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Review

Review: Unraveling the less explored flocking technology for tissue engineering scaffolds

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Clinical translation of the scaffold-based tissue engineering (TE) therapy still faces multitude challenges despite intense investigations and advancement over the years. In order to circumvent clinical barriers, it is important to analyze the current technical challenges in constructing a clinically efficacious scaffold and subsequent issues relating to tissue repair. The major limitations of current scaffolds are lack of sufficient vascularisation, mechanical strength and issues related to the osseointegration of the scaffold in case of bone tissue engineering. Hence, this review accentuates the main challenges hampering widespread clinical translation of scaffold-based TE, with a focus on novel scaffolds fabricated using flocking technology. Flock technology is a well known method used in textile industry. Flocking application for scaffold fabrication is less explored yet they offer promising solutions for creating anisotropic scaffolds with high compressive strength despite of high porosity. Critical insights on the current researches of the fabricated flock scaffold and future directions for advancing flocking to next-generation TE scaffolds into the clinical realm are discussed. This review will serve as a one stop arrangement for understanding the vital pre-requisite properties of scaffolds, principle as well as factors governing flocking of scaffolds and the improved properties of flock scaffolds. Further, this will promulgate flocking technology as a plausible candidate to spearhead TE scaffold fabrication.

1.Introduction

The deficiency of the organ has been a daunting challenge across the globe due to the increasing occurrence of organ failure and lack of organ donors for satisfying the growing demand for organ transplantation. There has been a drastic increase in the count of patients on the waiting list in the past decade. In America, one patient is added on to the national organ waiting list every 10 minutes, whilst an average of 18 deaths occurs each day owing to the reason of organ donor shortage. Statistics by New York Organ Donor Network delineates that a total number of 28,535 organ transplanted in the year 2011, with 79% of the transplants were obtained from deceased donors and the number of living organ donors was found to decrease every year.[1] In order to circumvent the dire shortage and long patient wait time for organ transplantation, novel tissue engineering strategies can be exploited as a putative strategy to replace, repair, restore diseased tissues and improve the quality of lives of patients.

At present, United States contributes 48.6% of the global market revenue for tissue engineering solutions and it allocates 60% of its global tissue engineering expenditure for research and development (R&D).[2] As there is a significant increase in active lifestyles, accidents, obesity and ageing population, the orthopaedic solutions encompassing joints and bone, osteoporosis and bone tumours repair remain to be at highest demand. Bone is the second most transplanted tissue globally and there is an immense need for bone grafts and substitutes.[3] Global statistics dictates that an annual incidence of nearly 15 million fracture cases, of which up to 10% are made severe by non-unions.[4, 5]

Whilst addressing the challenges encountered at the time of bone TE therapies, it is necessary to understand the underlying cause resulting in non-union repair and then tailor these TE strategies accordingly. Typically, non-union fractures fail to heal even after 3–6 months. This is mainly caused due to different factors like surgical technique, pathological conditions and/or fracture types that differ between patients. These fractures can be subdivided as

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hypertrophic, oligotrophic and atrophic non-unions. These conditions occur as a result of insufficient mechanical stabilization, poor fracture apposition and poor vascularity respectively.[6] The Fig. 1 depicts the different types of non-union fractures and the associated with each type of fracture.

The general feature of non-union fractures is the presence of significant gap between the fractured bone ends. For bridging this gap, a platform is essential to serve as a temporary support at

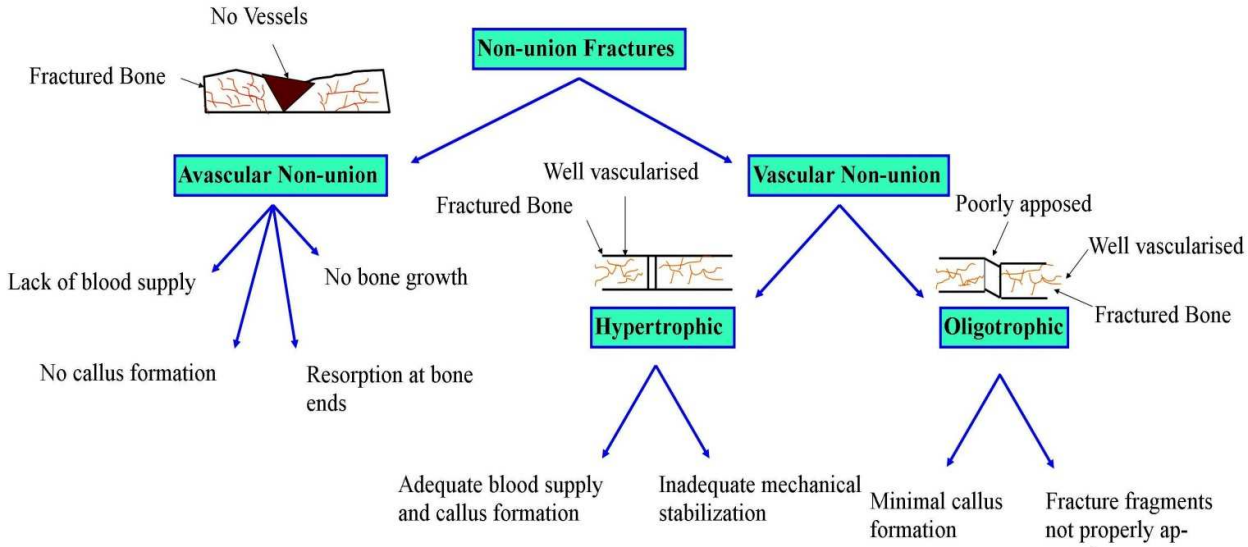

Fig. 1 Different types of non-union fractures and the associated problems

defect area.[7] Current strategies implemented for bone grafts are autografts, allografts and synthetic grafts. The autografts are bone harvested from the patient's own body where it still remains to be gold standard owing to its osteoconductive and osteoinductive environment and non-immunogenicity.[8, 9] Conversely, there are drawbacks associated with inadequate quantities for harvest and donor morbidity which called for the need of alternative solutions.[10] Whilst allografts and synthetic grafts circumvent these problems, they do not confer the sufficient osteoinductive signals and vascularity, hence resulting in poorer bone healing in comparison to autografts.[5] Moreover, allografts may experience a possibility of graft rejection due to the host immune system and disease transmission from donor to host, whereas artificial grafts suffer from fatigue and wear over a period of time.[5, 9]

At present, the lack of integration of grafts with bone substitution occurs often only at the ends of grafts, thereby resulting in non-unions with late graft fracture occurring at a rate high as 60% in 10 years.[11-13] With the advent of novel engineering techniques like TE hold a great potential as an alternative for fracture repair. On this regard, the scaffold plays an indispensable role in TE. Predominantly, the scaffolds produced by electrospinning were very well explored and there are more than 3000 papers (Scopus) published till now.

The application of flocking is not only limited to the non-union fractures, but it may be used in different kind of bone scaffolds, cartilage tissue engineering, skin tissue engineering, extracorporeal organ replacement or intervertebral disc tissue. Tissue engineering load-bearing parts of the body depends on either scaffold adhesion or integration with the surrounding tissue to avoid dislocation.[14] Thus, the tissue engineered scaffold is a cornerstone in regeneration of the intervertebral disc (IVD). Lower back pain (LBP) is a common problem across the globe, affecting 80% of adults at certain point in their lifetime and results in loss of approximately $100 billion annually.[15] The disk degeneration occurs due to a decline in the viable cell content of the central nucleus pulposus (NP) of the IVD, results in reduced rate of matrix synthesis. This leads to dehydration and inability to bear compressive loads. The compressive loads therefore placed on the outer annulus fibrosus (AF), tear and allow the migration of the NP via AF. Ultimately, the NP can impinge on nerve routes leading to LBP.[16] Flocking technology offers a promising solution to create scaffolds with a high compressive strength despite of high porosity for IVD.[17]

On the other hand, injury or disease of articular cartilage usually incurs damage, but it has a very limited ability to regenerate. In chondral defects, where a lesion is present within the articular cartilage, the vasculature would be absent. As a result, progenitor cells in blood and marrow will not be able to enter the damaged region to initiate or support the healing process. Resident articular chondrocytes do not migrate to the lesion, thereby leading to lack of production of reparative matrix. Hence, the defect is not filled or healed and remains permanently.[18] Tissue engineering strategies that transport a matrix seeded with chondrogenic cells (chondrocytes or progenitor cells) have been investigated experimentally and clinically.[19, 20] Even though there is good in vitro data utilizing different techniques, the reasons for the failure of cell-based cartilage repair techniques to form hyaline repair tissue *in vivo* still remains obscure. It may be coupled with unorganized nonphysiological orientation of fibres in the scaffolds utilized for cartilage repair.[21] The demanded technology must confer the possibility to create anisotropic matrices with a high compressive strength in spite of high porosity and flock technology can be considered as a putative candidate for solving this complication.[22]

Despite scaffolds produced using the flocking technology possess better mechanical properties, porosity and etc when compared to electrospun scaffolds, the lack of exploration and utilization of this novel technology for TE is evident from the meagre publications available till today on flock scaffolds for TE. Hence, the prime motive of this article is to shed light on novel flock technology utilization so that it may be further explored by researchers for the TE applications. Thus, the significant pre-requisite properties for ideal scaffolds are discussed prior to the introduction of flock technology. This is done for better understanding as well as tailoring the properties of the scaffolds to function with optimum efficiency. Since all the works done so far utilizing flock technology in biomedical engineering field are for scaffold fabrication, an thorough attempt is made to summarize all the works done using flocking for scaffold fabrication in TE. This review will focus on the key challenges, considerations and unmet demands of current scaffold designs for the TE application that are currently lacking success, by underscoring the scaffolds fabricated through novel flocking technology.

2.Scaffolds for tissue engineering

Even though there are more than 5000 publications and 100,000 citations in the area of scaffolds and TE in last twenty years (using the Web of Science® database, 2012 Thomson Reuters), the ubiquitous clinical translation of scaffold-based TE therapies still found to be at infant stage. At present, the success of scaffold based TE is still confined to small defects and it requires long term monitoring. For achieving a more clinically feasible scaffold, the right design and material properties needs to be investigated intensely. Moreover, scaffolds also need to be tailored to fix the different types of bone defects and fracture sites. Hence, the material must possess excellent biocompatibility and should not elicit physiological response from the host. The ideal scaffold must confer a provisional structure which degrades in tandem with the formation of new tissue in course of time. For designing a suitable scaffold for successful TE needs meticulous consideration of different factors like type of material used for developing the scaffold, its architecture and porosity, surface chemistry, osteoinductivity and mechanical strength. In filling up the gap defects, considerations like the ease of manufacturability and clinical handling are also important.

Hence, the criteria necessary for scaffold architecture and microenvironment such as porosity cell proliferation and tissue formation, vascularisation, cell entrapment via capillary action and mechanical property requirement for skin, IVD, cartilage and bone scaffold TE where flocking may be widely utilized are discussed in the subsequent sections.

3.Scaffold architecture and microenvironment

3.1 Porosity, cell proliferation and tissue formation

The cell behavior is inter-reliant with the scaffold architecture as the extracellular matrix (ECM) confers cues that influence the specific integrin–ligand interactions between cells and the surrounding.[23] Thus, the scaffold environment plays a vital role in influencing cell proliferation or direct cell differentiation.

The study carried out to investigate the role of 3D silk fibroin scaffolds on cell proliferation and migration of human foreskin fibroblast showed that pore sizes of 200 to 250 mm as well as porosity of nearly 86% improved the cell proliferation.[24] Yet, cell

proliferation of these scaffolds which has smaller pore sizes of 100 to 150 mm can be increased by having higher porosity of almost 91%. Therefore, by altering the either pore size or porosity the cell viability and proliferation can be improved significantly.[24-26]

In a recent study, Lien et al. demonstrated that chondrocytes displayed preferential proliferation and ECM production for scaffolds having pore sizes in the range of 250 to 500 mm.[27] It was found that this pore size range is capable of maintaining the phenotype of cells, whereas pores ranging from 50 to 200 mm resulted in cell dedifferentiation.[27] On the contrary, synthetic human elastin scaffolds possessing an average porosity of 34.4% and mean pore size of 11 mm facilitated infiltration of dermal fibroblasts, whilst a lower average porosity of 14.5% and mean pore size of 8 mm only enhanced cell proliferation across the scaffold surface.[28]

Recent findings in cellular and molecular biology have paved an exciting approach to disc regeneration that focuses on the delivery of viable cells to the degenerative disc. Adult mesenchymal stem cells (MSCs) are multipotent stem cells equipped with self-renewal ability and have the ability to differentiate into diverse specialized cell types, including chondrocyte lineages. Thus, the stem cell therapy is used widely in disc degeneration to repopulate the disc with viable cells that are capable of producing the ECM and restoring damaged tissue.[29] In a work done by Yang et al., mesenchymal stem cells were used to cease IVD degeneration via chondrocytic differentiation and stimulation of the endogenous cells.[30]

The scaffolds serve to mimic the actual *in-vivo* microenvironment whereas cells interact and behave according to the mechanical cues obtained from the surrounding 3D environment. Hence, the role of porosity and interconnectivity in scaffolds is necessary to permit the cell migration within the porous structure thereby facilitating the cell growth whilst overcrowding is avoided.[31]

The presence of porosity in the scaffold is a pre-requisite property of scaffolds for inducing osteogenesis and the in-growth of bone tissue. This is evident from the study done by Kuboki et al., where there was no new bone formation was observed on solid scaffolds that lacked porosity, whereas bone formation was observed in porous HA scaffolds with BMP-2 following implantation in an ectopic rat model.[32] Karangeorgiou et al. demonstrated that when the porosity is smaller, it limits the volume of space for cellular proliferation, thereby forcing cells to aggregate and differentiate.[33] Thus, the presence of smaller porosity stimulated the osteogenic differentiation of the cells which expressed higher alkaline phosphatase (ALP) activity and other osteogenic markers.[33, 34] Conversely, when there is higher porosity, it provides more space for cell proliferation and improves mass transport that increases cell viability, ultimately facilitating bone tissue in-growth.[34]

The pattern of which the bone in-growth occurs needs to be given a high priority in TE. It was found that the bone in-growth pattern varies according to the scaffold architecture, which is palpable from a study by Simon et al.[35] Here, the bone forming capacity of different scaffolds was studied following its implantation in a rabbit cranial defect model. The result of the study dictates that continuous bone-forming pattern was found in case of scaffolds with a random pore size whilst scaffolds with pores of similar size but with solid walls, led to the formation of discontinuous bone islets throughout the scaffold. However, when the walls of the scaffolds were made porous but the pores were of the same size, discontinuous and continuous bone formation was observed.[35] By utilizing electrostatic flocking the fibres are arranged perpendicular to the base material and parallel to each other. At the time of incubation, cells were found to settle prevalently on and between the fibres and build an extra-cellular matrix. The cell colonization as well as construction of the extra-cellular matrix was directed by the flocking fibres. Thus, the end result is an oriented tissue structure, where cells and the extra-cellular matrix are aligned relative to one another almost identical to natural tissue.[36]

Recently, the significance of adequate scaffold porosity to permit vascular infiltration, tissue in-growth and efficient mass transport for maintaining cell survival in thick grafts was underscored.[7] Moreover, it was also found that large pore size favours cellular migration.[33] Based upon the application, the most favourable porosity and pore size may vary, hence necessitating consideration on a cut-off range with desirable properties for scaffold-based TE. Architecturally, the interconnectivity between scaffold filaments and pores is vital in helping nutrient transport, promoting cellular migration, cellular bridging and in-growth of tissue.[33, 37, 38] The

continuity of filaments aids the even transmission of the shear stress along its filaments throughout the scaffold when a biomechanical force is exerted.[39] In addition to that, scaffold stiffness can also be increased by altering its structural architecture via modification of its porosity. Zein et al. demonstrated that change in PCL filament orientation in fused deposition modeling technique, can aid in tailoring the scaffold stiffness.[40] Williams et al. also showed that PCL scaffolds which was fabricated through selective laser sintering resulted in varying stiffness values from 52 to 67 MPa. This varying stiffness was found to be depending on the degree of porosity. In case of scaffolds produced by flocking, a pore is defined as the space between the fibres. The flock scaffolds possess large pores and they are highly interconnected. The pore size can be changed easily by the varying the flocking time. The flocked scaffolds possess huge pores as well as oriented fibres must facilitate cell infiltration make the flock scaffolds superior when compared to scaffolds made by other textile technologies like electrospinning.[41]

3.2. Vascularisation

The scaffold microarchitecture also plays a vital role for supporting vascular formation and growth. In general the pore size for TE scaffolds used is 150–500 µm for providing necessary vascularisation.[37] There has been recent evidence showing the exceptional role of scaffolds in supporting extensive vasculature formation within macroporous scaffolds in *in vivo* implantation conditions. In a work done by Zhang et al., and Rai et al. dictate the formation of extensive vasculature within the PCL/TCP scaffold following its implantation with mesenchymal stem cells (MSC) and platelet-rich plasma (PRP) respectively in a critical-sized rat femoral model.[42, 43] Whilst many have performed this *in vivo*, only less number of studies has been performed on the *in vitro* vessel formation in 3D scaffolds. Recently, Liu et al. showed formation of a complex *in vitro* vascular tree within the 3D scaffold in conjunction with co-culture of endothelial progenitor cells (EPC) and MSC.[44] This observation is attributed due to the inherent vasculogenic potential of EPC, supported by their pericytes besides suggesting the importance of a vessel development *in vitro* condition.[44] The mean distance between the fibres of the flocked scaffold varies between 40 µm and 250 µm, based on the cell types for which the scaffold is to be used. Hence, interspaces formed between the fibres allow optimal colonization of the cells in the scaffold.[36]

Conferring a blood-vascular network for promoting survival and integration of cells in thick dermal substitutes is important for the successful outcome of skin tissue engineering. However, promoting vascularization also suffers a critical setback in today's skin tissue engineering practice. Different cell types have been contemplated and tested, widely in preclinical studies, to enhance vascularization. When the clinical condition allows delayed reconstruction of the defect, an autologous approach is preferable, yet in acute cases allogeneic therapy is required.[45]

When the IVD is considered, at birth the human disc has some vascular supply present in both cartilage end plates and the anulus fibrosus. Nevertheless, these vessels soon recede leaving the disc with little direct blood supply in the healthy adult. As the age increases, water is lost from the matrix, and the proteoglycan content also varies and diminishes.[46] Since cartilage does not require vascularization, vascularization of cartilage is not discussed here.[47]

3.3. Cell entrapment via capillary action

The microarchitectural design of the scaffold plays a pivotal role in aiding the cellular entrapment within the scaffold, which is mediated by capillary action. The basic principles of capillary action ascertain the ability of liquid to flow against gravitational forces into narrow spaces without assistance. This scenario happens due to the intermolecular attraction forces between the liquid and its surrounding environment. Mathematically, the capillary pressure can be calculated by using Washburn equation $Pc=2\gamma\cos\varphi/r$, where Pc denotes capillary force, γ denotes surface tension, r denotes the radius of the capillary, and φ is the contact angle of the liquid on the capillary material.[48] When there is a porous filamentous network, cell suspension can flow with ease and get trapped within the scaffold following the cell loading. Thus, the porous filamentous network acts as a temporary support for the growth of cells. Furthermore, when the scaffold was implanted into a critical-sized rat calvarial defect, it has led to the observation of scaffold acting as a hemostat which absorbed blood via capillary action.[48] Hence, all these results recommend that by leveraging the capillary pressure

difference, it may improve cell-nutrient transfer and hence may result in enhanced cellular survivability within the scaffolds.

3.4 Mechanical properties requirement

Despite higher porosity and pore sizes may enable nutrient and oxygen delivery or allow more cell ingrowth, the mechanical properties of the scaffolds will be compromised because of the large amount of void volume.[49] Therefore, there is a limit to the porosity extent or pore sizes that could be included into a scaffold without compromising its mechanical properties to a great extent. Generally, scaffolds must have necessary mechanical strength to maintain integrity until new tissue regeneration occurs. There should also be adequate space for cells to proliferate and to facilitate the transport of nutrients and removal of wastes.[50] It is also essential to make sure that material property of the scaffolds matches the native tissue *in-vivo*, for instance, scaffolds fabricated for bone tissue engineering must have comparable strength to the native bone tissue to endure physiological loadings.[50]

The Young's modulus (E) of the skin varies between 0.42 MPa and 0.85 MPa for the torsion tests.[51, 52] The stress values fluctuates between 4.6 MPa and 20 MPa in tests carried by using mechanical equipment, whereas it ranges from 0.05 MPa and 0.15 MPa in the suction tests.[51-53] Large discrepancies in the results may dictate the corroboration for the variation occurring in the skin during the process of ageing, and delineates the differences in the skin properties, depending on its anatomic location.[54]

Similarly, a recent analysis of mechanical property of artificial IVD was performed by Kannan et al. In this study, the significance of distinguishing loads was depicted. For example, load that frequently occur during everyday life, like walking and lifting small weights, is different from rare extreme loads, i.e. those that occur whilst lifting heavy objects or falling. The first type of load is the IVD fatigue strength, while the second determines the maximum strength of the IVD, both of which are vital failure criteria. It was found from the study that, minimum IVD failure strength in compression is 8 kN, in lateral and sagittal shear 2 and 3 kN, respectively.[55]

The mechanical properties of cartilage differ with respect to its fluid content, hence making it essential to know the stress–strain history of the tissue to predict its load- carrying capacity. Besides, the material properties also vary with pathology. The compressive aggregate modulus for human articular cartilage corroborates in a vice versa manner with the water content and in a direct manner with proteoglycan content. However, there is no correlation with the collagen content, hence signifying that proteoglycans are responsible for the cartilage tissue's compressive stiffness. The Young's modulus of the cartilage is 1-10 MPa under tension and 1MPa under compression.[56]

Despite scaffolds developed nowadays for TE have been shown to yield promising results for supporting osteogenesis *in vitro* or *in vivo*.[43, 57-59] However, it still lags in terms of structural integrity of scaffolds to endure biomechanical forces over longer duration, where they suffer from creep and fatigue upon clinical implantation in orthopaedic applications. Moreover, when the porosity and pore size in TE scaffolds is increased, it reduces the mechanical strength of scaffolds. In spite of numerous advances were been introduced in the area of scaffold development, their limitation of inadequate mechanical properties within the range of the human cancellous bone still exist.[60] Devoid of an appropriate stiffness and material strength at the site of implantation, this implanted scaffold may lead to consequential resorption of native tissue.[7, 61, 62] This critically blocks the progression of bone formation, remodelling and functional union repair which may cause the bone recovery progress prolong to several years.[63] Successful clinical translation of scaffold-based TE majorly depends on improvement of the material properties to mimic the key mechanical properties of bone, such as compressive strength, toughness, and stiffness of the material.[61]

Besides that, the mechanical integrity of bioresorbable scaffolds for prolonged period of weight-bearing tissues has also been identified a serious problem of scaffolds. The design of scaffold architecture through different fabrication techniques seems to be a putative candidate to circumvent the problems associated with the scaffold vital properties to serve as a viable load bearing tissue. Via electrostatic flocking, the fibres are aligned perpendicular to the surface of the substrate. High fibre pull-out resistance achieved by electrostatic flocking hampers the detachment of the fibres from the substrate material. Surprisingly, the flock scaffold confers an elastic growth lattice, which is stable against compression. Combined with the base material, fibres nearly mimic the function of the extra-cellular matrix which occurs in natural tissue.[64]

3.5 Ideal Flock scaffold

One of the major requirements of any tissue scaffold is biocompatibility. Biocompatibility is defined as ability of the implant to support normal cellular activity including molecular signalling systems without eliciting any local as well as systematic toxic effects to the surrounding tissue.[65] Ma et al., studied the pore size of a 3-D polyethylene terephthalate (PET) nonwoven fibrous matrix for tissue development of human trophoblast ED27 cells. In this work, two types of matrices were evaluated, which are low porosity (LP) and high porosity (HP). The LP matrices had a porosity of 0.849 as well as an average pore size of 30 µm, whilst HP matrices had a porosity of 0.896 and an average pore size of 39 µm. As the trophoblast ED27 cells were cultured on these matrices, the initial cell proliferation rate in the LP matrix was found to be higher compared to the HP matrix. Moreover, cells cultured in the LP matrix spread across adjacent fibers more easily, which lead to higher cell proliferation rate. Nevertheless, the smaller pore sizes of LP matrices restricted the formation of large cell aggregates and decreased cell differentiation. On the other hand, cells cultured in HP matrices had a higher degree of cell differentiation and aggregation. This dictates that nonwoven matrix encourages the cell proliferation even at a lower pore size.[66] Similarly, in a work done by Mandal et al., the freeze-dry technique which is not a non-woven technique was utilized, and it was found that human dermal fibroblast cell proliferation and migration was promoted at 200-250µm.[24] Thus, flocking being a non-woven technique for scaffold production, can serve as almost ideal scaffold. This is because, in flock scaffolds, a pore is defined as the space between the fibers and it is highly interconnected facilitating cell infiltration. The pore size can be easily adjusted by altering the flocking time. Thus, this fact makes the flocked scaffolds superior to scaffolds made by other textile technologies like electrospinning, melt blown, weaving, knitting and etc.[41, 67, 68] The above said fabrication techniques did not provide sufficient physical strength and porosity for the scaffolds. Spefically, the novel flocking technology is relatively very less explored for scaffold fabrication in comparison to electrospining despite the flock scaffolds demonstrates better viability by improving the cell adhesion and providing the required biomechanical support.[41] Thus, an unmet demand exists for the development of scaffolds with enhanced properties. Hence, a succinct insight is given on the flocking technology and current applications followed by how it can be exploited for TE-scaffold fabrication in the upcoming sections.

4.Flock Technology

The fibres are being used as material for sutures in surgery for long period of time and this is a simple application of textiles in the field of medicine. Inspired by this simple application, there are certain textile technologies which can be leveraged to fabricate complex structures and three dimensional designs. The prime reason for the exploitation of textiles technology in regenerative medicine and tissue engineering is that they exhibit multitude of advantages in comparison to other available methods to fabricate scaffolds. One of the palpable advantages is the outstanding surface-to-volume ratio of fibres as well as the resulting textile structures. With their larger surface, these fibres possess the propensity to offer a huge area for cell adhesion. Hence, this makes them to be an ideal choice for effective cultivation of cells. By varying the fibre diameter or distance, and surface properties of the fibres, the properties of the resultant scaffolds can be changed very easily. The different textile technologies available nowadays are electrospinning, weaving, knitting, flocking etc allow for the alteration of the properties and the adaptation to suit for a particular application.[69-71] In conjunction to scaffold production, Ramakrishna has reviewed the various textile technologies which may be used for scaffold production.[72] Amongst all the available textile technologies, the scaffold fabricated by flocking confers exceptional properties, yet it still remains in an infant stage owing to the lack exploration in the novel flocking technology for tissue engineering applications.

It has been known for a long time that flocking can be utilized on all kinds of items for creating special surface design.[73-75] Flocking was first demonstrated by French researchers to develop flocked wallpapers almost two centuries ago, and it has gained attention by leap and bounds during the last two decades following the inclusion of electrostatics in flocking, resulting in so-called electrostatic flocking.[76, 77] Electrostatic flocking is a technology which is implemented to apply short length fibres on a substrate which is covered with adhesive in an applied electrical field thus the fibres were placed vertical to the substrate. When an electrostatic field is applied, the fibres are aligned and accelerated heading towards the adhesive coated substrate. Reaching the adhesive layer the fibres will be stuck perpendicularly to the substrate and thereby

conferring the surface a velvet-like look. Flocked fabrics are special textile-based products utilized in outer-wear as well as home textiles where it is composed of substrate, adhesive and flock fibre.[78-81] Whilst applying textile, velvety or brush-like surfaces to almost any material, we can obtain fancy surface characteristics. Moreover, flock applied materials are also utilized in the technical area and the household products.[82] Some uses of the common flocked materials are on clothes, sheets, curtains, packaging for perfumes, car seats, car glove boxes, car headliners, floor coverings, eye liner brushes and scrubbing pads, where the consumers are always expecting for something different and unusual.[83, 84] This is represented in Fig. 2.

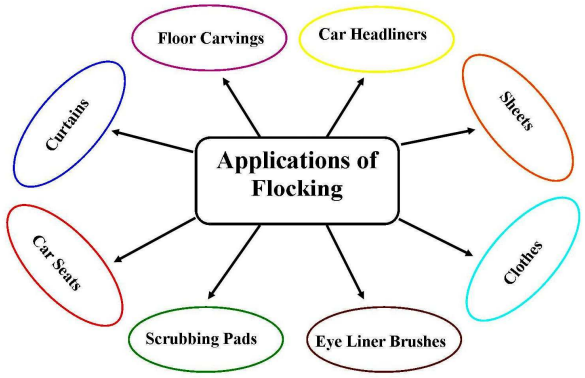

Fig. 2 Common applications of flocking technology.

In a recent work, flocking was utilized in leather finishing surface modification with flock fibres for improving its surface characteristics.[85] This study was performed as leather is such a valuable product which cannot be scrapped or cut out due to its surface defects. Despite conventional finishing recovers the defects to a certain extent by concealing them, fashionable surface effects are always preferred by consumers. Hence, flocking is considered as a reasonable technical approach for achieving this. Likewise, in other recent studies mechanical characterization of flocked fabric was performed for flocking application in automobile seat cover.[86, 87] Regression models were developed in these studies for investigating the relationship between rubbing and the tensile strength of the flocked fabrics. It was concluded that the regression models developed are viable and reliable tool and flocked fabric can be considered as putative candidate for seat cover material in automotive industry.

Initially, flocking was developed for improving the aesthetic value and haptics of textiles material. However, nowadays flocking can be found in diverse technical applications. Apart from flocking implementation in textiles field, flocking was also proposed for biomedical application. Recently, Chen et al., performed multiple microparticles flocking with automatically controlled optical tweezers.[88] In this study, an efficient technique for achieving microparticles flocking using robotics and optical tweezers technology was introduced. All particles trapped by optical tweezers can be automatically moved toward a predefined region without collision. In addition to that, several solutions were proposed in this work for the flocking manipulation of microparticles in microenvironments. Flocking controller was proposed for generating the desired positions and velocities for particles' movement. A velocity saturation technique was also implemented to avoid the desired velocities from exceeding a safe limit. Finally, two-layer control architecture was also proposed for controlling the motion of the optical tweezers. Thus, this architecture can help make many robotic manipulations achievable under microenvironments. Hence, the proposed technique with these solutions can be implemented in many bio-applications particularly in cell engineering and biomedicine. Tests were conducted on yeast cells with a robot-tweezers system to verify the effectiveness of the proposed approach which yielded desirable results.

The rationale of a scaffold for TE is to mimic the properties of the extracellular matrix (ECM) of the tissue that ought to be restored as well as to adopt its function. For satisfying this requirement, a scaffold must be mechanically stable, serve as a matrix for cell adhesion, proliferation and differentiation, allow nutrient and oxygen support and ultimately confer enough space for the newly synthesized matrix and blood capillary in-growth. Since the short fibres were aligned parallel to each other in scaffolds fabricated using flocking, therefore pore spaces were formed for seeding of cells, tissue formation and in-growth. Different cell types require different pore volumes for adhesion as well as proliferation. By varying the fibre's diameter, the flocking time, or the acceleration voltage, the distance between fibres, the pore size of the flocked scaffold could be controlled.

In a nutshell, the flock technology provides the possibility for easily adjusting the pore size to suit different types of cells, tissues and

medical applications. Moreover, scaffolds produced by flock technology possess highly anisotropic properties. They are stiff in the direction of the fibres, yet flexible whilst bending and tension (in case of substrate possessing elastic properties). On the flip side of the coin, most of the porous scaffolds fabricated with conventional techniques like freeze drying, gas foaming or particle leaching exhibit an isotropic porosity and mechanical properties. Since majority of tissues in the bone tissues in body exhibit anisotropic properties, hence anisotropic replacement materials are better suited than isotropic ones. Hence, to cater medical applications it is necessary to replace conventional materials for flocking with biocompatible and degradable ones. There are different pre-requisites concerning the flocking technology such as substrate, adhesive, fibres and etc which are discussed as follows.

4.1 Principle of electrostatic flocking

To transfer flocking technology to medical applications, it is important to substitute all components such as substrate, adhesive and fibres commonly used with biocompatible and resorbable materials.[22] Apart from biocompatibility another vital factor to be contemplated is the electric conductivity of the materials which needs to be suitable for the electrostatic flocking process. In majority of studies, mineralized membranes (''tape'') were utilized as model substrates.[89] The Fig. 3 depicts the principle of electrostatic flocking.

The basic principle of flocking is to apply short fibres to a substrate covered with adhesive. The fibres are aligned in an electrostatical field and accelerated towards the adhesive covered substrate. Reaching the adhesive the fibres become stuck perpendicular to the substrate or base material and results in flock coating. Finally, the crosslinking is performed using crosslinking agent to strengthen the bonding between fibres, adhesive and base material.[64]

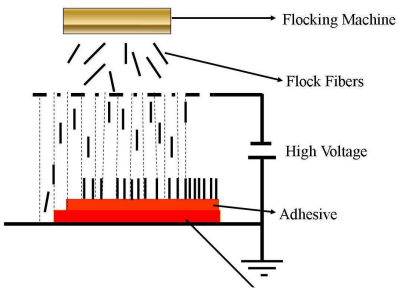

Fig. 3 Principle of electrostatic flocking.

Acid-soluble collagen type I, obtained from calf skin, need to be dissolved in hydrochloric acid prior to the addition of $CaCl_2$ and phosphate buffer (KH_2PO_4/ K_2HPO_4). For the purpose of fibril assembly of collagen as well as its mineralization, the used solutions have to be kept for a period of 12–24 h at 37ºC. A membrane of mineralized collagen ought to be produced through vacuum filtration with a glass filter plate, later crosslinking have to be done with N-(3-dimethylaminopropyl)-N0-ethylcarbodiimide (EDC), and ultimately freeze-drying.[90] Commonly 20 wt.% gelatine which is dissolved in aqueous 0.5M NaCl solution at 55ºC can be used as adhesive. Prior flocking, the tapes have to be moisturized with water and then coated with the gelatine solution. The plane surface can be achieved via removing the overlaying gelatine from the template using a coating knife. For performing electrostatic flocking, polyamide fibres (SwissFlock, Inc., Emmenbrucke, Switzerland) with a length of 1mm and a diameter of 30 mm (equals 6.7 dtex) and a Maag RF 400/500 flocking machine (Maag Flockmaschinen, Gomaringen, Germany) can be used. The parameters which can be altered are the acceleration voltage which can be 60 kV, flocking time 20 s, and flocking distance 12 cm. For stabilizing the adhesive and make it bind covalently with the substrate, a cross linking step with 1% EDC in 80 vol.% ethanol need to be done for 20h. In the last step, flock scaffold must be washed in acetone for removing potentially toxic components from the fibres, later rinsed in water, freeze dried, and sterilized with gamma irradiation prior to its usage in the cell culture experiments.[73]

The pivotal relationship or difference between the traditional flocking technology and flocking for biomedical application is the material selected for developing flock scaffolds comprised exclusively of biocompatible materials, which are the materials that are non-toxic and compatible with biological tissue. The materials are selected meticulously to make sure that they are as identical as possible to the body's own materials and structures which do not elicit immunogenic or allergic reactions.[64]

In traditional flocking technique, the substrate used would be woven fabric made of cotton, cotton polyester, or some similar material whereas recently more number of knitted and non-woven fabrics has been used.[91] In biomedical application of flocking, the base material is preferably a resorbable material which may be a membrane or tape developed from collagen or collagen derivatives.[36]

On the other hand, the adhesives commonly used for traditional flocking technology are acrylic based systems which contains 50%-65% solids content in water, yet for certain applications aqueous polyurethanes, epoxies, PVCs and other adhesive systems are used.[91] For the choice of resorbable adhesive preferably gelatine, collagen gel, alginate gel, hyaluronic acid solutions, or chitosan solutions can be employed for flocking technology application in tissue engineering.[64]

The flock fibres utilized for traditional flocking technology are generally fabricated via grinding and cutting from various natural and synthetic fibres, like cotton, artificial silk, nylon, polyester and etc. These fibres have varying colors, thickness, softness, touch and chemical structures. Here, thin and short fibres, and the thickness of the adhesive layer, would improve touch and softness of the flocked surface. Albeit working with short and thin fibres is intricate, such fibres were chosen for retaining the natural appearance and feeling of leather.[75] In case of flocking technology utilized for biomedical application, different fibre materials are used. The resorbable fibre materials are comprised of aliphatic polyesters like polylactide (PLA), polycaprolactone, polyhydroxybutyrate and polyglycolide, or their derivatives and copolymers. In case of non-resorbable fibre materials, polyamide, polypropylene, polytetrafluorethylene, cellulose, viscose or other cellulose derivatives, non-resorbable or slowly resorbable polyesters can be used.[36]

In the traditional flocking used in textiles industry, the electrostatic field used was 40,000 V whereas in the flocking for scaffold production, the electrostatic field required is 60,000 V.[73, 75] For stabilizing the flock scaffolds, they were crosslinked with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) followed by freeze drying whereas in case of traditional flocking technology, foam stabilizer is utilized.[64, 75]

4.2 Parameters concerned for flocking in tissue engineering

For production of flocked scaffolds, the substrate, adhesive and/or fibres used consist of resorbable material. Resorbable material is a material that degrades slowly following its implantation by biological and biochemical processes.[36]

4.2.1 Substrate/base material

The base material preferably a resorbable material is a membrane or tape developed from collagen or collagen derivatives. This includes mineralized collagen also or components of the extra-cellular matrix. Likewise, the base material can be a membrane or a fibre material, non-woven material or woven material comprised of resorbable biopolymers like chitosan, hyaluronic acid, mineralized and/or non-mineralized collagen or collagen-hyaluronic acid composites, resorbable aliphatic polyesters such as polylactide, polyglycolide, polyhybdroxybutyrate, polycaprolactone or their copolymers and derivatives.[36]

4.2.2 Adhesive

For the choice of resorbable adhesive preferably gelatine, a collagen gel, alginate gel, hyaluronic acid solutions, or chitosan solutions can be used. Nevertheless, other biocompatible materials are conceivable to function as adhesives if they exhibit a certain electrical conductivity and meet the demands of the electrostatic flocking process. Besides that, they should also be apt for the production of a suitable adhesive bond between base material and flocking fibre.[36]

The adhesive is preferably crosslinked after flocking. This is performed to avoid any detachment of the fibres and release into the bloodstream. The bonding between fibres, adhesive and base material is bolstered by the crosslinking agent. Crosslinking can be performed by using chemical crosslinking agents such as EDC (N'-(3-dimethylaminopro pyl)-N-ethylcarbodiimide hydrochloride) or via other methods by means of UV- or gamma irradiation. Crosslinking is done in particularly while using gelatine, a collagen gel or hyaluronic acid as an adhesive, to prevent quick dissolution of these adhesives in an aqueous environment.[64]

4.2.3 Fibre

The base material and the fibres should possess hydrophilic surfaces for attaining good wetting with the adhesive. The fibre material can be either resorbable or non-resorbable material. The resorbable fibre materials are comprised of aliphatic polyesters like polylactide (PLA), polycaprolactone, polyhydroxybutyrate and polyglycolide, or their derivatives and copolymers. In case of non-

resorbable fibre materials, polyamide, polypropylene, polytetrafluorethylene, cellulose, viscose or other cellulose derivatives, non-resorbable or slowly resorbable polyesters can be used.[36]

The fibre materials must demonstrate the prerequisite characteristics for electrostatic flocking where they should possess sufficient electrical surface resistance of 10^6-10^8 Ω. The electrical resistance of the flocking fibres influences their flight behaviour and the evenness of the flocked surfaces.[92]

Geometric relationship between fibre diameter and fibre length has a significant influence on the processing characteristics in flocking process. Individual fibres with a length between 0.3 mm and 3 mm preferred, specifically between 0.5 and 1.5 mm. The individual fibres diameter is preferred between 10 µm and 200 µm. The shorter the fibres used, the greater the compressive stability of the produced flock scaffold.[64]

4.3 Selection of the adhesive

The role of the adhesive is to offer a durable connection between the flock fibres and the substrate. When medical application of scaffold is chosen, a biocompatible and resorbable adhesive must be utilized. By comparison to the composition of the natural ECM of most tissues, it is evident that gelatine can play a viable role as adhesive. Gelatine is obtained from tissues like bone, skin and cartilage and comprised mainly of collagen type I and II. It was found that 10–20 % of the latter is part of the cartilage ECM.[93] Apart from gelatine, there are other available biopolymers like hyaluronic acid, chitosan or starch. They were also tested, yet gelatine yielded the best results. In order to utilize an adhesive for flock technology it needs to be electrically conductive. The conductivities of three types of gelatines were compared and they were found to be present in the same range. The conductivity was observed to be a little lower than normal flocking adhesive (Mecoflock D 303, KIWO GmbH, Wiesloch, Germany) with about

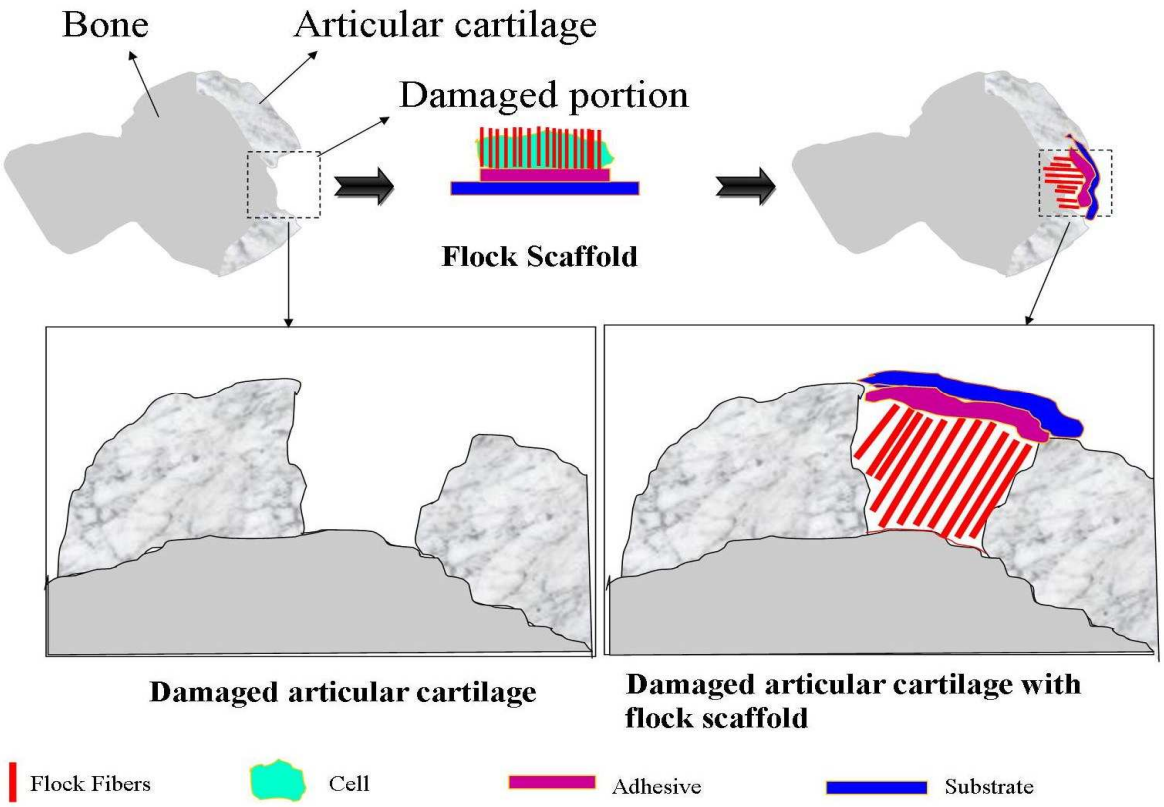

Fig. 4 Flock scaffold in cartilage tissue engineering (adapted from Fig. 1A, Steck et al., Tissue Engin. A 2010).

4.61 mS/cm.[73] Hence, the conductivity of the gelatin solutions was altered by adding NaCl to the solution. Moreover, it was also observed that the conductivity of gelatine solutions increased with the temperature. When 0.5 M NaCl solution was utilized, it was found that the electrical conductivity approximated that of normal flocking adhesive.

Even though the conductivity of the gelatins improves in tandem with increase in temperature, gelatine melts at a temperature of 37 °C and it starts gelling when the temperature decreases some degrees below. Thus, the gelatine ought to be used as a warm solution at the temperature range of 40–60 °C so that it can be processable and act as an effective adhesive for flocking. In addition to that, gelatine solutions at different concentrations levels were also tested.[73] The solution with 5 % gelatine was a very thin fluid. It was observed that just few fibres stuck perpendicularly to the substrate, however most of the fibres toppled down in the adhesive layer. In case of 10 and 20 % solutions were found to yield fair results for flocking, but the best results were achieved using the 20 % solution. The 50 % solution was found to be very viscous and started gelling quickly once after it was withdrawn from the heater. Hence, the 50 % solution was not at all processable. Apart from gelatine, other substances were studied as adhesives and experiments making use of hyaluronic acid, alginate, starch and chitosan were done in the same study. To attain a stable bond between fibre and adhesive on one side and adhesive and substrate on the other, it is very significant that the glue must have good wetting properties concerning both partners.[73]

4.4 Flock density

In a recent work done for the fabrication of scaffolds by Walther et al., two different flocked fibres (varying in length and diameter) and different flocking times were studied.[41] With the help of different fibre lengths structures, we can achieve variable heights of the flocked fibres. It was found from the study that varied flocking time resulted in distinct flock densities (the distance between each fibre in the flocked scaffold). In this study, flocking time was altered between 5 and 15 s. After the flocking was performed, the samples were sectioned with a razor blade. Microscopic analysis depicted that shorter flocking times of 5 s resulted in scaffolds with either less dense structure or less oriented fibres in comparison to the scaffolds that were fabricated using 15 s flocking time. For either short or long fibres, the orientation of fibres was found to be increased with longer flocking time. The best result of alignment of fibres was observed in case of the scaffolds fabricated with 1 mm fibre length as well as 15 s flocking time. The parallel alignment of the fibres results in scaffolds with an oriented pore structure. This may be useful especially for engineering of articular cartilage since this tissue shows a column-like orientation of cells. The cross-section of the scaffolds provides information regarding pores and pore range of the samples. The result depicts that flock scaffolds which possess larger pores are highly interconnected. The pore size can be easily be adjusted by the changing the flocking time. In addition to that, the less known fact is that there are huge pores combined with oriented fibres will facilitate cell infiltration that makes flock scaffolds performs better than the scaffolds made by other textile technologies like electrospinning. Hence, the properties of flocked scaffolds outperform the scaffolds fabricated using other techniques is discussed in the following section. The Fig. 4 shows the application of flock scaffolds in bone tissue engineering and Fig. 5 shows the stages involved in the implementation of flock scaffolds.[94]

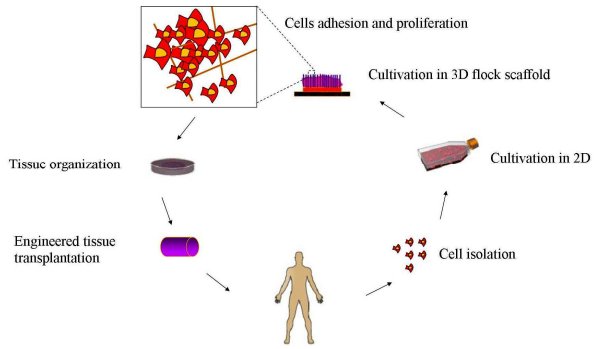

Fig. 5 Stages involved in the implementation of flock scaffolds (adapted from Fig.1 Dvir et al., Nature Nanotechnology, 2010).

4.5 Comparison of flocking with other textile technology

Similar to flocking there are other textile technologies available for scaffold fabrication like electrospinning and meltblown technique. The electrospinning is one of the most popular and versatile technique that allows the production of continuous fibres, with diameters at a range of micro to nanometres.[95] Similar to flocking, this electrospinning technique involves utilization of a strong

electric field. The electric field of 5-50 kV is applied to a droplet of fluid which would be the polymer solution coming out of the tip of the needle serves as one of the electrodes. This is in contrast to the flocking technology where the flock fibres were applied with high electric field of 60 kV.[92] Thus, in electrospinning when the applied voltage overcomes the surface tension of the fluid in the spinneret, a charged jet is ejected from the cone-shaped tip heading towards the oppositely charged collector placed on the other side. Here, the solvent evaporates leaving behind a dry fibre on the collecting device.[96] The collected random electrospun fibres on a plate produce connected porous mats with high porosity and high surface area. Despite the nanofibrous mats fabricated by electrospinning demonstrate high porosity and resemble the extracellular matrix of tissues in morphology, they possess pores at the range of lower micrometer that can hinder deep cellular infiltration.[97] This makes the flock scaffolds superior compared to electrospun scaffolds. Through the modification of the spinneret and/or the type of solution in electrospinning technology, it allow for the production of fibres with different structures and properties using different modes like side by side, multijet, multi-layer, co-axial, emulsion electrospining and surface immobilization.[98] Similar break through and exploration in flocking technology is needed to promulgate flocking technology for tissue engineering applications. The major parameters like the flocking time, flock fibre length, materials used for adhesive, substrate and fibre needs to be investigated exhaustively in order to conquer the flocking technology for the tailor-made tissue engineering applications.[41]

Melt blowing is another textile technique used for fabricating fibrous webs or articles directly from polymers or resins. When compared to flocking, the principle of melt-blown technology is completely different. In melt-blown process, molten polymer is extruded from the die holes later stream of high velocity hot air is used to attenuate polymer films to form microfibres. These microfibres were collected in the collecting screen to form self-bonded nonwoven web. The extruder and hot air are used as a significant component in melt blown technology whereas in flocking technology, the high electrostatic field is used. In melt blown technology, fibres thickness depends on parameters like melt-temperature and viscosity as well as velocity and temperature of hot air.[67] Moreover, Rom et al., demonstrated that the lower the diameters of fibres fabricated using melt blown technology, the smaller were average pores sizes. However, the flock scaffolds possess better porosity even if the fibre diameter is less to encourage cell growth for tissue engineering application.[36, 41, 67]

In a recent work by Jakub et al., a novel scaffold was produced by combining meltblown and electrospinning technology with in-situ particle integration in between fibres. The scaffold produced was found to possess satisfactory surface properties and porous structure.[99] When the flock scaffolds are compared, they possess good porosity encouraging cell growth as well possess exceptional mechanical properties to serve as a putative scaffold for tissue engineering.[41]

The scaffolds fabricated using textile materials specifically non-woven materials, fibre textiles or knitted textiles exhibit a very low compressive stability owing to the reason of horizontal alignment of fibres. A typical example for this is the production of three-dimensional air-inter mingled non-woven substances.[68] The flock scaffolds produced by Walther et al., with 1 mm fibre length with a flocking time of 15 s have a Young's modulus of about 250 kPa which was the highest value for investigated novel material.[41] On the flip side of the coin, compression tests of scaffolds produced by electrospinning revealed that the Young's modulus of the electrospun scaffolds rises up to about 17 kPa after 42 days of culture, which is lower in comparison to the Young's modulus of flock scaffolds without cells.[100]

5 Properties improved by flocking for tissue engineering

5.1 Improved porosity and capillary action of the flock structures

Porosity is a vital property of scaffolds for tissue engineering applications. When the porosity of the flock scaffold is higher, it results in higher capillary action.[41] The porosity of the fabricated scaffolds was estimated in this work using the following equation:

$$\text{Porosity} = \frac{V_s - V_f}{V_s} 100\%$$

Porosity was calculated by determining the volume of the scaffold (V_s) which is given from the fibre length and diameter of substrate,

as well as the volume of all fibres in the scaffold (V_f) which is nothing but the volume of a single fibre multiplied by the number of fibres per area that is given by the flock density. For all the tested scaffold types, their porosities were calculated. For every flock scaffolds the values for the calculated porosities were more than 90 %. This dictates that these scaffolds are highly porous structures. This was further delineated by the microscopic images of the embedded scaffolds in that study. It was observed that the prolonged flocking time of 15 s possess lower porosities in comparison to the shorter flocking time of 5 s.

The highest porosity was observed for the case of scaffolds with longer fibres (3 mm) and a shorter flocking time which is 5 s. Scaffolds with the higher porosities are contemplated to be suitable for the TE. The pores must be big enough and highly interconnected so that it may provide enough space for cells to adhere and to facilitate migration of cells into the scaffold. It has to be ensured that cells are supplied with adequate nutrients and oxygen but also to enable the export of metabolic waste products. Looking on the porosity and its relationship with the size of the pores, flock scaffolds are superior to nanofibrous mats fabricated by electrospinning, which is a textile technique commonly studied nowadays for tissue engineering application. Different materials and shapes are exploited as substrates for flocking such as polymer membranes, fabric, metal, plastic, ceramics, glass, and even EPDM (ethylene propylene diene monomer (M-class) rubber) component was recently utilized in automotive applications.[36, 73, 85, 101, 102] Besides higher porosities, mechanical strength is another vital characteristic need to be considered for scaffolds for TE application to provide at least an initial stability of the construct after implantation.

5.2 Enhanced mechanical strength of the flock structures

In a work done by Walther et al., compression tests were done for determining the mechanical strength of flock scaffolds in wet state under unidirectional compressive loading.[41] The study revealed that different scaffold type demonstrates different compressive strength as a function of fibre length and flock density. The result of the study dictates that the scaffolds made of 1 mm fibres and high flock density (15 s flocking time) were observed to be the most pressure-resistant. In addition to that, scaffolds with 1 mm fibres that were flocked for 15 s possess a Young's modulus value of about 250 kPa which is the highest value among the investigated types of novel materials. The almost parallel arrangement of the fibres that are aligned perpendicular in the substrate forms a mechanically stable structure. The fibres undergo Euler buckling and make possible the fabrication of anisotropic structures with a high compressive strength in spite of their high porosity.

On the other hand, in another work Janjanin et al. studied the electrospun scaffolds seeded with cells.[100] Compression tests were carried out in the study where they assessed the mechanical properties of these structures. The result of this study delineates that Young's modulus of the scaffolds rises up to about 17 kPa after 42 days of culture, which is comparatively very much lower than the Young's modulus of flock scaffolds without cells. Similarly, in a work done by Reiband et al., flock technology was utilized to create scaffolds with a high compressive strength in spite of high porosity.[17] A new type of scaffold for intervertebral disc tissue engineering was developed via flocking technology using mineralized collagen I as substrate, gelatin from porcine skin as adhesive and electrostatic flocking machine.

In order to ascertain the biomechanical properties of cell seeded flock scaffolds, the investigation of the hardness of the flock scaffold seeded with human MSCs over a period of 42 days was performed by Steck et al.[22] The work was performed to develop anisotropic scaffolds with parallel fibre orientation that are capable of supporting a cellular cartilaginous phenotype *in vitro*. Scaffolds that were produced by flock technology were comprised of a membrane of mineralized collagen type I as substrate, gelatine as adhesive, and parallel-oriented polyamide flock fibres at vertical position to the substrate. For the purpose of analyzing basic biomechanical properties of flock scaffolds, a comparison was performed between hardness and relaxation of flock scaffolds with a clinically applied collagen type I/III scaffold of the similar size. Flock scaffolds were found to possess higher initial hardness values at the beginning and even after 20 s of load application. When compared to collagen type I/III scaffold constructs, the flock scaffolds surprisingly shown a much faster recovery to initial shape in the load-free relaxation phase. In addition to that, for addressing the question of active chondrogenic differentiation of MSCs seeded on flock scaffolds and its effect on the mechanical properties of the

constructs, the hardness and relaxation of scaffolds under different duration of chondrogenic culture was characterized. It was palpable that, over culture time the hardness of MSC-loaded scaffolds improved in comparison to the day 0 controls. Hence, this result of the study shows that the hardness of the constructs improves drastically during culture due to the deposition of new matrix by the cells. Scaffolds for TE ought to be capable of protecting cells from early critical mechanical forces until enough matrixes are synthesized by the cells to bolster the structure. Moreover, whilst compression testing and cell culture experiments it was observed that no flock fibres detached themselves from the scaffold. This clearly indicates the stability of the novel flock scaffolds which is of prime importance for TE applications. These potential results further substantiate the fact that flock scaffolds represent a promising new matrix for TE, especially for load-bearing tissues like bone and cartilage.

5.3 Increased cell adhesion and cell stability of the flock scaffolds under cell culture conditions

In the same study discussed previously by Walther et la., the human MSC were seeded onto the flocked scaffolds and the cells adherence and cells stability after its adherence on the flocked scaffolds was investigated.[41] The adherence was microscopically studied with scanning electron microscopy (SEM) and confocal laser scanning microscopy (cLSM). The result shows that the cell were easily attached to the flock scaffolds and spread on between the fibres. Since the flock scaffolds have highly interconnected pores with necessary pore size, the cells can easily distribute over surface of the scaffolds. The distance between the fibres is also less thereby allows the cells to spread and build bridges between the fibres. SEM results after 28 days of cultivation delineates that the space between the fibres and the pores of the scaffolds were filled with cells. Only a very little space, predominantly at the top of the fibres, was still visible. In addition to that, the flock scaffolds were imaged at different time points in tenure of the culture. This revealed that the amount of cells in the scaffolds increased in course of time. Subsequent to 7 days of culture most portions of the fibres were still visible. The sporadic cells were palpable along and between the fibres. During culture, progressively it was found that the fibres got covered by cells. Following 21 days after seeding, surprisingly more cells were observable in the scaffolds compared to that of at the earlier time point.

The flock scaffold architecture with its high porosity and huge pores ascertained above as well as the almost parallel arrangement of fibres enables an easy cell infiltration and a homogenous distribution of cells in the flocked scaffolds.[41] The cells stretch between the fibres and fill up the pores. Difference in cell distribution was found between the scaffold types. For the flock scaffolds with the densest structure (1 mm fibre length, 15 s flocking time) a large amount of the cells were found at the top of the fibres directly after seeding. From there they migrated towards the scaffold and finally distributed evenly, filling the scaffold from top (fibre top) to bottom (adhesive layer). Owing to the reason of bigger pore size in case of the other three investigated scaffold types, the cells easily reached the adhesive layer at the time of seeding itself. Therefore, the cells filled the scaffold from the adhesive layer (bottom) to the fibre top and also attained a uniform cell distribution over the whole scaffold in course of time. Apart from SEM visualization, some samples were stained and analyzed through fluorescence microscopy. The overview from the top of a cell seeded flock scaffold subsequent to 28 days of cell culture delineates that only the tips of the fibres were visible (red spots) owing to auto-fluorescence and the gap between fibres were completely filled with cells.

Likewise, in another work done by Steck et al., the combination of matrices as a guiding structure as well as chondrogenically differentiated MSC were used with flock scaffolds to offer new possibilities in the treatment of cartilage defects.[22] In this study, electrostatically flocked matrices were used and it is comprised of a collagen substrate, gelatine as adhesive and polyamide flock fibres. The aim of the study was to determine whether anisotropic flock scaffolds are capable of supporting cellular cartilaginous phenotype *in vitro*. The flock scaffolds were embedded with chondrogenic cells as well as MSC. Adherence, vitality as well as proliferation were assessed via cLSM. Chondrogenic induction was done in presence of TGF-beta 3. Accumulation of proteoglycans was quantified through alcian-blue stain and collagen type II synthesis following the extraction of the newly synthesized matrix. cLSM result dictates that the vitality of embedded cells remained high over time. Articular chondrocytes as well as nucleus pulposus cells synthesized

proteoglycans and collagen type II in the scaffolds. Interestingly, the MSC filled in the flock scaffolds differentiated and increased their chondrogenic phenotype over time. The biochemical analyses performed using cLSM also showed that cells adhered well in the new flock scaffolds. Moreover, it was shown that the flock scaffolds are capable to support induction and maintenance of the chondrogenic phenotype. Hence, the flocking technology can be considered for fabrication of scaffolds for cell cultivation and TE.

5.4 Increased cell proliferation and osteogenic differentiation of the flock scaffolds under cell culture conditions

In the same work discussed previously by Walther et al., the proliferation of the cells in the flock scaffolds was studied through quantification of the DNA content on days 1, 7, 14, and 21 of culture following lysis of the cell-seeded samples.[41] DNA content was associated with the cell count and found to increase from day 1 to 21 after cultivation in all the tested types of scaffold. Subsequent to 21 days of cultivation the count of differentiated cells was observed to increase 5–6 folds in comparison to the initial amount of cells. ALP activity was measured from the scaffold which is dependent to the cell number. The cell number is determined from the DNA content of osteogenically induced cells in the flock scaffolds on day 14 and 21 of cultivation. For all the four different scaffold types the specific ALP activity was found to be significantly higher in the three-dimensional matrix of flock scaffolds compared to the control which was a normal polystyrene well plate. However, when the four flock scaffold types were compared and no significant difference in specific ALP activity was observed. This result clearly dictates that all investigated types of scaffolds support viability and osteogenic differentiation of hMSC. As there is no prominent difference in proliferation and differentiation of the studied structures, it was found that flock scaffolds produced with 1 mm fibre length and 15 sec flocking time demonstrated the best results when mechanical properties to considered and this scaffold type appears to be most plausible candidate for the use in TE. The almost parallel arrangement of fibres as well as their function as compression members must protect cells cultivated in the scaffolds from critical mechanical loads.

In a different study it was demonstrated that flock scaffolds are capable of supporting the chondrogenic phenotype.[22] The flock scaffolds were seeded with articular chondrocytes obtained from porcine knees and porcine nucleus pulposus cells. It was observed that primary chondrocytes deposited a proteoglycan and collagen type II-rich ECM in flock scaffolds which dictates that the cells retain their chondrogenic phenotype during cultivation. As a continuation of the same study, flock scaffolds were seeded with hMSC embedded in a collagen type I gel. It was shown that chondrogenesis of MSC determined by proteoglycan synthesis and collagen type II deposition of cells loaded on flock scaffolds, was notably higher than that of MSC, cultivated only in collagen gels.

In another work done by Walther et al., it was observed that during cultivation for seven days on flocked scaffolds, 7F2 osteoblasts proliferated with higher rates, which was evident from 15-fold increase of Lactate Dehydrogenase (LDH).[73] In addition to that, SEM images of flock scaffolds seeded with cells demonstrated a significant increase in cell number in comparison to day 1. It was found that the fibres were covered up with a thick cell layer and the cells filled the pores between the fibres. The membrane bound ALP activity was investigated following cell lysis and related to the cell number. This specific ALP activity was found to be more for cells on flock scaffold against cells on polystyrene, specifically following seven days of cultivation. The three-dimensional arrangement of the cells in the flock scaffold may have enhanced their osteogenic differentiation. This improved osteogenic differentiation might be the reason behind the decreased proliferation rates of the cells in comparison to those cultivated in polystyrene culture dishes. For attaining an even distribution of cells all over the scaffold, a collagen type I/cell suspension was produced and allowed to polymerize to form a gel after application to the flock scaffold. The result indicates that cellular vitality of hOB and hMSC improved in the fabricated flock scaffolds, as in the collagen gel over 21 days. Following 21 days of osteogenic induction, membrane bound ALP enzyme activity was found to be increased 5-10-fold in hOB and nearly 10-15-fold in hMSC. In conjunction to this, it was also found that secreted ALP-activity was elevated almost 3-fold of starting activity in culture supernatants of hOB and hMSC. Hence, when the results were contemplated together, this shows that both hOB and hMSC proliferated and undergone osteogenesis independent of whether cells were seeded in the flock scaffolds or in collagen gel alone.

6.Conclusion

To conclude, it is high time for researchers to further investigate the flocking technology for scaffold fabrication, to cater the tremendously growing demand for tissue engineered scaffolds. The improved properties of the flock scaffolds are shown in Fig. 6.

The flock technology-based scaffolds is a boon for tissue engineering, as they provide anisotropic orientation of fibres with higher porosity that enabled cell adherence, proliferation, and high vitality of cells; and comprised of superior biomechanical properties. The adhesive as well as substrate were successfully substituted with biocompatible and degradable materials. The scaffolds were found to be stable even under cell culture conditions and it has been demonstrated with different cell types like murine 7F2 osteoblasts and human osteoblasts and primary human mesenchymal stem cells. Moreover, the cell adhered and proliferated well in this novel scaffold. The cells demonstrated their typical behavior expression of ALP as typical osteogenic marker. Thus, we can conclude that the flock structure can be exploited for tissue engineering especially for TE.

The future improvement of this flock technology will be the construction of a flock scaffold where beyond substrate and adhesive, the flock fibres are fully biodegradable. In order to achieve this, the current flock scaffold technology can be further extended in experiments using fibres, which were polyamide, can be replaced with biocompatible and resorbable materials like polyhydroxybutyrate (PHB), polylactid (PLA), chitosan or collagen. Similarly, a wide spectrum of flock scaffolds can be fabricated through other substrates like membranes of PHB or chitosan or adhesives like chitosan, starch or hyaluronic acid and fibres made of PHB, PLA or PGA and a combination thereof. Moreover, the flocking was utilized so far for bone tissue engineering as well as cartilage tissue applications. This may be further exploited for fabrication of scaffolds for other biomedical applications like artificial skin, extra-corporeal organ and etc as represented in figure 7.

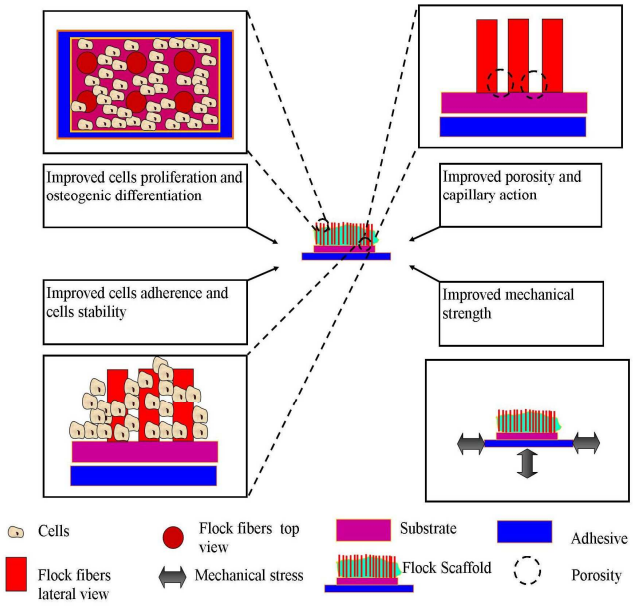

Fig. 6 Improved properties of flock scaffolds.

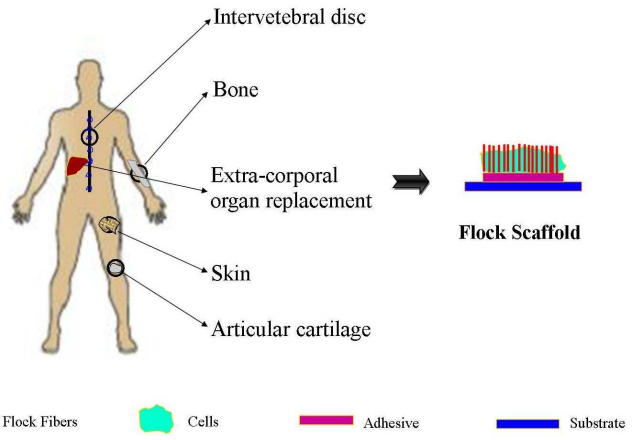

Fig. 7 Potential applications of flocking technology in tissue engineering.

Acknowledgements

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