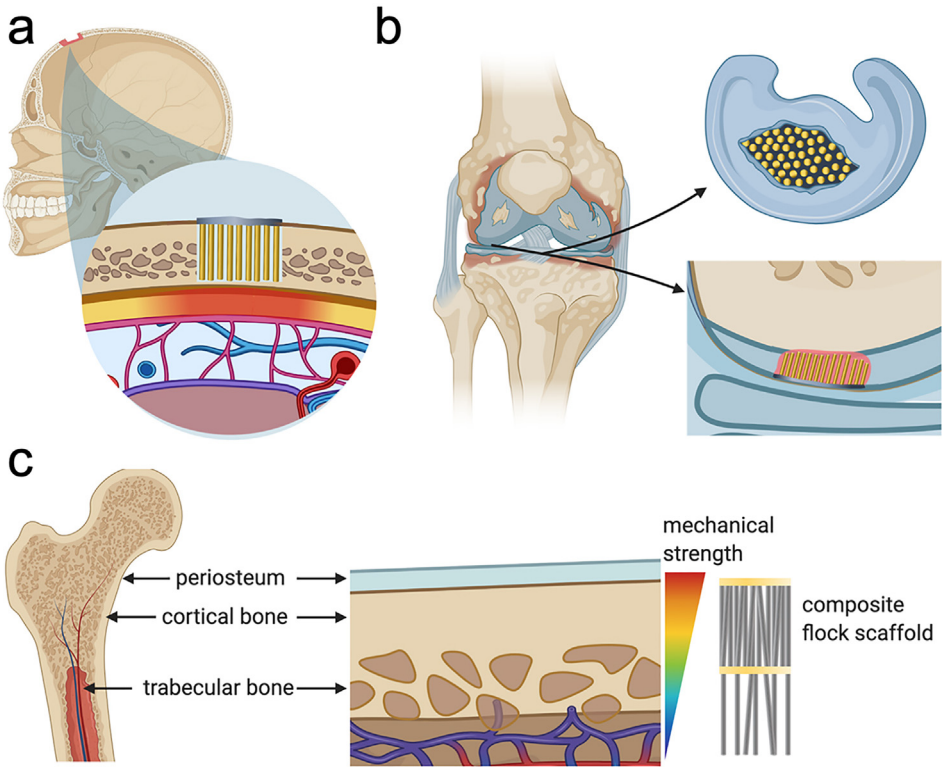

FIG. 10. Improving orthopedic outcomes with electrostatically flocked tissue scaffolds. Proposed uses of flocked tissue scaffolds for (a) repairing cranial defects and (b) osteochondral engineering of joints to treat arthritis and cartilage defects. (c) Illustration representing composite flock scaffolds with gradient mechanical properties matching different regions of cortical and trabecular bone.

is not subject to mechanical loads, there is little need for scaffolds with high mechanical strength and relatedly high surface area-to-volume ratios. A flocked scaffold may be an ideal cranial bone defect scaffold as it is very porous, can have gradient changes in mechanical properties, and may have a thin film substrate that may seal the most superficial part of the defect from the periosteum, preventing migration of non-bone cells into the defect that may leave visible indentations requiring further plastic reconstruction [Fig. 10(a)]. Additionally, flocked scaffolds for cranial bone defect healing can fit a variety of defect sizes simply by cutting the substrate to match the defect geometry, a shortcoming that other approaches, such as mold casting, face in implementation.

In a recent unpublished dissertation study, McCarthy *et al.* investigated the bone regenerative capabilities of 0.5% AgNP/PCL fiber and chitosan adhesive/substrate scaffolds in an 8-mm rat cranial defect model.[117] Flocked scaffolds were custom prepared for each animal model to fit an 8 mm surgically induced total cranial bone defect. During surgery, the scaffold substrate was laid flush against the in-tact dura mater, with the fibers filling the empty volume of the defect [Fig. 11(b)]. The periosteum was sutured along the top of the scaffold fibers. Micro computed tomography images show that, in both flock scaffold treatment groups, total bridging of the defect was achieved, while no significant bone volume was regenerated in the negative control [Fig. 11(a)]. These results demonstrated a fiber-dependent and significant recovery of bone volume over a 7-week period compared to a negative control [Fig. 11(c)]. As the first *in vivo* model investigating flocking as bone tissue scaffolding, these results warrant larger, more comprehensive animal studies to definitively elucidate any substantial bone regenerating effects flocked scaffolds possess.

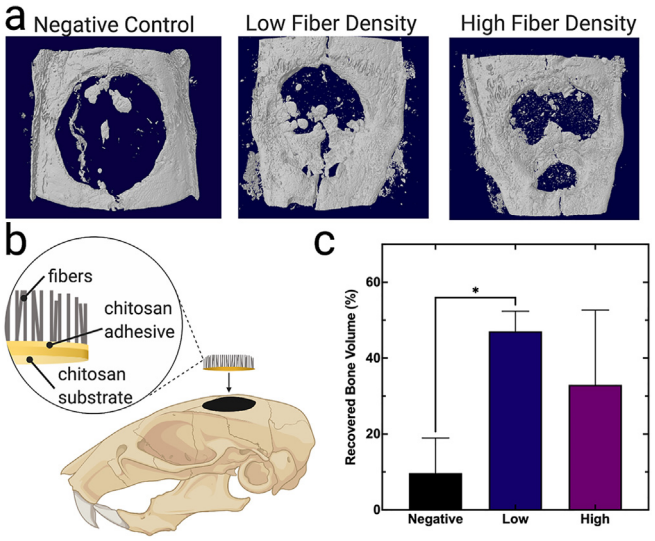

FIG. 11. Flocked AgNP/PCL fiber and chitosan adhesive/substrate scaffolds demonstrate a modest and significant cranial bone regeneration effect in a rat cranial model. (a) Micro computed tomography images of rat cranial bone defects untreated and treated with low and high-density flocked scaffolds for 7 weeks. (b) Scaffold orientation *in situ* (substrate flush with dura mater). (c) Graph comparing recovered bone volume (%) between each group ($P < 0.05$, $n = 4$). Reproduced from A. McCarthy, unpublished Ph.D. Dissertation (2021). Copyright 2021 University of Nebraska Medical Center.

3. Osteochondral & chondral repair

At joint sites, there exist different, complex tissue types that are often subject to trauma and require repair. Articular cartilage, the smooth tissue that buffers the interface between bones, is a prime area of focus in regenerative medicine.[118] Through normal wear and tear, articular cartilage often deteriorates at injury sites. Many techniques have been suggested to restore cartilage in defected sites, including stem cell therapy, anti-inflammatory drugs, volume-filling injections, hydrogels, and 3D tissue scaffolds.[119] Imparting significant compression resistance, serving as a buffering interface between bones, and encouraging proliferation and differentiation of chondrocytes into a seamless transition into native cartilage tend to be the primary areas of articular cartilage engineering. Given the fibrous and aligned morphology of articular cartilage, flocked tissue scaffolds could easily mimic the architecture of native cartilage tissue while meeting the aforementioned requirements for cartilage TE. In fact, nearly all reports on flocked tissue scaffolds have indicated their use as repair scaffolds for articular cartilage degeneration.[23,26,27,73,120]

One notable application of electrostatic flocking related to osteochondral engineering was recently reported by Gossla *et al.* and introduced the use of flocked fiber to reinforce a hydrogel matrix. In this use case, chitosan flock fibers were embedded in an alginate hydrogel.[120] The hybrid system exhibited anisotropy imparted by the flocked fibers as well as cell-encapsulating abilities enabled by the alginate hydrogel. At 40 and 50% strain, flock-reinforced alginate hydrogels scaffolds demonstrated significantly higher strength. Additionally, the hybrid scaffolds seeded with primary human donor chondrocytes exhibited regionally distinct cellular morphologies depending on the spatial orientation within the scaffold. Cells in direct contact with flocked fibers appeared as spindles, while cells suspended in the alginate were spherical. Most importantly, Gossla *et al.* showed that hybrid scaffolds had biological characteristics of both flocked and alginate hydrogel-only scaffolds. Hybrid scaffolds had significantly higher expression of most genetic markers for chondrogenic genes and significantly higher amounts of glycosaminoglycans compared to flocked or alginate-only scaffolds. The deployment of flocking to reinforce an osteochondral scaffold while retaining chondrogenic differentiation ability shows the versatility enabled by flocking.

Given their ability to sustain formation of pre-chondrogenic cells, differentiate into chondrocytes, and maintain cell viability and scaffold alignment after long periods of cell culture, there is compelling evidence to warrant the investigation of flocked scaffolds as regenerative therapies in arthritic animal models. Fiber length can be tuned to match that of the cartilage in the host site, and flock-covered substrates may be bound to the exposed bone such that the fibers align with native cartilage [Fig. 10(b)]. It is crucial to evaluate a suitable animal model to determine how flocked scaffolds respond to constant and repeated loading cycles, ensuring scaffolds resist abrasion until cells are able to re-populate the defect site.

B. Wound management

Treating and maintaining wounds is one of the most fiscally burdensome processes in healthcare.[121] Wounds may be difficult to adequately treat because they vary dramatically in size, shape, cause, rate of healing, and susceptibility to infection.[122] For example, chronic wounds fail to progress through the main stages of wound healing in a

timely manner and may persist for months or even years. Approaches to treating chronic wounds range from surgical debridement, antibacterial therapies, drug delivery systems, hydrogel wound dressings, tissue constructs, or combinations of the aforementioned.[59,77,123–127] Conversely, acute wounds progress through the stages of wound healing at expected rates, but may have problems ranging from blood loss to necessitating skin grafts for large area wounds.[128,129] Obviously, treatment approaches must consider the long-term goal of healing and managing wounds for the best clinical outcome. Often, wounds are approached with a one-size-fits all mentality where bandages are applied repeatedly, and wounds are cleaned. Wet wounds may macerate after long periods of bandaging, while dry wounds may dry out. Assisting wounds at different stages of healing may offer the best clinical outcomes for all types of wounds, such that the wound conditions remain attenuated to the requirements of the body. Flocked devices, serving as either scaffolds or hemostatic devices, may have a wide range of application regarding wound healing [Figs. 12(a) and 12(b)]. Section IV B 1 offers a perspective look at applying flocked constructs to wound healing, and hemostasis is considered.

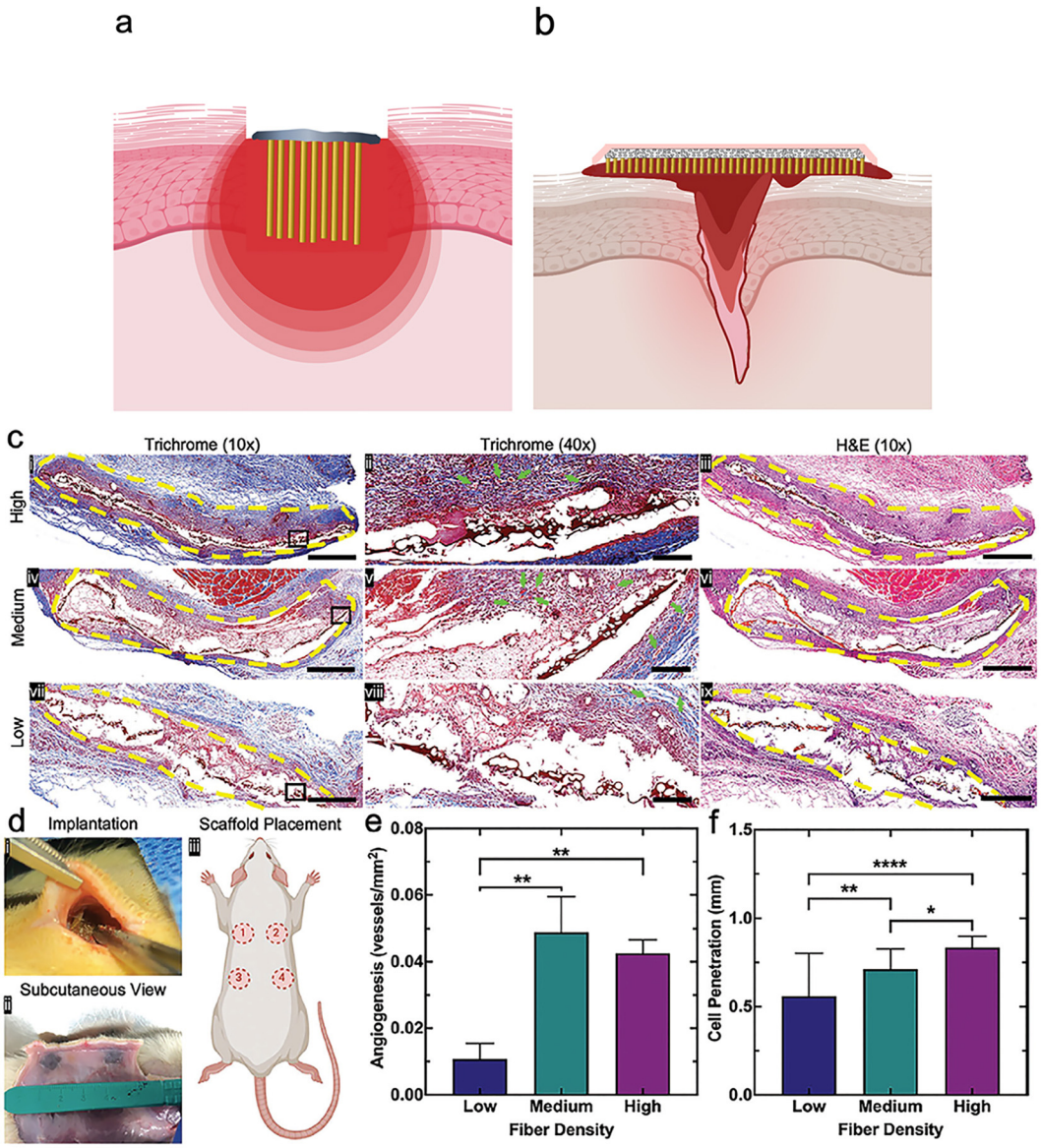

FIG. 12. Proposed methods to incorporate flocked constructors for wound healing and skin tissue engineering. (a) Flock implants form granulation tissue or (b) are paired with absorptive materials to facilitate blood absorption and hemostasis in acute wounds. (c) Subcutaneous implantation of AgNP/PCL scaffolds shows a fiber-dependent granulation tissue formation. Scale bar = 1 mm and 200 um for 10× and 40× images. (d) Schematic outlining the surgical procedures for flock scaffold implantation. (e and f) The fiber-dependent angiogenetic and penetration response from flocked scaffolds implanted in a mouse model. Reproduced with permission from McCarthy *et al.*, Adv. Healthc. Mater. **10**, 2100766 (2021). Copyright 2021 Wiley.[32]

1. Wound repair

As previously mentioned, chronic wounds are wounds that fail to self-heal. For decades, managing chronic wounds has been a massive area of concentrated healthcare efforts. Chronic wounds are one of the most costly injuries to treat and manage, contribute significantly to mortality, are highly susceptible to infection, and typically effect already at-risk populations (elderly, diabetic, malnourished, etc.).[130–133] Generally, wound healing is considered to occur in four phases: hemostasis phase, inflammatory phase, proliferative phase, and maturation/remodeling phase.[134] Under normal physiological conditions, a wound would progress smoothly through each phase. However, chronic wounds typically fail to progress through at least one stage, with many chronic wounds struggling to move past the proliferative phase. Causing granulation tissue formation, or the synthesis of new connective tissue and blood vessels, is perhaps the most important factor during the proliferative stage of wound healing.[135–139] Research toward utilizing microneedles, *in situ* drug delivery methods, nanofiber scaffolds, and other biomaterial-mediated treatments are continually advancing although improvement or synergistic treatments are still an area of interest.[123,137,140–142] To this end, a flock-based wound healing scaffold may serve as an important step in granulation tissue formation.

Previous studies have demonstrated that biocompatible and biodegradable fibers embedded in a wound causes granulation tissue formation and thus accelerate wound healing.[89,140] Since these fibers degrade and their by-products are biocompatible, implantation into the wound bed would not necessitate surgical removal. In theory, degradable flock fibers flocked onto a wound-covering substrate could be directly applied to a cleaned wound, with the fibers penetrating into the wound bed and the substrate sealing the outside of the wound from the surrounding environment. Since fibers remain aligned after implantation, granulation tissue could form from the wound bed toward the surface of the wound, while simultaneously allowing re-epithelialization at more superficial layers of the wound. By uniformly inducing granulation tissue formation, new layers of tissue should be able to form between fibers, with aligned fibers acting as mechanical guides for proliferation. Similar concepts have demonstrated this concept have gained Food and Drug Administration (FDA) approval, such as Integra® Dermal Regeneration Template, which uses multiple layers of cross-linked fibers to serve as bases for granulation tissue formation.[143–146]

One advantage of using flocked scaffolds for wound healing is the tunability of the substrate. It is conceivable to flock nearly any surface where an adhesive may be applied. This may offer a huge advantage in treating chronic wounds as it has been widely demonstrated that many different sheet-type dressings increase the rate of wound healing. For example, aligned electrospun fibers have been demonstrated to increase the rate of re-epithelialization, while others have been doped with antibiotics, silver, or other antioxidants to help promote natural wound healing.[88,138,147–149] Selecting a bioactive or biomechanically designed substrate can help to concurrently seal, protect, and re-form the outer layer of the wound, while the flocked fibers induce granulation tissue formation below. Such a design may prove successful because the wound healing process is assisted from multiple directions (from the wound bed to the superficial layer; superficially, from all sides).

To date, only one report using flocked scaffolds *in vivo* is reported. McCarthy *et al.* showed the formation of granulation tissue in rats with flocked scaffolds implanted subcutaneously.[32] The scaffolds, composed of AgNP/PCL fibers and using a gel/chitosan adhesive, demonstrated enhanced cell migration and antibacterial effects *in vitro* and showed a similar fiber density-dependent *in vivo* response. McCarthy *et al.* also investigated the effects of subcutaneously implanted scaffolds with low, medium, and high densities in a healthy rat model and found a fiber density-dependent angiogenic and cell penetrative response [Figs. 12(c) and 12(d)].[32] Vessel formation was greatest in medium density scaffolds while cell penetration was highest in high density scaffolds. Low density scaffolds had poor cell penetration and vessel formation [Figs. 12(e) and 12(f)].[32] Scaffolds with medium and high fiber densities showed improved skin tissue regeneration in the defect site, increased cell penetration into the scaffolds, and improved angiogenesis. Further studies using flocked scaffolds in cutaneous wounds, applied superficially, are warranted to investigate the practical application of such a device.

2. Flocked bandages

Unlike chronic wounds, acute wounds are generally easier to treat and typically do not require the same level of concentrated care. However, there are still problems associated with acute wounds. Blood loss, pain, infection, and clotting are typically areas of concern regarding acute wound care. Typically, bandages and antibacterial ointments may suffice in treating smaller wounds. For wounds with persistent bleeding and difficulty clotting, additional pressure or highly absorptive dressings may be employed. While these may manage blood and eventually lead to thrombosis, creating a bandage or fiber-based device that can stop bleeding much faster, without the need for painful removal, may impact millions of people with small to medium-sized acute trauma wounds.[150,151]

An interesting phenomenon previously mentioned about flocked bandages is their inherent microcapillary action and its relatedness with surface wetting. At superhigh fiber densities, flocked articles may become superhydrophobic. In fact, several reports have investigated the induction of superhydrophobicity by flocking.[152,153] In this case, a superhydrophobic flocked material can be placed directly onto a wound, with the fibers facing the wound bed. As blood is released, the microcapillary action of the fibers traps the blood without absorbing it, essentially holding it in place and allowing fibrin to accumulate, forming a clot. A similar theory proved that using a hydrophobic wound covering could rapidly induce clotting by immobilizing blood released from the wound.[154] Additionally, at lower fiber densities, a Janus-typed, biphasic dressing can be fashioned where the flocked fibers use microcapillary action to move blood and exudate from the wound and into a superabsorptive substrate.[124] As with chronic wounds, the substrates selected for flocked bandages may range in composition and bioactivity, offering substrate design flexibility for different wound types (incorporating thrombotic factors for faster clotting or antibiotics for particularly infection-prone or immunocompromised individuals). Since it has been demonstrated that the surface wetting of articles may be controlled with the application of flocking fibers, it is easy to conceptualize how designing bandages with controlled absorptive properties may be a useful tool in controlling blood loss and facilitating healing in acute wounds.[154]

Another interesting use case for flocked bandages may be the implementation of electrical stimulation via conductive flocking fibers.

A plethora of studies have demonstrated the effect of *in situ* DC or ionic electric fields/currents in wound healing.[155,156] Notably, the application of electric fields has been shown to increase wound closure rates,[157] modulate fibroblast proliferation,[158] and promote the formation of granulation tissue.[159] To this end, a variety of devices, ranging from bioceramic plasters to *in situ* electrodes, are used to stimulate wound healing via electrical stimulation.[157,160] Given predicated studies demonstrating the ease of fabrication and use of flocked fabrics for electrodes in biomonitoring applications, it is extremely fathomable that flocked bandages or wound healing scaffolds can deliver electrical stimulation, particularly in fibers that have bulk conductivity or adequately conductive surface finishes.[161,162] Unlike polar two-point electrodes, the discharge of electrical stimulation from individual fibers may stimulate different regions of the wound including the wound edges and wound bed, which would not only facilitate faster wound closure, but increase perfusion at the wound edge and cell migration from the wound bed.

C. Collection of biological specimens

With the sudden onset of Sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019, the importance of swabs for specimen collection was reemphasized. Perhaps one of the least mentioned but most frequent medical application of electrostatic flocking is for the creation of flocked swabs. Flocked swabs show several advantages over traditional cotton swabs and have been used for clinical and self-administered nasal, vaginal, rectal, oral, and pharyngeal tests, DNA extraction, and viral and bacterial extraction, largely due to their excellent capillary action-enabled absorption.[25,163–171] While most research compares flocked swabs to traditional swabs in a variety of applications, there seems to be no innovative research focused on enhancing the efficacy or application of flocked swabs by design improvement. Several commercially available flocked swabs, such as the Puritan PurFlock® and HydraFlock,® showcase how flock innovation can lead to improved medical devices.

1. Bacterial swabbing

The incorporation of anisotropic collection fibers allows flocked swabs several advantages over traditional swabs, perhaps the most obvious advantages being the improved surface area-to-volume ratio, such that samples can contact the length of the fibers, similar to how cells may penetrate and move along the flocked fibers. Probst *et al.* quantitatively and qualitatively highlighted the advantage of flocked swabs in bacterial culture using Nylon-flocked swabs and cotton swabs.[25] Nylon-flocked swabs recovered 45.4 ± 1.2% of inoculated B. atrophaeus while cotton swabs recovered only 13.2 ± 1.%. Based on SEM images, Probst *et al.* captured how the fiber orientation of flocked swabs, compared to traditional swabs, allows for more bacteria to adhere along the flock fibers' surfaces and for more bacteria to release from the swab during extraction [Figs. 13(a)–13(f)]. In another study, Rock *et al.* compared nylon-flocked swabs and cellulose sponges for carbapenem-resistant Enterobacteriaceae recovery, finding that flocked swabs improved the overall gram-negative bacteria recovery (100%) compared to cellulose sponges (21%), but were similar for the recovery of carbapenem-resistant Enterobacteriaceae.[172] Similarly, Walker *et al.* showed that anatomically designed flocked rectal swabs were able to detect the same amounts of bacteria as stool samples, where flocked swabs detected 168 bacterial samples, while stool samples detected 167 samples.[167] While the flocked swabs did not exhibit enhanced detection, it performed similarly to the standard stool sample in a cleaner and faster technique that can be employed in a wider range of climates and clinical settings.

2. Viral swabbing

In addition to improving overall bacterial swab yields, flocked swabs show promise in viral extraction. During the initial SARS-CoV-2 outbreak, flocked swabs were brought to the forefront of viral swabs, with other swab types contributing to failed diagnostic tests due to low sample isolation. To date, flocked swabs have shown improvements in a variety of viral extraction applications ranging from oral, nasopharyngeal, vaginal, and anal swabs. Viviano *et al.* compared cellular retrieval of cotton and flocked swabs in self-administered vaginal sampling for the detection of human papillomavirus (HPV).[166] Detection of HPV by cotton swabs was 29.7%, while detection by flocked swabs was 38.1%, representing a significantly increased simple detection rate. Additionally, the mean number of cells collected per ml was only 96 726.6 using cotton swabs, and 425 544.3 using flocked swabs. Finally, flocked swabs released more cells (17 503.36) than cotton swabs (13 130.42). Taken together, the study demonstrated a significantly higher detection rate by extracting over four times as many cells and releasing more of the extracted cells. A similar study by Wiley *et al.* investigated the use of flocked swabs for anal histological intraepithelial lesion (hHSIL) identification in transgender women and gay or bisexual men who have sex with men.[173] The study sought to extract viruses related to hHSIL and cells for lesion identification, finding that flocked swabs offered improved specificity compared to Dracon swabs (76% vs 69%), though both swab types had similar sensitivities (47% vs 48%). A plethora of studies investigating and comparing different clinical swabs for the extraction of viruses via nasopharyngeal aspirate have repeatedly demonstrated that flocked swabs have higher yields, higher sensitivity, and higher viral detection rates.[165,169,174,175] Flocked swabs have also demonstrated the ability to detect antibodies from common pathogenic viruses via oral swabbing, detect SARS-CoV-2 via nasal swabbing, and extra and release DNA from isolation studies.[38,170,171,176,177] Puritan Medical Products has lead the way innovating a variety of flocked swabs including PurFlock ULTRA® and HydraFlock® swabs that have standard or nonstandard flowering fiber tips, which are created using an island-in-sea fiber flocking methodology [Fig. 13(g)].

V. CONCLUSIONS, INDUSTRY PERSPECTIVE, & FUTURE DIRECTIONS

Electrostatic flocking is a unique textile engineering technique that applies a surface finish of vertically aligned microfibers onto an adhesive-coated substrate. Since flock fibers may be applied to any surface that an adhesive may be applied to, it has wide-reaching applications. Characteristics associated with aligned fibers include anisotropy and high compressive strengths, tunable microcapillary action, high surface area-to-volume ratios, and tunable surface wetting. There are many material considerations to evaluate when determining the feasibility of flocking. Fiber geometry, conductivity, elastomeric and thermal properties, susceptibility to post finishing treatments that are all important considerations to create flock fibers. Additionally, creating conductive, viscous adhesives that either thermally or chemically cured without

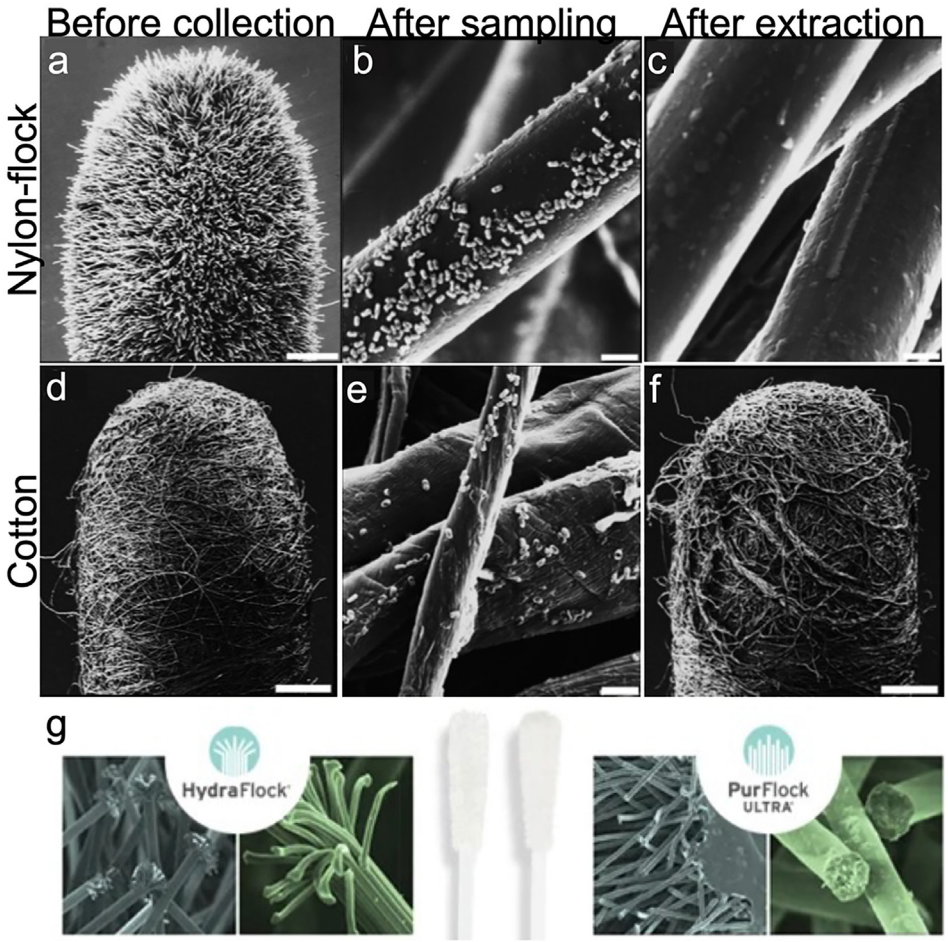

FIG. 13. Flocked swabs for sample extraction. (a–c) SEM images of nylon flocked swabs before collection, after sampling (presence of bacteria), and after extraction (absence of bacteria). (d–f) SEM images of traditional cotton swabs before collection, after sampling (presence of bacteria), and after extraction (absence of bacteria). Scale bar in (a, d, f) = 1 mm. Scale bar in (b, c, e) = 5 um. Reproduced with permission from Probst *et al.*, Appl. Environ. Microbiol. **76**, 15 (2010). Copyright 2010 American Society for Microbiology.[25] (g) Photograph and SEM images of two FDA approved flocked swabs developed by Puritan Medical Products, with the HydraFlock® on the left and the PurFlock® on the right. Photograph supplied and reproduced with permission from Puritan Medical Products.

disrupting the alignment of fibers is a necessary consideration. The many design considerations have perhaps hindered the progression of flock technology in the biomedical field although the recent progresses reported in this review demonstrate a renewed and growing interest.

While flocking may enhance or functionalize many materials for a variety of applications, it offers several distinct advantages in medicine. Utilizing the high relative porosity and excellent mechanical properties, flocked TE scaffolds may be suitable for bone TE. Tuning flocked or flock-reinforced scaffolds may show promise as standalone or hybrid scaffolds for load-bearing or static bone TE applications. Additionally, flock bandages and wound healing devices may act through different mechanisms inherent to flock-based structures (microcapillary action, granulation tissue formation) to enhance clinical outcomes related to wound healing. Nevertheless, more measures must be taken to use flocking for biomedical applications, though. Ensuring that the scaffolds/bandages are degradable, biocompatible, non-cytotoxic, and immunological inert are minimum requirements for their use in TE/wound healing.

One sizable consideration when choosing to utilize an engineering technique for clinically relevant biomedical research is the feasibility of scale-up and reproducibility. To this end, flocking is at a sizable advantage compared to other technologies, as the United States flocking industry is thriving. With multiple large-scale flock producers and manufacturers, nearly any conceivable application worth scaling is practical. Research and development efforts in the flock industry are beginning to blossom, with flocking companies initiating or partnering on projects related to patient comfort, moisture management, fabric breathability, liquid sampling, anti-slip properties, protective barriers, smart sensors, and filtration devices. With the manufacturing infrastructure in place to use flocking at a large scale, we anticipate an increase in innovative flock technologies in the coming years.

Overall, this comprehensive outline reviews the fundamentals required for flocking and describes different methods to create biologically relevant flocking components: fibers, adhesives, and substrates. Flock-based scaffolds offer distinct architectural advantages over other kinds of TE scaffolds (higher porosity, anisotropy, biomimicry) while retaining biological relevancy by being biodegradable and bioactive. Further, different applications, future studies, and directions of research are considered, with industrial perspectives on the feasibility of scaling and reproduction reported. As an under-investigated technology, there are still many avenues of research utilizing flocking technologies that have not yet been explored and we hope to see an increase in this technology's use in the coming years.

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AUTHOR DECLARATIONS

Conflict of Interest

We have no conflict of interest to disclose.

Author Contributions

A.M. prepared the figures, wrote, and edited the manuscript. R.S. and J.V.J. contributed to writing the manuscript. D.B. and J.X. reviewed and edited the manuscript.

DATA AVAILABILITY

Data sharing is not applicable to this article as no primary data are presented.

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