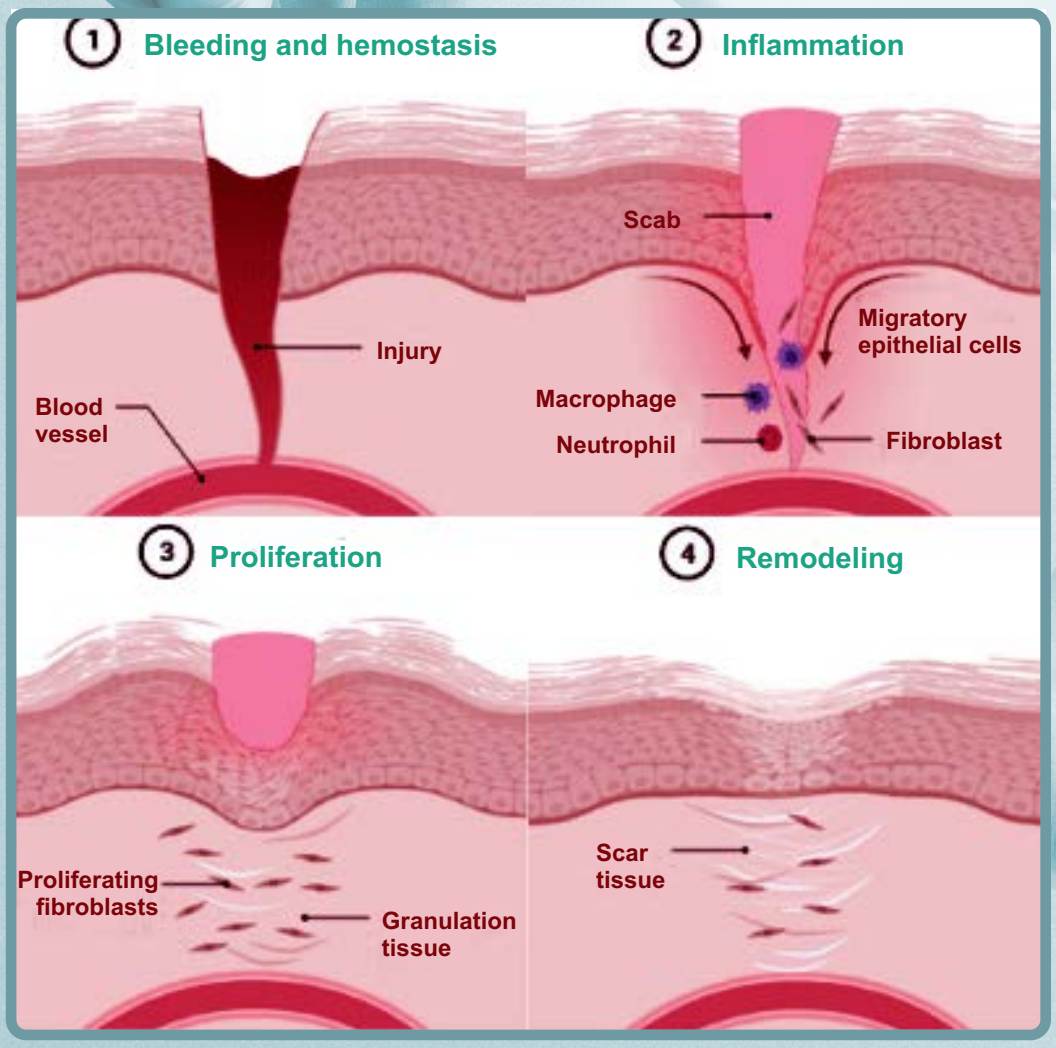


OMSTH

OPINIONS IN

Medical Sciences, Technology and Health

- Challenges in Disease Modelling
- Challenges in Scarless Wound Healing
- The Human Hippocampal Formation

June 30, 2025

Volume 3, Issue 1

OMSTH

OPINIONS IN Medical Sciences, Technology and Health

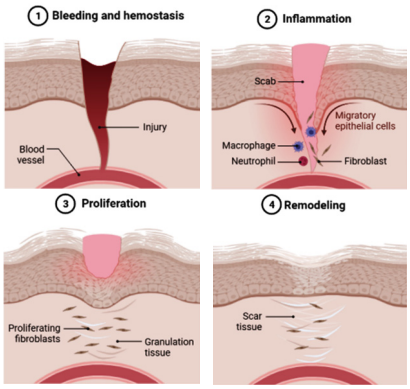
Scope

Technology has been breaking boundaries in medical sciences. We here at SCTIMST were the torchbearers of innovations in medical sciences in India by the amalgamation with engineering, basic science and public health and were responsible for the development of the first indigenous aortic valve in India. The Chitra blood bag is another success story. At this juncture, as we complete 44 years of dedicated innovations to society, the knowledge and expertise we possess on the development, validation, clinical trials, translation and commercialization of medical devices, and at the same time delivering high-end medical care in neurological and cardiological disciplines and public health, we think it is time to share and hence this Journal.

The Journal will publish reviews in the field of medical sciences with a focus on cardiological and neurological sciences, biomedical technologies, its translation and commercialisation and public health.

Cover Photo

Different Phases of Wound Healing



Adapted from *G. Perumal et al., Engineering Biomaterial Scaffolds for Scarless Wound Healing: Opportunities and Challenges, e25004.*

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Volume 3, Issue 1, 2025

Editorial

Maya Nandkumar

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Today, the rapid and significant technological advancements in medicine have a profoundly positive impact on the lives of millions. From the humble microscopes of the past to today's MRI and PET scans, we can now image the inner depths of the human body. With advancements like robotic surgery, complex and complicated surgeries have seen remarkable success. This journal, Opinions in Medical Sciences and Technology, is dedicated to sharing these advancements with all readers and positively influencing opinions and policies.

In the third year of our publication, we are starting with an article in Systems Biology, an understanding of which would lead to targeted and personalized medicine, as it views the individual as a whole, with the complex interactions between the organs, organs systems and the genes, proteins and various metabolic pathways as interconnecting components as against the earlier view of one gene one disease approach. In this complex scenario, Artificial Intelligence plays a key role.

There is a technology landscape for lint-free wound dressings. The technology landscape is a versatile business tool for understanding the current and future perspectives of technologies of interest. This helps in making informed decisions that would impact the progress of the individual and the Institute as a whole. In line with wound dressings is an essential concept of scarless wound healing, and there is also a comprehensive review on Biomaterial scaffolds for scarless wound healing. This review guides the reader through the various scaffolds currently in use and those in the developmental phase, as well as the impact of AI on personalised approaches to treating complex wounds.

Research without statistical significance is meaningless, and why we need to understand statistics and whether the 'p-value' should be the major yardstick to measure one's research significance is debated in the perspective and commentary on the subject.

As Systems Biology becomes the bedrock of medical technology development, the technology landscape offers a panoramic view of the technology that will be impactful tomorrow, paving the way for personalised medicine. Genomics, proteomics, and metabolomics will enhance our understanding of complex life interactions and the development of disruptive technologies, such as the CRISPR-Cas9 gene editing tool, which will aid in treating specific diseases, as described in this issue. All these technological advancements, with AI at their core, will enable personalised medicine, and SCTIMST is rightly poised to ride this wave to success. We thank all contributors for partnering with us in this journey and solicit continued contributions towards creating impactful opinions .

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PERSPECTIVE

Moving to a World Beyond 'p≤0.05' – The Requiem for 'Statistically Significant'

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The American Statistical Association (ASA) sounded the death knell to the 'p' value in its statement issued in 2016 on scientific reporting of results. (1) In the period from 2016 to 2019, while the rest of us were mulling about the ways and means to a 'p' free world, we also have to say that in reality, the world has not become 'p' free after all. (2) Not everyone reads the guidance of the ASA or is aware of its existence. The words 'statistically significant' or 'otherwise' continue to dictate publication, policy statements and decisions. There were efforts to explain the importance of the ASA's guidance across disciplines, and some of us did try, including this author (3).

While some of us were engaged with explaining what the 'p 'means, and why it is inadequate to scientific decision making, the ASA has also continued to mull over further action about the ubiquitous 'p' value and what is going to take its place. Thankfully, we have measured language, a variety of choices that emerge out of their engagement with finding alternatives, and a list of potential 'dos' to replace their long list of 'don't ' that echoed through the statement issued in 2016.

The ASA's editorial is written in simple language that even an undergraduate student with a modicum of statistics would get the salient points being made. It prohibits the use of words 'statistically significant' or 'significantly different' or 'pd"0.05' or 'non-significant' or the use of single or multiple asterisks in tables to indicate the same. The ASA takes this step following its statement of 2016, attempting to explain what the "p" was and how it was misunderstood and misused and providing guidance on how to undertake better research embracing uncertainty, which is the hallmark of much of statistical analysis.

In its 2019 editorial, the ASA goes beyond the litany of don'ts it subscribed to and has moved forward to provide a long list of 'do's' that provide the way forward. It urged that conclusions not be based on associations of effects being statistically significant based on some arbitrary threshold value of 'p'. It cautioned that such associations could be spurious. It also pointed out that the lack of an association indicated by the absence of an arbitrary threshold value of 'p' is not indicative of an absence of association. It said that the 'p-value' did not give the probability that chance alone produced the observed association or effect, and lastly, required that no scientific or matter of practical importance be based on the statistical significance or its absence.

However, for want of an alternative, the 'p-value' has not been replaced in scientific thinking in most processes of knowledge production where it was used earlier. The reasons for this could only be speculated upon – the dissemination of the ASA statement was limited, it did not resonate with many editors, and it did not

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make sense in the biological sciences except for a few where the numbers were larger than or nearing 30 or more.

The ASA continued its confabulations and has come up with a long list of dos following the abandonment of the 'p-value' threshold to indicate significance. This can be summarised using the Acronym ATOM. It sets forth on its "atomising" revolution by explaining what it means. The way to a better understanding of data does not lie with summarising it with data and finding some arbitrary threshold to determine the scientific significance through a heuristic statistical significance. It also showcases the plurality of alternative actions that one can take to replace the use of the 'p-value'. The editorial summarises the 43 voices in its special volume by providing a specialised list of dos and don'ts from each of the 43 sets of authors. These are easy reading as they are written in a language that can be understood by most.

What does the ASA's atomising process call for? It asks for researchers to recognise and accept the varying degrees of uncertainty in the analysis and consequently the results - accepting uncertainty (the A in ATOM). The next requirement is thoroughness, specifically statistical thoroughness in examining alternatives to the null hypothesis and in testing a pre-specified alternative. The requirement of thoroughness extends to multiple options for action (the T in ATOM).

The third requirement, 'Be open', transcends mere analysis and also encompasses communication of the various steps or stages of analysis. However, such a requirement, which only applies to researchers, cannot change reporting standards across journals. For this reason, the ASA's statement does provide a requirement that the researcher provide sufficient analytical information to enable replication. This definitely has implications for the routine Introduction, Methods, Results and Discussion (IMRaD) format for reporting that is the backbone of scientific reporting (the O of ATOM).

The fourth, and most frequently used word by faculty teaching students who use statistical analysis strategies and make pronouncements of 'interesting statistical findings', is the requirement 'be modest'; (the M of ATOM). It enjoins researchers to describe model specifications, their limitations and the messy process of diagnostics and inference using statistical approaches. It also requires that truth claims be juxtaposed with the limitations of method, model specification and understanding of underlying distributions.

Lastly, it excellently summarises the advice from each of the set of authors of the 43 supplementary papers in terms of potential 'dos'. This brought home to me why it was always difficult to explain to my colleagues what to do instead of using the 'p'. That would take us down multiple pathways depending on the nature of the question being asked, the data used and its description. That is what renders the ASA statement truly great. It reflects upon the multiple points of subjectivity in statistical analysis, which is obscured by the fig-leaf of 'objectivity that the 'p-value' is assumed to provide. It calls for recognising the potential for subjectivity in the various stages of analysis and calls for careful elimination of bias. The statement also provides significant guidance to journal editors and reviewers. It urges editors to send the ASA's statement to reviewers and asks them to give comments using the framework provided by the statement. It calls for results-blinding reviews and recourse to a series of steps editors could take (in addition to banning use of words like 'statistical significance') to manage the move to a 'beyond-p-p' world.

The move to a world beyond the frequentist approach that avoids the use of the 'p' value is not going to be easy, and every effort made by researchers, academicians, academic institutions, professional guilds and journal editors will con-

tribute to taking a step forward. Recently, a blog post by Bayesian Spectacles reported on the University of Amsterdam's decision not to teach 'p-values' to first-year students at the Faculty of Social and Behavioral Sciences, marking a small step towards a broader reform (4). Hopefully, many others will join this effort and strengthen it.

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REVIEW ARTICLE

CRISPR Genome Editing Technology: Applications and Challenges in Disease Modelling

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Abstract

CRISPR-Cas9 technology has revolutionised the field of genome editing, providing a powerful tool for researchers to precisely modify the genomes of various organisms. This review article explores the applications of CRISPR-Cas9 technology in disease modelling, focusing on its ability to generate cell lines and animal models with specific genetic mutations. The article discusses the CRISPR-Cas9 technology, mechanism and its advantages over traditional methods of disease modelling, including its efficiency, flexibility, and versatility. Additionally, the review addresses the challenges and limitations associated with CRISPR-Cas9 technology in disease modelling, such as off-target effects, mosaicism, and ethical considerations. Finally, the review provides insights into the future directions of CRISPR technology in disease modelling, including its potential for advancing personalised medicine and drug discovery.

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Introduction

Genome engineering is a powerful tool for a wide range of applications in biomedical research and medicine, whereby the genomic material of any organism can be manipulated. DNA can be edited by inserting, deleting, or replacing nucleotides, enabling a complete transformation of the genome content (1), and it is a powerful tool used to correct nucleotide sequences in cases of mutated inherent disorders. Gene editing is done by utilising specialised nucleases that are programmed to carry out cleavages at specific sites of interest. Many programmable nucleases are employed these days in biomedical laboratories like, ZFNs or Zinc finger nucleases, Meganucleases, TALENs or Transcription Activator-like Effector Nucleases and the most popular and affordable technique of CRISPR/Cas or Clustered Regularly Interspaced Short Palindromic Repeats and other specific nucleases along with CRISPR gene locus [1]. Nuclease enzymes, such as Cas9, have the ability to create double-strand breaks in DNA, which are typically repaired by cellular machinery later. Repairing or editing is by either of the two mechanisms; non-homologous end joining (NHEJ) or homology-directed repair (HDR) [2]. The CRISPR/Cas technology utilises a guide RNA that specifically directs Cas, the nuclease enzyme, to act on target DNA and cleave it. The technology has gained tremendous popularity due to its simplicity in usage and efficiency, even in treating difficult-to-manipulate organisms. Presently, CRISPR/Cas9 is employed in various

model organisms including, yeasts (*Saccharomyces cerevisiae*), plants (*Arabidopsis thaliana*), *Caenorhabditis elegans*, *Drosophila melanogaster*, Zebrafish (Danio rerio), Mice (*Mus musculus*) and Humans (*Homo sapiens*) [3].

Discovery and Evolution of CRISPR/Cas

Initial discovery of CRISPR was made in the *Escherichia coli* genome (1987). Ishino et al. first reported the presence of a cluster of repetitive DNA sequences interspersed with variable spacer regions in *Escherichia coli* [4]. Subsequent studies by Mojica et al. identified similar repeat-spacer arrays across diverse bacterial and archaeal species, leading to the designation of these elements as Clustered Regularly Interspaced Palindromic Repeats (CRISPR) [5]. It is a series of 29-nucleotide-long repeats interspersed with 32-nucleotide-long variable sequences. Also, several short repeating palindromic sequences, usually 24-40 nucleotides in length, are present. Each of the repeated sequences is interfered by unique variable sequences 20-58 nucleotides long. The acronym CRISPR was coined in 2002 [6, 7]. Each of the CRISPR loci had an adjacent CRISPR-associated gene with inherent nuclease activity. This enzyme, called the Cas enzyme has a mutual association with the CRISPR locus. It was proposed that CRISPR-Cas is an adaptive defence system that might use antisense RNAs as memory signatures of past invasions [8]. It was hypothesised that the 20-22 long nucleotide sequences intercalated between the repeating sequences were mostly derived from plasmids and viruses. The addition of new spacer sequences in the CRISPR/Cas locus in the gram-positive bacteria *Streptococcus thermophilus* was discovered in 2007 [9]. In 2008, mature CRISPR RNAs (crRNAs) were demonstrated to function as guide molecules in complex with Cas proteins, enabling interference with viral proliferation in *Escherichia coli* [10]. In the same year, the DNA-targeting activity of the CRISPR-Cas system was also reported in the pathogen *Staphylococcus epidermidis* [11]. In 2012, for simplification in usage, the dual RNA system of crRNA-tracrRNA was replaced by a single RNA fragment, usually 20 nucleotides in length, termed as the single guide RNA or sgRNA. This made the technology a lot simpler as here, instead of using and constructing two RNAs, only a single gRNA needs to be designed [12]. This technique is widely used today to induce mutations in various live organisms and cell lines for multiple applications.

Mechanism of the CRISPR/Cas9 system

The CRISPR/Cas9 system is an adaptive immune mechanism found in most bacteria and all archaea, protecting against phages and other foreign invaders by preventing the uptake of plasmids. CRISPR loci are non-contiguous direct repeats separated by nonhomologous spacer sequences—predominantly of captured viral and plasmid DNA—usually adjacent to Cas (CRISPR-associated) genes. The Cas genes code for a multifarious family of proteins that have nuclease, helicase, polymerase, and polynucleotide-binding activities, constituting the CRISPR/Cas system [13]. There are six core Cas genes that have been identified, i.e., the universal markers cas1 and cas2, and subtype-specific genes and repeat-associated mysterious proteins (RAMPs), which are functionally associated with specific CRISPR repeat sequences.

While CRISPR systems are quite diverse, they are generally classified into two broad classes - Class 1 and Class 2 - depending on the structural arrangement of their effector complexes. Class 1 systems, which comprise Types I, III, and IV, have multi-protein effector complexes and are further divided into 15 subtypes. In contrast, Class 2 systems utilise a single, large effector protein and are classified into

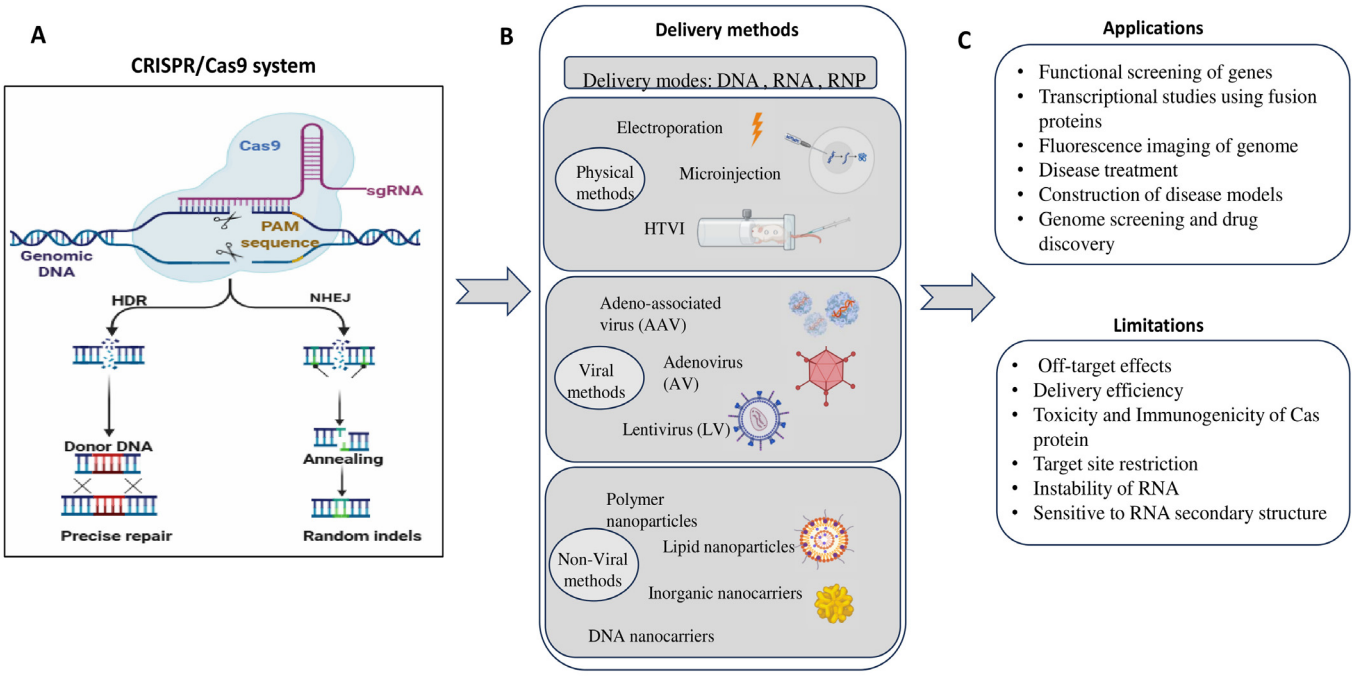

Figure 1: Schematic of A) CRISPR/Cas9 system , B) Different delivery methods for gRNAs, C) Applications and limitations

Types II, V, and VI [14]. Class 2 systems have only a single protein effector domain and are commonly used. Among the Cas proteins from Class 2 systems, most Type II Cas9 variants and Type V Cas12 variants possess RNA-guided DNA endonuclease activity, while Type VI Cas13 variants exhibit preferential RNA-targeting and cleavage activity [15]. Cas9 is the enzyme used in CRISPR technology as it is versatile plays a major role in processing of crRNA a property displayed due to its role in various steps in bacterial and archaeal adaptive immunity and adaptation. The Type III system is different from either of the former types. It can target and cleave both the DNA and the RNA. It consists of the nuclease enzyme Cas10 [16,17]. There are 45 different Cas gene families distributed among various subtypes of CRISPR. Cas1 and Cas2 are universal associated with all CRISPR types, while Cas enzymes of types 3, 9 and 10 are respectively for CRISPR Types I, II and III [17]. In benefit of the ability to precisely create DSB in any target dsDNA, the Type II system is the most relevant and most studied one [18].

Genome editing experimentation at minimum needs to have three necessary factors. Initially, a host cell needs to be chosen, in which the genomic alterations will be carried out. Next, a guide RNA (gRNA), this RNA molecule is actually transcribed in the cell and is engineered such that it is specifically able to recognize and bind the target genomic sequence by way of complementary base pairing. Lastly, Cas proteins; either endogenously expressed or exogenously delivered are required to recognize the gRNA-bound DNA target and cause a site-specific double-strand break, thus initiating the process of genome editing.

The most commonly used protein in genome editing techniques is *Streptococcus pyogenes* Cas9 (SpCas9), which mediates targeted genome alteration through the generation of double-strand breaks (DSBs) within a specific genomic locus. The

3D analysis of the spCas9 enzyme revealed its bilobed structure: a recognition lobe (REC) and a lobe with nuclease activity (NUC) [12]. The cleavage action depends on SpCas9 recognizing and binding to a 52 -NGG-32 protospacer adjacent motif (PAM), where "N" represents any nucleotide (A, T, C, or G), found immediately 32 to the target location. Upon binding to the PAM, Cas9 induces local DNA strand separation adjacent to the motif, creating a window for the single-guide RNA (sgRNA) to hybridize with the complementary DNA sequence. If the sgRNA successfully base-pairs with the target DNA, Cas9 undergoes a conformational change that activates its nuclease domains, resulting in a site-specific double-strand break at the target locus [5]. Importantly, the order and specificity of the PAM can differ based on the type of Cas protein utilized and the microbial species that it is isolated from, thus affecting the targetable regions of a given genome.

In SpCas9 genome editing experiments, guide RNA may be designed in two modes: as a two-component system of a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA), or as a single guide RNA (sgRNA), where both components are combined into one molecule. Within the native bacterial system, tracrRNA has a 52 complementary segment to the repeat-derived region of the pre-crRNA, which allows the two RNAs to hybridize and create a functional guide complex. The crRNA consists of a 20-nucleotide guide sequence, typically directing Cas9 to the target genomic location. For genome editing, this guide sequence is fused with an optimized tracrRNA scaffold through a linker loop to form a chimeric sgRNA. The format of the sgRNA is generally preferred because it is simpler and more efficient, since the individual crRNA and tracrRNA components might not consistently anneal in heterologous systems. Notably, only the guide region of the sgRNA specifies the genomic target, and this results in a high level of programmability and flexibility of the CRISPR-Cas9 system. Moreover, the DNA encoding the sgRNA does not have a PAM motif, and this prevents the recognition and cutting of the DNA by Cas9, thus maintaining the stability and integrity of the guide RNA throughout the editing process [19].

DSB created by the action of Cas9 is generally repaired in either of the 2 ways: Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR). The former one is error-prone and usually introduce indels in the site that is cleaved. Therefore, repair by NHEJ results in point mutations and there by frame shift mutations. While the latter repair mechanism of HDR is usually less prone to errors. So, CRISPR system requires only a gRNA corresponding to the target DNA and the endonuclease involved has a generalized activity and therefore can act on any genome. Therefore, it is one of the most popular tools used in genome editing [20-22].

After genome editing, verification of the intended modification is an essential procedure regardless of the experimental setup or DNA repair pathway utilized. PCR amplification of the genomic area covering the target position is generally the first step towards this verification. The obtained amplicon may be inspected using Sanger sequencing or next-generation sequencing technologies to evaluate editing results. After acquiring sequencing data, the edited sequence needs to be compared with an unedited control sequence to determine any off-target effects that are not intended and detect insertions or deletions (indels) in the target locus. Numerical scores that represent editing efficiency and specificity are provided by the majority of the computational tools designed for CRISPR data analysis, providing a good measure for comparing the success of the genome editing experiment. When the presence of the desired edit is verified with minimal off-target effects, the system is available for downstream functional studies as well as additional scientific investigation.

Gene knockout using CRISPR/Cas9 technology

CRISPR gene knockout signifies a technique whereby the expression or function of a gene is totally disrupted on a permanent basis. Here, rather than considering a specific mutation in the selected gene, the whole gene is picked and knocked out. The guide RNA forms a ribonucleoprotein complex with Cas9 or Cas 9 nickase enzyme and thereby target the 5' exon or the crucial protein domain. The complete inactivation or deletion of a gene utilizes the non-homologous end joining repair mechanism. In NHEJ or Non-Homologous End Joining, DSBs are sealed by the pathway on utilizing proteins that recognize and further lead to polymerization and ligation od DNA ends. Joining the DNA in this way would lead to frame shift mutations. This would as a whole render the gene non-functional enabling the complete knockout of the target. Thus, through NHEJ pathway, CRISPR knockout functions to eliminate the gene under consideration on introducing a DSB. Translation of this gene would lead to production of a truncated protein, lacking the amino acids represented by the gene.

CRISPR/Cas gene knockout need not always work, even after introduction of DSB by Cas9 followed by NHEJ could render the gene functional. One among the possibility for such an output could be the presence of an alternate start codon in the same gene that could further initiate transcription and protein synthesis to produce a totally functional protein. Another possible chance is when, even with shifted nucleotide sequence, protein synthesised was completely functional. CRISPR/Cas technology is utilizing the error prone DNA repair mechanism of NHEJ to knockout and thus inhibit a gene on creating a site specific DSB through RNA guided Cas9 nuclease enzyme.

The accuracy of genome editing mainly depends on the effective delivery of the CRISPR-Cas system into the biological system. It is also critical for reducing the immunogenic risk of CRISPR components, which may trigger immune responses in some organisms. The main delivery methods currently in use include viral vectors (such as AAV and lentiviruses), non-viral approaches (such as lipid nanoparticles, electroporation, and cell-penetrating peptides), and physical methods (such as microinjection). Each method has its own advantages and disadvantages, and the choice largely depends on the specific biological system being used.

Disease modelling

Historical methods of disease modeling, particularly the use of animal models such as mice, rats, and non-human primates, have been the hallmark of biomedical science for many years. These models have provided a great deal of understanding concerning the mechanisms of disease and the creation of therapeutic agents [23,24]. While animal models have been a key driver of advances in contemporary medical science, their use has also been beset by problems for many years. These are the restricted "translatability" of results into human applications, difficulties in precisely forecasting efficacy and toxicity, as a result, many therapeutics that show efficacy in animal models fail in human clinical trials, highlighting the limited predictive value of these models [25], and concerns related to the welfare of animals - specifically pain, distress, and the sheer numbers of animals killed and used [26-29]. The use of different regulations and laws regulating animal research is based in part on the 3Rs principle - Replacement, Reduction, and Refinement - a set of guidelines intended to advance more responsible and ethical use of animals in scientific studies. Developed by Russell and Burch in 1959, the 3Rs seek to replace animals with alternative approaches wherever practicable, re-

duce the number of animals used to the minimum required for statistically reliable results, and refine experimental techniques to minimize pain, distress, and suffering [30].

Additionally, animal models often fail to capture the full complexity of human diseases, especially those that are multifactorial, such as neurodegenerative disorders and cancers, which involve intricate interactions between genetics, environment, and lifestyle. In response to these challenges, CRISPR-Cas9 genome editing technology has emerged as a transformative tool for enhancing the precision and relevance of animal models. CRISPR genome editing is widely used for different disease modeling studies such as neurodegenerative diseases, cardiovascular disorders, autoimmune diseases and different types of cancers. Studying the genetic mechanism behind these disorders allows researchers to understand the disease associated mutations, mechanism and potential therapeutic interventions. Subsequent to the publication of Jinek et al. in 2012 [12], three studies that were independently conducted in January 2013 illustrated that the CRISPR-Cas9 system is a potent system for editing the human cell genome [31-33]. In these investigations, "humanized" variants of *Streptococcus pyogenes* Cas9 [31-33] and *Streptococcus thermophilus* Cas9 [31] were co-expressed with either designer single-guide RNAs (sgRNAs) [31-33] or with tracrRNA and designer crRNAs [31]. These systems were efficiently utilized in diverse human cell types, such as human embryonic kidney cells, chronic myelogenous leukemia cells, and induced pluripotent stem cells [31-33], and mouse cells [31].

CRISPR-Cas9 genome editing has been shown to be an effective method for modeling cardiovascular diseases (CVDs) through the precise and efficient modification of genes that relate to cardiac development, function, and pathology. Hereditary heart disease, including congenital heart disease (CHD), hypertrophic cardiomyopathy (HCM), and Duchenne muscular dystrophy (DMD), which are triggered by a mutation in either a single gene or several genes [34,35]. CRISPR-Cas9 technology has been used extensively in in vitro and in vivo models to study the genetic causes of CVDs, including arrhythmias, cardiomyopathies, and atherosclerosis. For instance, CRISPR has been used to introduce mutations in genes like *MYBPC3*, *LMNA*, and *TTN*, which are linked to hypertrophic and dilated cardiomyopathies, in human induced pluripotent stem cells (hiPSCs) and mouse models [36,37]. These engineered models recapitulate human disease phenotypes, facilitating mechanistic studies and drug screening. Alankarage et al. (2020) created CRISPR/Cas9 gene-edited mouse models to functionally dissect novel missense variants of patients with congenital heart disease (CHD) and show the effect of multiple congenital abnormalities on CHD phenotypes [38]. Likewise, Jaffré et al. (2019) established successfully a model of hypertrophic cardiomyopathy (HCM) of Noonan syndrome using patient-derived cardiomyocytes and CRISPR/Cas9-made isogenic control iPSC-derived cardiomyocytes, allowing a more precise examination of disease mechanisms [39]. In addition, Moretti et al. (2020) used a Cas9-mediated excision of exons to revive the reading frame of the DMD gene such that a functional but truncated dystrophin protein could be expressed [40]. This therapy enhanced skeletal as well as myocardial function in Duchenne muscular dystrophy (DMD) pig models and decreased the sensitivity of DMD cardiomyocytes to arrhythmias.

The most prevalent neurodegenerative diseases are Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and spinocerebellar ataxia [41]. The CRISPR-Cas9 system has also demonstrated great promise in the treatment of these diseases. Konstantinidis et al., in a 2022 published study, showed that the CRISPR-Cas9 system was capable of specifically in-

Table: 1 List of disease modeling studies using CRISPR/Cas9 technology

Disease	Target genes	Cell/ Organism	Delivery method	Ref.
Huntington's disease (HD)	CAG^{EX}	Fibroblasts derived from patients and induced pluripotent stem cell-derived neurons	AAV	54
Amyotrophic lateral sclerosis	Superoxide dismutase 1 (SOD1)	Mouse spinal cord	AVV9	55
Alzheimer's disease (AD)	PS1 gene	Human fibroblasts	Electroporation	42
Alzheimer disease (AD)	APOE4 gene	Induced pluripotent stem cells	LV	56
Early-onset Parkinson's disease (PD)	DNAJC6	Human embryonic stem cells	Electroporation	57
Late-onset Parkinson disease (PD)	*VPS35*	Mice model	Microinjection	58
Clonal hematopoiesis of indeterminate potential (CHIP)	*DNMT3A, TET2, and ASXL1*	Human stem and progenitor cell	Ribonucleoprotein (RNP)-based delivery	59

activating the PSEN1^M146L allele, an Alzheimer's disease-causing mutation. The specific inactivation caused a partial normalization of the aberrant Aβ42/40 ratio found in mutation carriers, a major pathological feature of the disease [42]. In a similar vein, Ortiz-Virumbrales et al. (2017) demonstrated that editing the PSEN2^N141I mutation in neurons generated from patient-specific fibroblasts with CRISPR-Cas9 restored the Aβ42/40 ratio to normal and effectively revived the electrophysiological properties of the neurons [43]. Deng et al. reported a study with the application of CRISPR/Cas9-mediated editing for disrupting the hSOD1-G93A transgene that causes amyotrophic lateral sclerosis (ALS) in two lines of hSOD1-G93A transgenic mice. The results revealed that the in vivo gene-editing system was efficient in disrupting hSOD1 and brought about a disease-free condition in both the hSOD1-G93A transgenic lines (G1H and G1L), thus identifying its potential in therapeutic treatment for ALS [44]. Yet, a number of challenges lie ahead before CRISPR/Cas9 technology can be completely translated into clinical treatments for humans. Fundamental issues like safety, off-target effects, and effective delivery methods need to be carefully addressed and optimized. In spite of these obstacles, the therapeutic value of CRISPR/Cas9 for the treatment of neurodegenerative diseases is unquestionable. Further research and development are needed to fully realize this technology's potential and to offer significant hope to the millions of people suffering from these debilitating disorders.

Challenges and limitations of CRISPR-Cas9 system

Although the simplicity and accessibility of CRISPR-Cas9 are key advantages, some important concerns have yet to be addressed. Delivery of the programmable nuclease is a key problem in genome engineering. The choice of vehicle for the CRISPR system depends on the purpose of the experiment and can vary from viral to non-viral methods. Another major concerns about the CRISPR-Cas9 system since its development has been the high rate of off-target effects that it generates, immunogenicity and editing efficiency which needs to be improved for its biomedical applications.

Off-target effects continue to pose a major problem in CRISPR applications. In organisms with larger genomes than bacteria, sequence-specific off-target interactions are more significant [45,46]. Nevertheless, the frequency of off-target effects for CRISPR is much lower than RNA interference (RNAi), the commonly ap-

plied gene-silencing technique in previous generations of knockout technologies [47,48]. Off-target effects may cause genomic rearrangements and large deletions, leading to the disruption of normal gene function and, in severe cases, contributing to oncogenesis [49]. Therefore, minimizing off-target effects is a critical step in the development of CRISPR-based gene therapies. To minimize these effects, a combination of strategies is being used, mainly by the use of high fidelity Cas9 variants, such as eSpCas9, SpCas9-HF1 or SpCas9-nCas9 etc. and different approaches for delivery of CRISPR components, as ribonucleoprotein complexes (RNPs) [50]. This type of RNP delivery ensures the transient expression and minimizes the risk of off-target effects [51].

The fast-paced growth of CRISPR technology has brought tremendous industrial challenges, notably in the production of CRISPR-based treatments and products. Among the concerns is the astronomically high price tag for manufacturing, coupled with the requirement of having tight control over quality assurance of genome-editing tools as well as delivery systems. As the technology develops to be produced on a wider clinical and commercial scale, challenges become more demanding (52).

Recent advances and Future perspectives

Last few years have witnessed tremendous manipulation of CRISPR/Cas9 as a technology in fields of biomedicine, agriculture, dairy industry, textiles and much more. Much of awaited advancements in all these fields popularized the technique and is nowadays one among the most harnessed genome editing tool. Over 500 articles on CRISPR technology have been published and five times the number of researches is ongoing all around the world. The technology has been used in many different organisms and cell categories including lower as well as higher organisms.

One of the prominent developments in CRISPR is the dCas9, which is literally inactive Cas9, that is a specialized Cas9 enzyme with induced mutations in the H840A (HNH domain) and D10A (RuvC domain) positions [22]. This enzyme is catalytically inactive and thus cannot bring about a DSB, even though it is enough efficient as the wild-type Cas9 enzyme. dCas9 can be used in targeting the promoter in human cell lines and *Escherichia coli* cells and to activate or repress any gene on recruiting functional domains on to respective loci. This has led to the development of custom-made transcription factors that can turn genes on with high accuracy - a technique known as CRISPR activation (CRISPRa). Activation is achieved by using modified guide RNAs that recruit the dCas9 protein - now acting as a DNA-binding scaffold rather than a nuclease. These sgRNAs are either fused with an RNA-binding protein linked to an activation domain or designed to interact directly with dCas9 carrying the activation domain.

Transcriptional suppressor domains—preferably the KRAB domain, epigenetic modifiers, or the Krüppel-associated box—may be recruited by an inactivated Cas9 protein (dCas9) to transcription start sites within the human genome to repress gene expression [53]. This method, referred to as CRISPR interference or CRISPRi (also known as CRISPR jamming), allows for effective repression of genes without causing double-strand breaks. CRISPRi can be used to achieve efficient knockdown of coding and non-coding RNAs in human cells, providing a versatile and powerful tool for the investigation of the functional effects of gene silencing, including partial gene expression suppression.

Another important application of CRISPR technology is the drug screening assay for both genome-wide and targeted applications. It is commonly used to

modulate the expression of specific genes and gene mutations associated with various genetic diseases and create an in-vitro model of those specific diseases, which allows the researchers to assess the efficacy and toxicity of potential therapeutic compounds prior to advancing to in vivo studies. In addition to drug screening, CRISPR technology is widely used for developing personalized medicine, which is a specific treatment option depend on the patient's genes, their environment and lifestyle. Personalized cancer screening and treatment is widely used nowadays by the use of CRISPR technology which helps to identify the tumor growth, drug resistance, epithelial-to-mesenchymal transition, cancer initiation, metabolic reprogramming, and metastasis. Additionally, CRISPR-based screening has been applied to patient-derived tissues to pinpoint individualized drug targets and to model potential responses to various therapeutic regimens.

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REVIEW ARTICLE

Engineering Biomaterial Scaffolds for Scarless Wound Healing: Opportunities and Challenges

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Abstract

Wound healing is a complex biological process that involves a sequence of events aimed at restoring tissue integrity after injury. Scar tissue can frequently develop through conventional wound healing techniques, which cause practical constraints and aesthetic problems. This review primarily focuses on scarless wound healing, highlighting the types and stages of wound healing, the role of biomaterials in facilitating scarless repair, and the application of machine learning algorithms to optimise the selection of suitable biomaterials. In addition to that, we discussed the role of biomaterials in scarless wound healing and how they help in tissue regeneration without leaving permanent scars. Interestingly, the examination of machine learning methods using various criteria, such as biological responses, biomaterial qualities, and patient-specific aspects, is discussed. This involves using different algorithms to anticipate the best biomaterials for specific patients by identifying correlations and trends in the data. However, for faster development in this promising area, future research should focus on improving biomaterials and extending the use of machine learning. A series of biological activities are involved in the complicated biological process of wound healing, which is intended to restore tissue integrity after injury. Conventional wound healing methods typically result in the formation of scar tissue, which can create functional limitations and cosmetic issues. The focus of this review was specifically on scarless wound healing, emphasising the many types and stages of wound healing, the importance of biomaterials in enabling scarless repair, and the application of machine learning algorithms to optimise the selection of suitable biomaterials. Additionally, we reviewed the function of biomaterials in tissue regeneration without creating long-lasting scars and their involvement in scarless wound healing. It's interesting to see how machine-learning techniques are applied to a wide range of criteria, including biological reactions, biomaterial properties, and patient-specific features.

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Introduction

Scar formation, or fibrosis, is the primary method of wound healing in humans. It can significantly alter the appearance of the injured area, impacting the patient's physical and psychological well-being (1). Numerous treatments have been developed to address scar formation, including photodynamic therapy, laser treatment, microneedling, microdermabrasion, and fractional radiography (2-4). An alternative to traditional wound healing methods is scarless healing. In recent years, re-

searchers have discovered that human fetuses and several other animals can regenerate their skin after a wound. However, this regenerative property depends on various factors, such as the size of the wound and the age of the individual (5). The ability of a wound to heal without forming scars decreases with age, with adults having the lowest rate of scarless healing. Researchers have identified certain key differences between human foetuses and adults, but the main mechanism behind scarless wound healing remains unknown (6). Unlike humans, some animals can heal wounds without scarring, even in adulthood (7). Animals such as mice, deer, frogs, dolphins, and salamanders can regenerate broken limbs and skin (8,9). These animals have been used as research models to identify key parameters for complete regeneration of the skin and other body parts, to induce these processes in adult humans (10). The motivation behind this review article is to identify the factors responsible for scarless wound healing in foetuses and to apply these factors to adults to achieve scarless wound healing. Promising strategies for inducing scarless wound healing in adults include gene therapy, mechanical force induction, chemical inhibition, nanoparticle treatment, and genetic transcription (11-14). Biomaterial scaffolds have been a promising solution for inducing scarless wound healing in adults. FDA-approved synthetic polymers, such as PLGA, PCL, and PVA, are widely utilized in wound healing applications due to their excellent biocompatibility and biodegradability. In addition, several natural polymers, including collagen, gelatin, hyaluronic acid, chitosan, dextran, silk, alginate, and cellulose, are also extensively used for wound healing applications (15,16). Various tissue-engineered scaffolds with different geometries have been developed to mimic the extracellular matrix (ECM) of human skin. These methods include the nanofibrous structures by electrospinning, 3D interconnected porous designs, particulate leaching, solvent casting, gas foaming, fibre mesh, melt moulding, and freeze drying. These biomaterial scaffolds are intended to enhance healing and prevent scar tissue formation, promoting scarless wound healing (16,17). Amino acids such as arginine, glycine, and aspartate (RGD) are crucial for wound healing. Scaffolds containing RGD peptides have demonstrated accelerated wound healing (18). Scaffolds incorporating ZnO-curcumin nanocomposite, hyaluronic acid, chitosan, graphene oxide, and N-acetyl cysteine demonstrated enhanced wound healing (19-22). 3D bioprinted scaffolds are showing great promise in wound healing applications by providing a supportive framework for cell

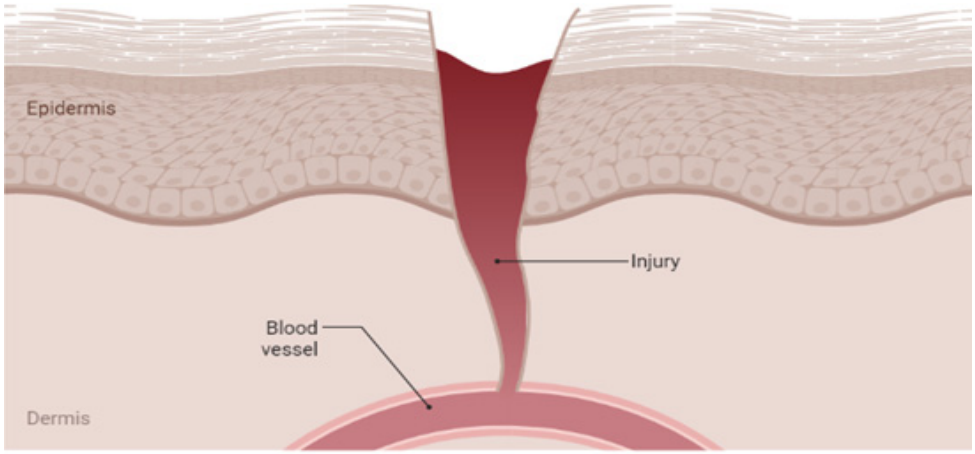

Figure 1: Pictorial representation of wound development in human skin

Table 1: Annual cost for various wound types in the United States of America

Wound type	Annual costs
Non-healable wounds	50 billion dollars
Surgical and traumatic wounds	12 billion dollars (35)
Burns	7.5 billion dollars

growth and tissue regeneration. These scaffolds, often mimicking the natural extracellular matrix, can be designed to deliver cells, growth factors, and drugs directly to the wound site, accelerating the healing process (23).

Nowadays, machine learning (ML) has become increasingly important in wound healing applications, enhancing diagnostics, predicting healing times, and personalising treatment plans. Artificial intelligence (AI)-powered systems can analyse wound images, patient data, classifications of burns, wound segmentation, prognosis models, and chronic wound healing predictions, among other relevant information. This analysis assists clinicians in making more informed decisions and optimising wound care strategies. These algorithms have demonstrated efficiency in supporting clinical evaluations, ultimately improving diagnoses (24-27). Recently, monitoring the wound microenvironment for biomarkers such as pH, glucose, temperature, and moisture using machine learning (ML) approach has shown effective wound management (28-29). Another study by Salem et. al, predicted nanofiber performance based on fibre diameter, water contact angle, mechanical strength, and interconnected porous structures for skin tissue regeneration (30). This review focuses on scarless wound healing with the primary objective of summarising current understanding and advancements in the field, specifically aiming for wound repair that results in minimal to no scar formation.

Wound and its Types

The skin, the outermost layer of the body, is prone to damage from a variety of sources, including physical harm, dangerous radiation, microbial infections, and more. These injuries cause problems for both the patient and the healthcare industry (31). Table 1 illustrates the annual costs accounting for various categories of wounds in the United States of America. Complex wounds are common, and some of the examples are lower limb ulcers, surgical ulcers, and surgical wounds. The treatment for these complex wounds would be costly, and it must be provided by medical guidance. It was suggested in 2015 that future work on complex wounds should be focused on developing insights and prognostic factors that are cost-effective (32). Grossly, wounds can be classified into acute and chronic, which vary in their etiologies. Chronic wounds take much more time to heal than acute wounds, requiring medical care and support for months, since they fail to heal in the normal phase. The most important factor is the accurate measurement of the wounds. Wound segmentation is a widely recognised method in recent years, classified as a computer vision and deep learning approach. The latter part of the literature discusses this method in depth. Also, it is highly expected that by 2022, the wound care market will exceed $15 billion, and by 2024, it will exceed $22 billion (33). A wound is damage that can happen externally, like a cut or damage to the skin, or internally, like clotting. However, an external wound is better since it is visible and can be treated at an early stage. An appropriate assessment of the wound is crucial for effective treatment, as distinct types of wounds exist

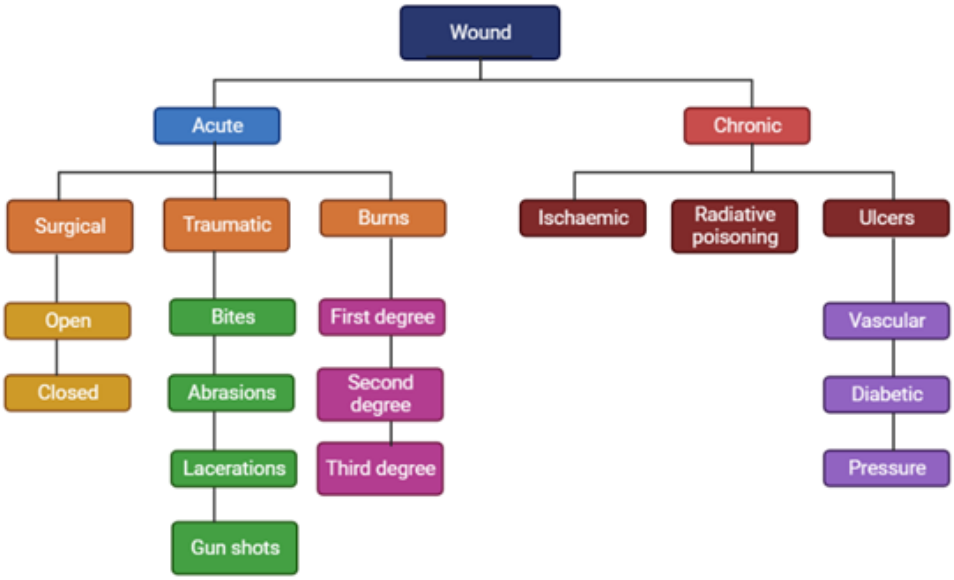

Figure 2: Flowchart representation of the classification of wounds

with varying etiologies. Figure 1 represents the wound occurring in human skin.

Based on their expertise and the manner of evaluation, clinicians' views on the different forms of wounds will differ from one another. Wound measuring is the approach most frequently used to determine the best course of therapy. The classification of wounds is crucial because it can provide answers to several queries about the origin, location, reaction and severity of the wound. Additionally, the categorisation of wounds organises them based on some shared characteristics; by defining the type of wound, this can help the doctor diagnose the wound.

Acute wounds

Surgical and traumatic wounds, superficial burns, and abrasions come under acute wounds. The majority of the acute wounds result from surgical recovery, and this has significantly increased the costs for post-surgery care (36). In 2014, 17.2 million hospital visits occurred due to acute injuries, with 57.8% outpatients and the rest 42.2% inpatients (37).

The acute wounds can be further classified as open and closed wounds. The former one is dangerous due to the risk of microbial infection. The first underlying treatment for any acute wound should be the intake of antibiotics suggested by the medical professionals, and to irrigate the wound with suitable disinfectants (if it's an open wound) to avoid contamination. Colonisation of bacteria has been observed widely in open wounds. Microorganisms are transferred through contact with the contaminated water, health care workers, or fomites. *Staphylococcus aureus,* a gram-positive bacterium, and fungi like Candida can aggravate acute wound infections. Gustilo-Anderson et.al classified the open wound into 5 different types (figure 2), such as 1,2, 3A, 3B, and 3C, respectively (34).

Type 1: A clean wound with a diameter of <1cm, no soft tissue injury, appropriate soft tissue coverage of the bone, and absence of periosteal stripping.

Type 2: A wound that is moderately contaminated, has moderate soft tissue injury larger than one cm, has enough soft tissue coverage of the bone, and has not had the periosteum stripped away.

Type 3A: Periosteal stripping, severe soft tissue injury, significant contamination, and appropriate soft tissue coverage of bone are present in the wound.
Type 3B: Periosteal stripping, considerable soft tissue injury, significant contamination, and inability to replace bone with soft tissue without transplant.
Type 3C: Similar to types A or B, but with damage to the artery that needs to be repaired.

Chronic wounds

A chronic wound is providentially identified as a wound that would be arrested with the normal stages of inflammation. Chronic wounds persist for more than a month and can also remain open (36). Considering diabetic wounds, most of the wounds that haven't healed at least 50% within the first 4 weeks probably undergo arrested healing and convert to chronic wounds, as studied by Sheehan et al.

The primary objective in chronic wound healing is to identify the underlying cause of the wound that is not responding to conventional healing methods. If a wound escapes the normal phase of healing, then it is said to be chronic. There are several reasons to determine the chronic nature of a wound. These reasons must be analysed for the treatment of the wound/wound healing (34). Wound assessment is to be performed only after the determination of the issue/cause for the halt of wound healing. These factors play a key role in determining the chronicity of a wound.

- *Arterial*: This is based on the level of blood flow. Usually, ABI should be higher than 50mm Hg. If this condition fails, then there would be a failure of wounds for normal healing.
- *Venous*: Pressure changes in the walls of a blood vessel led to the leakage of fibrin, and this accumulation causes effects on the healing since it reduces the synthesis of collagen.
- *Infection*: Processes with infections, like cellulitis, can inhibit the healing process.
- *Pressure*: The increased pressure in the wound area would destroy the normal growth of the new tissue. It also prevents the profusion of blood at the site of a wound.
- Oncologic: Biopsy areas are considered non-healing wounds with atypical malignant presentations.
- Systemic: Multiple systemic diseases, like diabetes and microvascular disease, can inhibit the healing process.
- Nutrition: Vitamins and mineral deficiencies can suppress wound healing.
- Pharmacological: Certain medications, like Hydroxyurea, have been reported in multiple instances for the development of non-healing ulcerations.
- Self-inflicted/psychosocial/self-inflicted: There are some instances where the patient might be the cause of ulceration, either knowingly or unknowingly. This could be a tough factor to identify, but this factor must be accounted for in wound assessment.

As previously reviewed (33,38), chronic wounds are a great challenge for wound care professionals for both the assessment of the wound as well as the treatment. Hence, it is essential to know the pathophysiology of these chronic wounds. Chronic wounds would be treated in such a way that the patient should be hospitalised according to the type of wound and the impact that he/she had. Some common features observed in chronic wounds, as compared to acute wounds, are the excess levels of protease, ROS, and cytokines. Diabetic and obese

patients have a high risk of developing chronic wounds. A prolonged open wound indicates that the patient is suffering from major health conditions. The review confirms that chronic wound affects the elderly population maximally. By 2020, the US government estimated that chronic wounds would be a persistent problem in the surviving population (36). Diabetic ulcers are complicated and are often found in patients having Diabetes Mellitus (39). The treatment for diabetic ulcers remains a challenge to clinicians due to complex wound healing, which features bacterial infection, chronic wounds, exacerbated inflammation, persistent pain, and impaired angiogenesis (40). CDC stated that more than 100 million people have diabetes or pre-diabetes in the US. People with pre-diabetes are untreated, leading to the development of Type 2 diabetes in the upcoming 5 years. The prevalence of diabetes has increased with age. It is estimated that 400 million people worldwide have diabetes. Diabetes is highly found in Foot ulcer patients, which indicates a neuropathic origin. The risk of these ulcers in diabetic patients is estimated at 15% to 25% (36).

Foot ulcers are nothing but sores that are open on the foot. It may be shallow or deep. As already reviewed, foot ulcers are not only common in patients with diabetes but also in people with compromised blood circulation. The prevalence of foot ulcers has not changed, despite advanced medical techniques. DFU prevalence was found to be 6.3% globally. It is said that around 70% of ulcers in the lower extremities are caused due to chronic venous insufficiency. The venous ulcer has a major socio-economic impact on its medical care and treatment. Long-term therapeutics are needed. The understanding of pathogenesis is vital for the management of venous ulcers. Recently, it has been shown that the pathogenesis of foot ulcers is associated with microcirculatory abnormalities, leading to the generation of an inflammatory response (41). Pressure ulcers are complex, chronic wounds. It is really difficult to prevent or manage, and has devastating comorbidities for the patients (42). Pressure sores, also called decubitus ulcers, are primarily caused by pressure and shear. The estimated occurrence of pressure ulcers ranges from 3% to 14% in hospitalised patients and 25% in nursing homes. The two groups that are at major risk of this type of pressure ulcer are the elderly and spinal cord injury patients. Also, people with neurological disorders get affected in the ankles, sacrum, trochanters, ischium, and heels, which are some common sites of infection (43). PU care is not human-friendly, as the annual cost in the U.S is around $11 billion. A pressure ulcer can be lethal if not taken care of (36).

Burn injuries are those that arise due to heat, exposure to radiation, the sun, fire accidents, etc. The complications and severity of the burns depend on the degree. There are four different degrees of burn injuries. Figure 3 represents the clas-

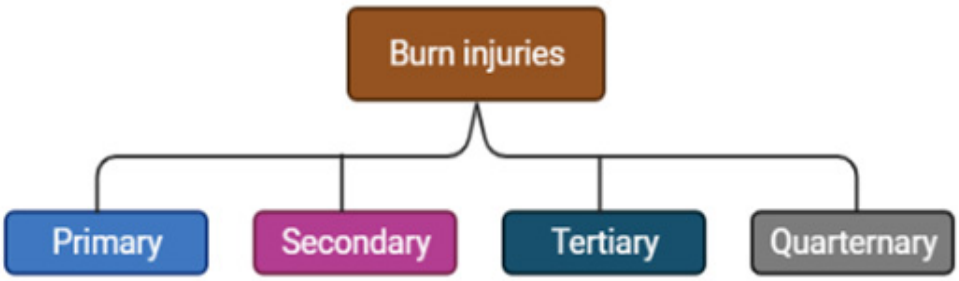

Figure 3: Classification of burn injuries

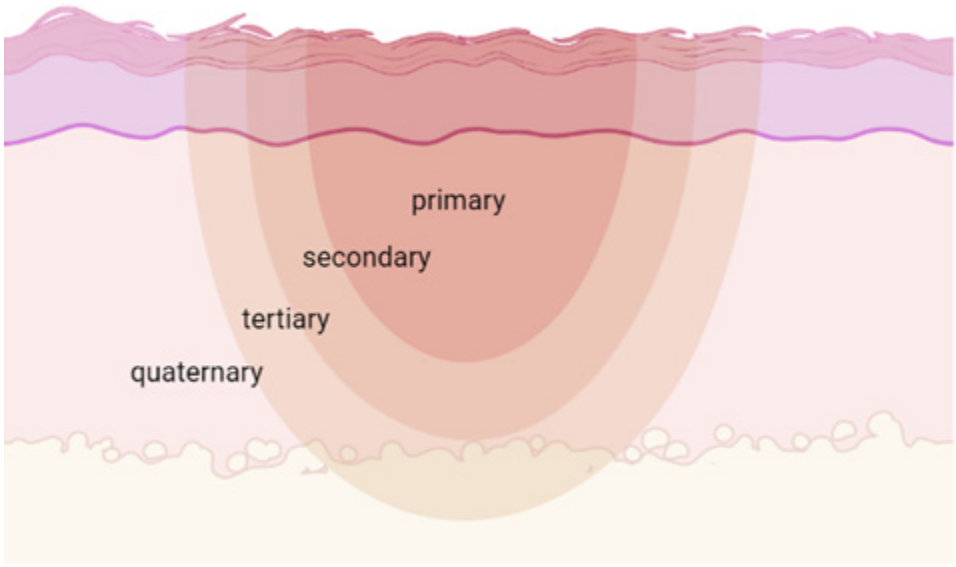

Figure 4: Zones for the different types of burn injuries

sification flowchart for burn wounds. Primary or first-degree burn injuries include reddening and swelling of the epidermis with slight pain. The secondary or second-degree burn injuries are split into two subcategories. IIa involves blistering and reddening of the superficial dermis, and IIb involves blistering and reddening of the subdermis. Tertiary or third-degree burns destroy the dermis completely with black necrosis. Quarternary or fourth-degree burns may extend to the bones and joints, resulting in their carbonization (44). Figure 4 represents the zones for the different types of burn injuries. Humans have been provided with another mode of survival, collagenous "glue" formation in response to injury, and production of this scar tissue is the primary means of repair for all vertebrates (45). A significant portion of the healthcare burden is attributed to scars. Though the wounds heal, the burn wounds leave hypertrophic scars. There is a high risk for the face to undergo scarring, resulting in aesthetic changes and functional deficits. Scarring is a substantial problem in the healthcare domain, except for the face (36).

Wound Healing Stages

Tissue injury goes through a set of events in an ordered fashion, starting from coagulation, through inflammation, and reaching the final stage, repair. While the initial stages are similar in almost all kinds of wounds, the subsequent course of events depends upon the tissue type and the degree of damage. Repair of the wounds mainly includes connective tissue replacement. Resolution, replacement, and regeneration are the three ways for cell repair. Many cells and inflammatory mediators (for example, cytokines) are involved in the repair process. There are many extrinsic and intrinsic factors influencing wound healing (46). Healing of a wound follows a chain of events. Each stage tends to overlap with the successive and previous stages. The five phases of wound healing are (1) Haemostasis, (2) Inflammation, (3) Proliferation, (4) Remodelling. Figure 5 represents the different phases of wound healing.

Haemostasis is the process of preventing and stopping bleeding. All external injuries are at the vascular level and thereby initiate the cellular and molecular re-

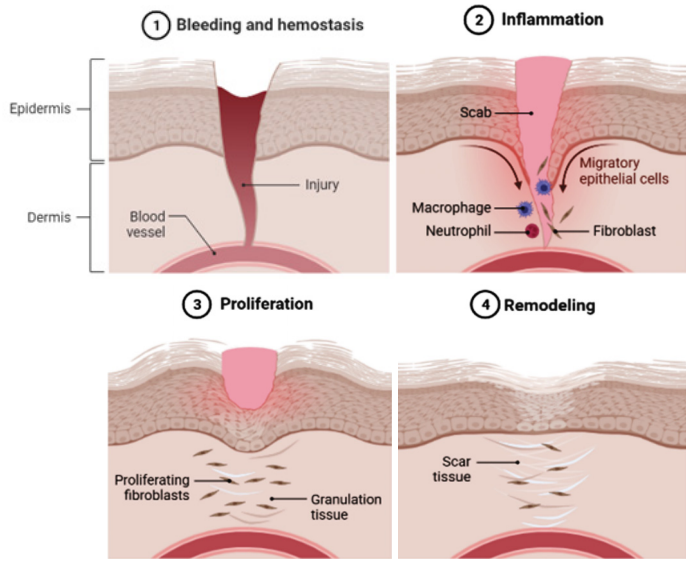

Figure 5: Graphical representation of wound healing phases

sponses that help to bring forth haemostasis. Vasoconstriction, fibrin deposition, and platelet aggregation contribute to haemostasis, which ultimately leads to clot formation. The composition of the clot is aggregated platelets, blood cells, and a fibrin mesh (47). The main reason for the formation of a clot is to prevent fluid loss and limit contamination from the environment. Figure 6 represents the role of fibrin in wound healing.

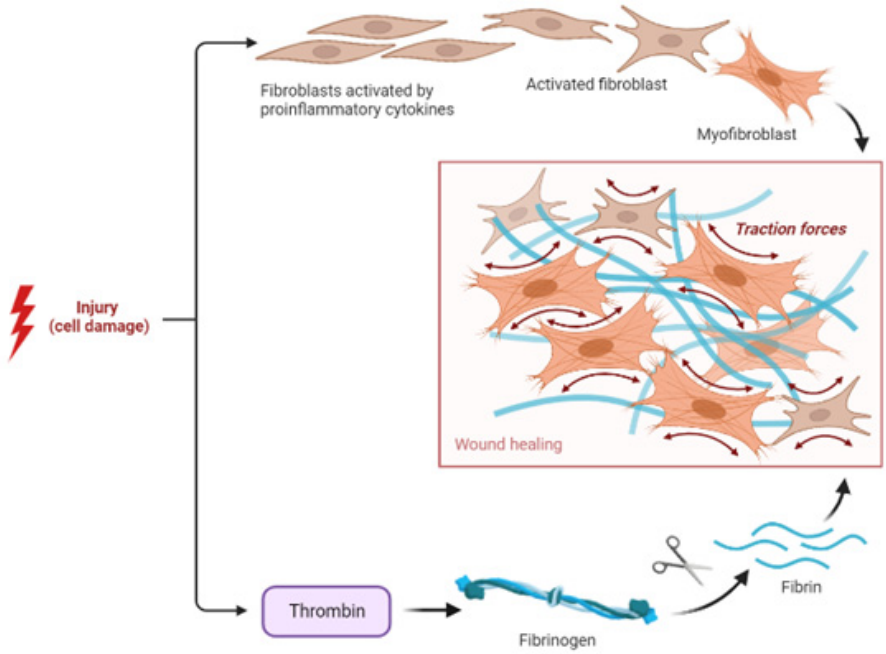

Figure 6: A depiction of the formation of clots by fibroblasts during wound healing

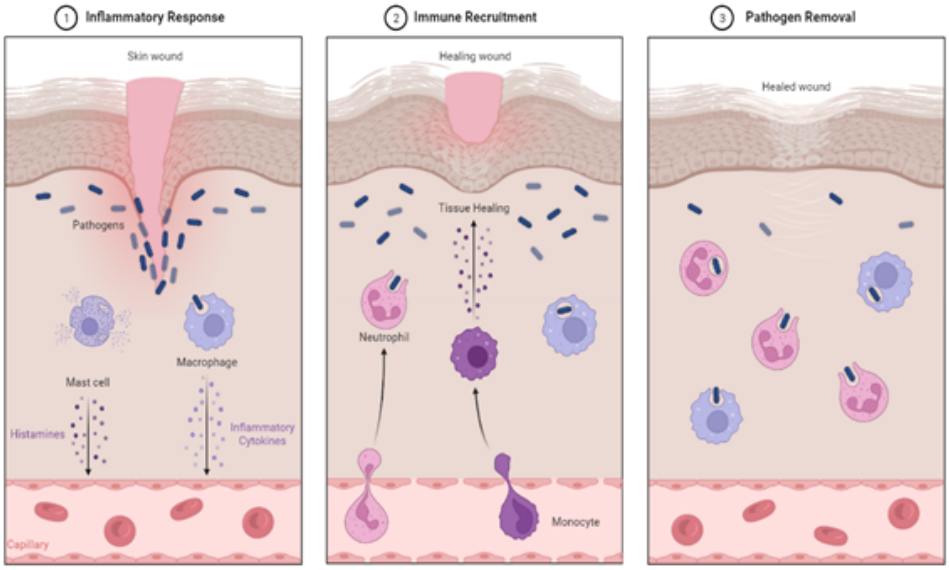

Figure 7: An illustration of the immune system's reaction to injury

The release of vasoactive amines initiates vasoconstriction, and some amines occur with dermal penetration. The release of epinephrine into the bloodstream occurs. Simultaneously, norepinephrine is released due to sympathetic nervous stimulation. Secretion of prostaglandins by the injured cells contributes further to vasoconstriction. The damaged cells release tissue factors that stimulate platelet aggregation. Using Von Willebrand factor and fibrinogen, the platelets adhere to the vascular sub-endothelium (48). Alpha granules, lysosomes, and dense bodies are released from the cytoplasm of platelets during their adhesion and aggregation. The early and late stages of healing are affected by a range of immunomodulatory and proteinaceous substances that are present in alpha granules, including fibronectin, albumin, IgG, fibrinogen, coagulation factors V and VIII, PDGF, TGF-a, TGF-b, FGF-2, EGFs, and endothelial cell growth factors (49). Dense bodies contain compounds involved in the initiation of the coagulation cascade (50). There are two phases in the inflammation stage, namely the early and late inflammatory phases, classified based on response duration, time, and inflammatory cell type. The activation of the coagulation cascade initiates the inflammatory phase. This is followed by infiltration of the wound with neutrophil granulocytes, which are attracted by several chemoattractants. Within a short duration, the leukocytes adhere to the blood vessel endothelium in close proximity. This process is also called margination. The leukocytes further start to move more actively through the wall of the blood vessel and the process is called diapedesis. Figure 7 represents the body's immune response to a wound.

In the wound environment, after the process of phagocytosis of foreign particles is completed, increased mitotic activity is exhibited by the cells present in the cut edge of the epidermis. Migration and proliferation of epithelial cells along the cut edges to the dermis is observed. Through the process of phagocytosis or extrusion to the surface of the wound as slough, the redundant cells are cleared from the wound surface. During the late inflammatory period, the conversion of monocytes to macrophages occurs upon arrival at the wound site. Monocytes get attracted to the wound by collagen and elastin products, fragments of immunoglobulins, and clotting components. The macrophages, which are the key repair

regulatory cells, are very important in this phase (48-72 hrs). They produce proliferation factors and aid in extracellular matrix production along with fibroblasts. They also release proteolytic enzymes that help in the wound debridement. Polymorphonuclear leukocytes and macrophages secrete additional growth factors, stimulating inflammation. The margins of the wound are lined with collagen fibers during the late inflammatory phase. As the proliferation of epithelium continues, it forms the thick epithelial cover (51). Proliferation is marked by granulation tissue formation, collagen deposition, epithelialization, and angiogenesis, even though they occur at varying time points in healing process. Cell detachment and mitotic division mark the beginning of epithelialization. The keratinocytes present at the wound-edge initiate the epithelialization process. This process is also initiated from hair follicles, sweat glands, and sebaceous glands, which are dermal appendages and is stimulated by growth factors like fibroblast, epidermal, transforming epidermal b, and multiple cytokines. Angiogenesis is new thin-walled blood vessel formation from existing vessels. Increased metabolic requirements caused by a healing wound are highly metabolically active and require oxygen (51). Without an adequate supply of oxygen for the hydroxylation of lysine and proline residues, the chemical bonds to create mature collagen and fibroblasts will not form. Fibroblasts produce elastin and organise the ECM. These critical elements serve as the basis for remodelling, and is the final part of wound healing (52).

After two to three weeks of onset of the injury, the fourth phase of healing begins with remodelling. It could last for a year or more, depending on the intensity of the injury. This stage of healing results in development of maximum tensile strength along with degradation and re-synthesis of the ECM. In the final stage of wound healing, regeneration of normal tissue takes place. This is effected by the gradual remodelling of granulation tissue, leading to less cellular and vascular scar tissue formation, with the increase in concentration of collagen fibres. When the surface is entirely covered by a keratinocyte monolayer, cessation of migration of the epidermis occurs with the establishment of a basal lamina and stratified epidermis (53).

During this stage, type 1 collagen replaces type 3 collagen as it gets degraded resulting in wound closure with greater tensile strength. For functionality restoration and tissue appearance, chemokine production by anti-inflammatory cytokines, such as IL-10 or TGF-b, is lowered. Due to the process of emigration, apoptosis, and other unknown mechanisms of cell death, blood vessels, inflammatory cells, and fibroblasts vanish from the wound area during the maturing and remodelling process. The result is a scar tissue formation with minimal cells. Later, the fibroblast phenotype changes and start to express the smooth muscle actin, and is called myofibroblasts. They move closer to wound borders and become responsible for the wound's contraction. These myofibroblasts are the main ECM producers in the process of fibrosis. The structural components, such as collagen, fibronectin, and fibrin, unleash specific cell activities at the wound site. For example, fibronectin makes adhesion and cell migration feasible by generating a framework. Vitronectin, an adhesive glycoprotein, aids in the contraction of the tissue. These processes can be influenced by exogenous and endogenous factors also. Due to the existence of such processes, the interactions of the cell/matrix have been targeted for therapeutic approaches. Systemic disorders, like diabetes, immunosuppression, etc, and those due to external agents like, smoking, can hinder early wound closure. One of the factors that is complicating this is the hypertrophic scars (54).

Primary wound healing or healing by first intention

When a wound closes within 12-24 hours of its occurrence (for e.g., clean surgical incision) there primary healing occurs. Using sutures, tissue glue, mechanical devices, or tapes, the wound edges are directed. Epithelial regeneration prevails over fibrosis because the incision only results in a limited epithelial disruption at the basement level and the senescence of a small number of epithelial cells. Faster healing and rapid closure occurs due to balance of all healing phases.

Secondary healing (healing by second intention)

In a wound with excessive loss of soft tissue (severe burns), secondary healing occurs. Since regeneration of the epithelial cells alone cannot result in restoration of the original structure of the skin, a granulation tissue from the margin of the wound, followed by ECM accumulation with collagen synthesis, is seen. Epithelialisation and wound contraction close the wounds (55).

Scarless Wound Healing

Since the skin aids in the protection of the body from the external environment, proper healing is an essential response to tissue damage. Defects in scarring can lead to excessive deposition of ECM, which could lead to pathological scarring. Overproduction of ECM could affect the functioning of internal organs and the skin (46). Abnormal skin wound healing can lead to scarring, fibrosis, and increases the chance of conversion to chronic wounds. Even though millions of people are being affected, there is no effective cure for adverse scarring.

Formation of Scar Tissues

As stated earlier, the final stage of wound healing occurs with collagen production. Collagen is produced primarily by the fibroblasts. The strength of the skin doesn't depend on the amount of collagen formed. Wound durability or the tensile strength depends on the microscopic welding of these filaments formed by fibrils and collagen fibres. Glycosaminoglycans are also synthesized by the fibroblast, which fills the space between and around collagen fibres and glycosaminoglycans, combined with water, provides lubrication. New cross-linkages are formed when this important buffer disappears, making the moving tissue stationary. The location and number of cross-linkages formed depend on the composition of the non-fibrous substance. Thus, the scar tissue's architecture is defined by the relationship between glycosaminoglycans and collagen. This allows the wound for tolerated early, motion with control devoid of disruption.

Under optimal healing conditions, wound healing proceeds to the next phase. Re-inflammation of the wound is possible due to oedema, infection, and rough handling of the wound. In case of a broken scar, it will create another wound, leading to the formation of another scar. Thus, it is understood that "The return of function is inversely related to the amount of scarring (56). It takes more than closing the wound for successful wound healing with enough tensile strength. For the scar to fit the tissue, remodelling of the scar tissue is required. This is done by rearranging the collagen fibres to permit motion between the parts. The repaired ligaments must have a firm scar for resistance to deforming forces. Such factors define the final physical characteristics of the scar (57).

Wound healing without scar formation

One of the major breakthroughs was the discovery that foetal skin heals without

Table 2: Comparison of adult and foetal wound healing

Components	Adult	Foetal
♦ Collagen in the extracellular matrix:		
Rate of deposition	Delayed	Immediate
Histological pattern	Dense parallel bundle	Fine, reticular
HASA	Low level	High level
♦ Cytokines		
IL-6 and IL-8	High level	Low level
IL-10	Low level	High level
♦ Growth Factors		
TGF-beta1 and beta2	High level	Low level
TGF-beta3	Low level	High level
♦ Stem Cell		
MSCs	Absent	Present

scarring. Similarly, the oral mucosa heals without any kind of scarring. Appreciation of fibroblasts has led to advancement in the mechanism of wound healing without scarring (12). As stated earlier, the dermal fibroblasts result in scar deposition after cutaneous injury. The papillary and reticular dermis contains two different types of fibroblasts (58). Another subpopulation is associated with the hair follicles. These fibroblasts failed to exhibit developmental and functional diversity when cultivated, but showed variances in growth, morphology, and cytokines. Driskell et al concluded that skin connective tissue has two separate fibroblast lineages with particular functional characteristics (59). The first lineage of fibroblasts gives rise to the upper dermis and forms hair follicles, whereas the second lineage of fibroblasts deposits the connective tissue. Epidermal B-catenin activation stimulates upper dermal lineage expansion, rendering wounds permissive to hair follicle growth. As this field is being further explored, cellular targets for scar formation modulation will emerge (60). Tissue adhesives play an important role in the natural regeneration of tissues. These materials provide an alternative approach to wound healing that is painless and does not form scar tissue. Octylcyanoacrylate is being used as a tissue adhesive to close the low-tension lacerations, such as those occurring during a caesarean operation (61). Nano silver-decorated mesoporous silica nanoparticles act as potential tissue adhesives that can be used to induce scarless wound healing. Also, this material is antibacterial due to the presence of silver. This material can be used for post-operative scar prevention and wound healing in case of dermal injuries (62).

Scope of Scarless Wound Healing

Nearly every year, 100 million patients acquire scars, among which 4 million are burn scars and 11 million are keloid scars. Further development in the field of scarless wound healing, also known as regenerative wound healing, would help millions. To revolutionise care of wounds like lacerations, burns, and incisions and to alleviate suffering, healing of cutaneous injuries without fibrosis should be encouraged (63).

Scarless wound healing in foetus and adults

A curious research effort has been undertaken focusing on unravelling the mechanism that underlie scarless wound healing in foetus as an attempt for quality improvement of the healing process in both children and adults. Wound healing in

Table 3: Factors influencing foetal wound healing

Modifying factors	Examples
Tissue specificity	Skin, intestinal, diaphragmatic, and
Wound size	Large wounds heal with scar, in foetal
Gestational age	Formation of scars increases with age

adults, predominantly by scar formation, is one of the physiologic processes that can affect patients in a devastating fashion. The foetus heals without the formation of scar, in contrast to adults, in the early gestation period, Researchers are trying to find the mechanisms for scarless wound healing in the foetus and trying to induce those changes in adults to carry out scarless wound healing in them. Table 2 shows the significant changes seen in the adult wound healing when compared to those of the foetus (6).

Comparison of parameters in foetal and adult wound healing

In the transition of foetal research to regenerative healing, there key differences in the foetal and adult skin that have facilitated scarless healing in the foetus, are important particulates like growth factors, cytokines, and extracellular matrix substituents. In recent times, cosmetic products that contain compounds that mimic foetal dermis have shown promising results (19). Early gestational foetal skin wounds heal quickly without scar formation, in contrast to adults. The precise mechanism of foetal healing is still unclear, despite ongoing research. Table 3 highlights the factors determining the scar/scarless wound healing (64). The table below displays the changes observed in the adult and foetal skin for some important parameters governing scarless wound healing (65).

Advances in foetal monitoring, open foetal surgery, and the study of foetal scar in wound healing, particularly for life-threatening congenital diseases, have provided opportunities to examine foetal wound healing. Different factors responsible for adult and foetal wound healing are given in table 4. In the foetal wound, TGF–beta and hyaluronic acid wound matrix play a pivotal role in scarless repair. This work concludes that these factors are considered important to induce scarless wound healing (66).

Stem cells are responsible for complete regeneration in scarless skin wound healing during early gestation. Adult wounds undergo repair via fibroproliferative response, leading to regeneration of the original tissue in an incomplete fashion, resulting in fibroblast deposition, ultimately leading to scar formation (67). The scarless wound repair in the foetus depends on the size of the wound and gestational age. An important characteristic for scarless repair is that the tissue is less differentiated. An understanding of scarless tissue repair is needed for clinical application in the modulation of adult fibrosis and preventing abnormal scarring. Amy et al. reported on fetal wound healing, indicating that the developing foetus

Table 4: The difference between factors for adult and foetal wound healing

Foetal Factor	Adult Factor
Amniotic fluid, sterile environment	Dry, contaminated environment
Little inflammation	Massive inflammation
No inflammatory effector cell	Macrophage is an inflammatory effector cell
Scarless	Scar

heals wounds with proper ECM restoration in terms of architecture, function, and strength. Induction of foetal wound healing in adults might involve manipulation of certain genes to induce scarless repair (5). Inflammatory response, scar formation, and granulation proliferation that are observed in adults are not observed in the foetus (68). The inflammatory mast cells contribute towards scar formation and they convert scarless healing to fibrotic scarring. The embryonic 15 and embryonic 18 skins differed in degranulation and cytokine levels, and this might encourage the formation of scar with the mast cells contributing to fibrotic healing during fetal development (69).

Techniques to induce scarless wound healing in adults

Humans and other animals in their early gestational ages heal without scarring. Enhancement or blockage of relevant genes can result in healing without scar formation. The distinct differences in the pattern of growth factor profiles and inflammatory reactions are observed between foetal and adult wound healing. Strategies have been proposed to focus on modifying adult wounds to mimic the cytokine profiles present in foetal wounds, hence decreasing the inflammatory reaction to reduce the scarring of wounds in adults (70). The study demonstrated the role of mechanical forces in cutaneous wound healing, thereby advancing therapies to reduce scar formation. We can focus and maximise mechanical forces to reduce the production of scars thanks to improvements in our knowledge of those forces (71). When compared to adult wounds, foetal wounds have significantly lower resting stresses, which may be why they recover without scarring. Adult scar size can be decreased by treatments that control mechanical forces in the wound environment. Mechanotransduction pathways are prospective targets for reducing excessive scar development (72). Scarless human foetal wound repair is inhibited in the presence of transforming growth factor beta. The study focused on understanding the relation between TGF-beta1 and scar formation. They showed that within fourteen days of wound formation, there was scarring in TGF beta 1 grafts, where the size varied directly with the TGF beta 1 amount. One of the reasons the foetus heals by regeneration rather than scarring is the relative absence of TGF beta, a cytokine known to cause fibrosis. These results suggest that anti-TGF beta and TGF beta inhibitor-induced treatment techniques may reduce scar formation in both children and adults. The skin has the potential to prevent scar formation at birth, but this becomes elusive in adults due to the complex interactions that result in our inability to recapitulate scarless wound repair (12). Table 5 represents the various miRNAs with their correlation to scar growth and their respective targets.

The development of nanoparticle-based therapies cleared the door for the regeneration of both acute and chronic wounds. Silver and gold-based metallic nanoparticles have sped up the healing of chronic and infected wounds. Based on a scratch experiment for dermal epithelial cells, newly synthesised Erbium borate nanoparticles (ERB-Np) were shown to have considerable impact on wound clo-

Table 5: miRNA and their correlation to scar growth

miRNA	Correlation with scar growth	Targets involved
miR-149	Negative	IL-1alpha, IL-6
miR-145	Negative	Smad3
miR-155	Positive	IL-1beta, Col I
miR-98	Negative	Col 1A1

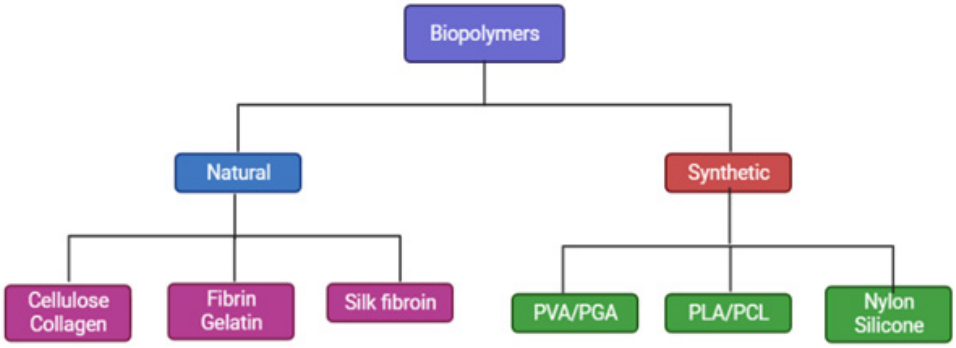

Figure 8: Classification flowchart for biopolymers

sure and is suitable agent for scarless wound healing (13). In chronic inflammation, where the wound healing response plays a crucial role, the research focused on substances that are connected to wound healing. Several treatment strategies that target various components of the wound healing process and fibrosis have recently been developed based on the cellular and molecular principles underlying tissue repair. Pharmaceuticals have the potential to address fibrosis and wound healing. In the future, the proliferation of numerous cell types involved in wound healing could be improved by molecular-level manipulation, such as DNA replication, enhancing tissue regeneration (73).

Biomaterial Scaffolds for Scarless Wound Healing

The scaffold, an important concept in tissue engineering, is a material that provides mechanical support for damaged tissues. The biomaterials used for skin regeneration must have good biocompatibility, biodegradability, and bioactivity to be fabricated into scaffolds (6-18,21). Figure 8 represents the different types of biopolymers used to make scarless wound healing scaffolds.

Several natural biopolymers like cellulose, collagen, fibrin, gelatin, silk, fibroin, etc., have been explored in the last 30 years. Similarly, synthetic polymers like PVA, PGA, PLA (74), PCL, nylon, and silicone have also been studied. These natural

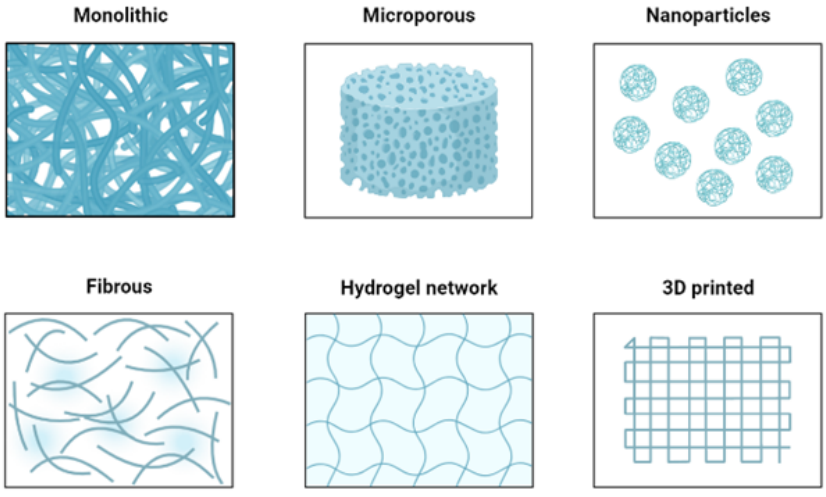

Figure 9: Different types of scaffolds are used in tissue regeneration

Table 6: Comparison chart for biomaterial polymers

Biomaterial	Source	Availability	Affordability	Merits	Demerits
Silk fibroin	Natural	Easily available	Relatively expensive	Thermally stable, Excellent mechanical strength, delayed degradation	Residual of sericin in the material can provide contamination
PLA	Synthetic	Widely used	Inexpensive	High mechanical strength and flexible features	Prolonged degradation, acid degradation, and biological inertness
PGA	Synthetic	Commercially available	Extremely expensive	Good biocompatibility and biodegradability	A high friction coefficient. Difficulty in processing and insufficient toughness.
PCL	Synthetic	Easy availability	Relatively inexpensive	Easy processability and nontoxic degradation product	low melting point and glass transition (~60°C and -60°C receptively)
Fibrin	Natural	Easily available	High cost	Reduce wound morbidity, prevent seroma formation at the subcutaneous level	Isolation is challenging
Gelatin	Natural	Commercially available source	Very low cost	Rich in Arg-Gly-Asp (RGD) sequences (Arg-Gly-Asp) that	Can cause allergic reactions and increase cell adhesion

and synthetic polymers greatly contribute to wound dressing (15,16). Perfect wound healing occurs when there is an absence of scar. Scaffolds are classified as porous, fibrous, particulate, and printed. Biomaterials in a sponge form possess randomly distributed pores on their surface that are needed for tissue ingrowth and vascularization. Fibrous scaffolds are made from biodegradable polymers, and nanofibers are the most advantageous biomaterials for wound care. Printed scaffolds have a high degree of precision in spatial parameters. Additionally, CAD provides suitable scaffolds since it has the advantage of being patient-specific. Scaffolds are proven to be the ideal solutions for the regeneration of the skin and for the misalignment of the skin architecture. Additionally, the biomimetic mechanical cues used in the scaffold fabrication procedure improve the results of wound healing and induce quicker regenerative processes. Figure 9 represents the different types of scaffolds used in tissue regeneration (16,21).

Among the various cell signaling molecules that act as scar inhibitors, GS-Rg3 is an ideal drug for the prevention of hypertrophic scar (HS) formation. Since GS-Rg3 could hinder the generation of the pro-inflammatory cytokines TNF-a, IL-6, and IL-1 and inhibit the expression of VEGF, it is regarded as an inhibitor of inflammation and angiogenesis. Bionic fibrous scaffolds coated with adhesive peptides can enhance fibroblast proliferation and maturation. Furthermore, it leads to formation of a basement that regulates space and fills the gap leading to early wound closure. In addition, the sustained release of GS-Rg3 stimulates *in vivo* wound healing. This is facilitated by improved cell-cell communication, skin regeneration, and subsequent reduction of angiogenesis and collagen deposition (17). Table 6 compares the different biomaterials based on their availability and cost.

ECM when used to construct multifunctional scaffolds, are ideal as they pocess the basic signals that promote cell adhesion, connects the cells in a tissue and leading to deposition of of fibrous proteins such as collagen, which helps in wound healing. Wang et al prepared poly(e-caprolactone)/gelatin nanofibrous structures doped with TGF-b1 inhibitors, reducing fibroblast proliferation to prevent HS (16,17).

Now, the polymer material (a basic component of bionic ECM) that was intro-

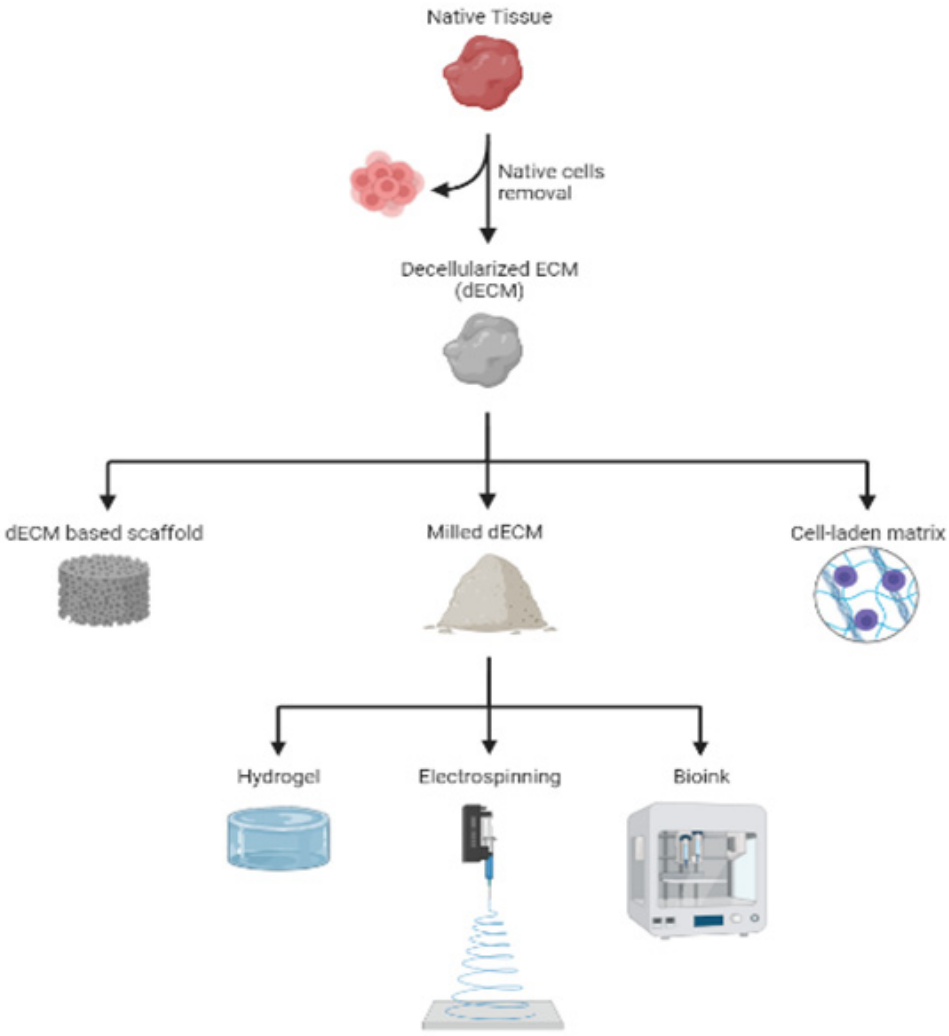

Figure 10: A graphical illustration of extracellular matrix -based biomaterials, techniques and methods

duced should have good biocompatibility and versatility to avoid immune risk to the patients. According to reports, figure 10 depicts PGA, which is linked to other molecules by peptide bonds and contains a great deal of carboxyl groups that make it easier to create scaffolds that are like the ECM. The excellent biocompatibility, biodegradability, and commercial accessibility of PGA are well recognized. It is also a potent hydrophilic polypeptide that creates a desired microenvironment that mimics the biological activities of glycosaminoglycans with a high-water content. Since glutamic acid, containing an N-terminal amine group, is recognized by the glutamyl transpeptidase found in cell membranes, PGA can facilitate GS-Rg3 medication release. As a result, PGA is considered an excellent polymer for bionic ECM with features of electrospun hydrogel fibers with nanostructure and the softness of the hydrogel. These properties, in turn, allow the cells to migrate onto the scaffolds to build 3D microvascular structures. Not only do they promote the early stages of wound healing, but they also prevent scarring at a later stage by on-demand releasing the GS-Rg3 (17). For tissue development, the growth and survival of the cells are vital. Scaffold material should be

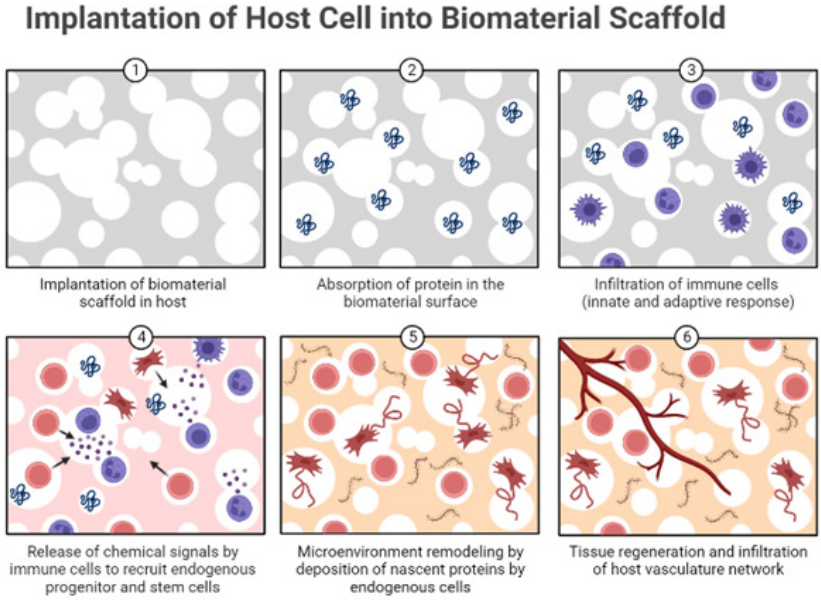

Figure 11: A pictorial representation of various procedures used for recruiting host cells into the scaffold

selected taking into consideration the chemical composition and assembly conditions, that address cellular viability and mechanical stability. Another material is RGDC, an RGD (arginine-glycine-aspartate) derivative used for surface fiber modification by click chemistry, which ensures long-term bioactivity by avoiding unstable adsorption through hydrogen bonds (75).

The PGA scaffold surface when chemically modified using RGDC improves the hydrophilicity of the fiber surface for better cell adhesion and proliferation in the scaffolds, as illustrated in figure 11. Another finding showed that the electrospun PGA fibrous hydrogel scaffolds can preserve the shape and function of biomolecules with a favourable chemical modification that creates a surface that is conducive to cell adhesion. Macroscopically, a preliminary assessment of wound healing and scar formation was conducted. To make observation easier, it was done on the dorsal skin of the rat. Within 16 days, the wound base had healed, and the wound edge disappeared. Hence, cutaneous wound healing can be improved by the functional g-PGA scaffolds that promote cell growth and proliferation, within 16 days. The healing rates for different scaffold materials are given in table 7 (17).

This indicates that the functional RGDC scaffold provides better repairability than the unmodified scaffold. It has been proposed that GS-Rg3, released from fibrous structures, inhibits the formation of HS. These results imply that photoresist-linked RGDC-peptide -PGA fibrous scaffolds loaded with the drug GS-Rg3 not only enhanced wound healing in the early stages but also inhibited the formation

Table 7: Corresponding biomaterials for wound healing applications and their healing efficiency

PNS	90%
PNS-G	86%
PNS-RGDC	95%
PNS-GRGDC	97%

of scars at a later stage. As per the histological studies, complete healing along with re-epithelialization of the damaged tissue is promoted by fibrous scaffolds. In addition, in the biofunctionalized fibrous scaffold a sustained release of GS-Rg3 without the burst effect promoted HS inhibition. With prospective possibilities for regenerative medicine and drug delivery, this technique is an effective method to speed wound healing and avoid the development of HS (17).

Scar development following burn injuries is a concern for the healthcare industry. The fibrous PGA scaffolds, which exhibit bionic ECM features in function and structure, can stimulate cell adhesion and proliferation (19). Evaluation of the histology and immunohistochemical reaction of the hybrid collagen scaffold with ZnO-curcumin nanocomposite reveals that the hybrid scaffold hastens the healing of scarless burns in albino rats by stimulating angiogenesis and triggering the expression of TGF-b3 (21).

The complex multistep wound healing processes including the formation of granulation tissue, inflammation, the production of new extracellular matrix, re-epithelialization, and tissue remodelling, makes it very difficult (19,20). Burn injuries are substantially worse than other injuries since they impose significant damage and can even be deadly in rare situations. There will be significant damage in full-thickness skin wounds and severe burns (21,23). The healing process of burn injury is more complicated as it is accompanied by metabolic, neurologic, and orthopaedic problems, leading to excessive scar formation and poor healing. The increased healing time also leads to scar formation. The most abundant structural protein in the ECM is collagen. Collagen-based scaffolds are the right option for tissue regeneration as they are similar to natural ECM (16,19,21). Fibrin is a component of a platelet plug and acts as a wound-healing adjunct. It is the most widely used agent and is an effective scaffold facilitating ingrowth of regenerative cells. Hyaluronic acid is another molecule gaining prominence as a wound-healing drug. Chitosan is another possible wound-healing candidate, a polysaccharide used to create porous scaffolds and 3D bioprinted scaffold. These polymers do not offer any side effects or allergies (19,20).

Hybrid scaffolds has gained prominence as a suitable wound healing biomaterial as it could be adapted to overcome the major problems in wound care. ZnO-Cur nanocomposite is an example. Zinc is said to be an essential cofactor in the activation of several matrix metalloproteinases (MMPs) involved in the wound healing process, making use in the production of hybrid collagen biomaterial favourable. The prepared hybrid scaffolds were characterized for their physical, chemical, and biocompatibility properties. Around 73% of amino acids in the hybrid scaffold were cross-linked in comparison to the native one. The maintenance

Table 8: *In vivo* studies with hybrid collagen curcumin ZnO nanocomposite scaffold response at different time intervals

Days	Hybrid Scaffold Treated Animals
On Day 7	Animals show less inflammation than others, along with new blood vessels and fibroblasts.
On 14th day	Formation of keratinized epidermis, unlike other groups. Adequate control of the inflammation phase and the basic skin structure has been seen on the 14th day of healing
After 30 days of maturation	Excision of the whole regenerated skin

of a moist environment in the wound bed and increased exudate adsorption depend upon the scaffold's swelling ability. It is interesting that adding ZnO-Cur nanocomposite did not alter the enzymatic and swelling stability of the scaffold. Neutralisation of bacterial toxin and ROS by antioxidant activity promoted faster re-epithelialization. It also demonstrated that the proliferation and viability of human keratinocyte cells were unaffected by the high ZnO-Cur nanocomposite (5 wt%). Another study proved that one of the most important factors necessary for rapid wound healing without scarring is the reduced inflammatory response. The investigation, was conducted using CD68 immunohistochemical staining (21). Various staining methods, notably H and E and Masson's trichrome, were also used to analyze the skin development and architectural improvement at various stages of healing (22).

These studies demonstrated perfect skin architecture for hybrid scaffold-treated animals in the case of burn wound healing, while controls and other combinations of scaffold failed to promote complete closure of the burn wound. Also, these groups had well-aligned collagen fibers, whereas the other combinations showed thick collagen deposition in a disordered fashion, leading to scar formation. *In vivo* burn healing experiments demonstrated that the hybrid biomaterial accelerated wound healing with minimal to no scarring, as shown in table 8. The study of ZnO-Cur nanocomposite in a hybrid scaffold also demonstrated regulation of angiogenesis and TGF-b3 expression, reducing scar formation. The thickness and SEI value determine scarless wound healing. SEI (the scar elevation index) value is normally 1, and the rise in value directly reflects the scarring in the regenerated skin. Based on *in vivo* and *in vitro* testing, the hybrid collagen scaffold is promising for scarless healing. Antioxidant NAC can reduce the ROS which influences wound healing. As already reviewed, collagen would be an ideal biomaterial due to its remarkable biocompatibility and biodegradability (21). But collagen-containing scaffolds degrade within 2-3 days and have lower mechanical performance. Graphene oxide (GO), a derivative of graphene, showed good potential for tissue injury healing. Fernández et al investigated a GO-based material combining PVA with other bioactive components, which showed great potential for the regeneration of skin. Kawamoto et al reported that collagen sponge coated with GO accelerates periodontal wound healing of class II furcation lesions in dogs. Here, synthesis of N-acetylcysteine-loaded graphene oxide-collagen hybrid membrane (N-Col-GO) was done using a 20 mm diameter EDC mold, and the charecterised. *In vivo* experiments on the rats showed that both N-Col-GO and Col-GO membranes had high water retention with high porosity and elasticity. Additionally, it displayed good biocompatibility. These studies showed that GO was non-toxic, despite deregulated cell adhesion and migration. These results show that the NAC-loaded GO-Col hybrid membrane is a possible candidate for wound healing applications (22).

Table 9: Various biomaterials and their contribution towards wound healing

PEGDA	Cutaneous tissue regeneration is facilitated
PHEMA-PMPC-PHEMA	Used as a wound dressing (65)
Gelatin	The wound gets closed quickly and completely
Collagen	Improved MSC regeneration capacity and wound healing
Hyaluronic acid	Enhanced vascularization or wound healing
PEG-PCL-PEG	Improved incision wound healing

Scaffolds as skin substitutes are divided into two categories: synthetic scaffolds and biological scaffolds. Cells can be incorporated into some scaffolds, as in 3D bioprinting. Hydrogels are functionally and structurally like natural ECM and three dimensional hydrophilic polymeric networks. Hydrogels are preferred in many case as suitable scaffold because of its biocompatibility and versatile approaches (17,18). Some of the hydrogel scaffolds that have been developed and have shown promising results are shown in table 9.

It is reported that host tissue integration can be promoted by vascularized scaffolds. When the hydrogel scaffolds of appropriate mechanical strength are applied to wounds, the hydrogel prevents wound contraction, thus reducing distortion and scar formation. Chen et al further demonstrated that hydrogel stiffness regulation can affect the secretion of TGF-b1 and bFGF, which additionally affects skin wound healing. Hanjaya-Putra et al. showed that reducing mechanical force by modulating the degeneration process can facilitate vascularization and promote wound healing. The term regenerative immunology was coined by Elisseeff et.al for the role of immunity in regenerative medicine. This review has developed Dex-IEME, which is a dextran-based hydrogel that is known for its biocompatibility, low pro-inflammatory response, and is also bioabsorbable. This hydrogel regenerated complete skin, and a perfect skin was obtained after healing, which creates a positive impact on Dex-IEME (18).

The relationship between macrophage phenotype and scaffold materials can be used to predict the outcomes of wound healing. Those biomaterials with high biocompatibility, which can direct macrophages to the M2 phenotype, are highly desirable for the regeneration of the skin. Hydrogels have been extensively studied in skin wound healing (16,75). The pig skin is more like human skin, and thus it is used as a preclinical model for wound healing. these studies showed that hydrogel-treated wound in pig contained more adipose tissue, and a full skin structure was formed. In contrast, non-hydrogel-treated wounds developed fibrotic collagen and thus showed more fibrosis. Newly regrown skin is fully regenerated with hydrogel-treated wounds; strongly explaining the potential of hydrogel in wound care (75). Over the last decade, elastin-based polymers have become popular as biomaterials for tissue engineering applications. This is because they are easily processed, designed, produced, and modified, and have strong cyto- and biocompatibility. ELPs and synthetic elastins are two significant groups of biomaterials. Tropoelastin and collagen mixtures have reportedly been used as fast wound closure scaffolds to enhance skin regeneration (16,17). ELP refers to polymers that are obtained by synthetic strategies. ELR-based hydrogels have proven to be an excellent platform for the growth and proliferation of fibroblasts. The ELR-based hydrogels with or without bioactive sequences behaved very differently under *in vivo* conditions. A successful wound healing process depends on the immune response of a scaffold and the generation of new blood cells to support cell proliferation within the scaffold. The method for biofunctionalization of glass substrates by chemically attaching ELR to the surface of the glass substrate. This suggests a possible use of ELR for ocular epithelial regeneration after trauma (76). The nanocomposite scaffold comprises PEGDA. This forms an auxiliary dynamic network with the scaffolds, formed between BGNC and ALG (PABC scaffolds). Experimental results confirmed that the PABC scaffold exhibits good self-healing properties. PABC scaffold can accelerate collagen deposition and exhibit excellent self-healing, viscoelastic mechanics, and injectability, as well as broad-spectrum antibacterial properties. PABC scaffold has good cyto-compatibility, significantly enhancing the angiogenic ability of EPCs *in vitro*. In addition, the PABC scaffold increases the formation of dermal appendage-like tissue, suggesting that

Table 10: The progress of the wound healing in different time points (20)

Day 1	Delayed wound contraction than spidroin treated wounds.
Day 7	No wound size difference is observed
Day 10	Complete wound closure
Day 21	Scar formation reduction after the treatment of MPs

our scaffold is likely to promote dermal tissue formation and reduce scar tissue (14).

By mimicking the extracellular matrix chemical composition and biophysical nanostructures, the bioactive scaffold was developed. The introduction of HA and natural SNFs, silk fibroin-based scaffold, was prepared by lyophilization. This led to a better cytocompatibility of the scaffolds. *In vivo*, SNF-containing scaffolds have been shown not only to accelerate wound healing (up to 98.2 ± 0.5% in weeks) but also to regulate collagen arrangement with nanofibers to prevent scar formation (77). Thus, it offers a useful strategy, to explore bioactive SF-based natural SNF-decorated scaffolds for novel wound dressings and artificial skin (14). A great solution for donor skin shortage is using biomaterial scaffolds to fabricate skin constructs. The technology of additive manufacturing can deposit cells, biomaterials, and factors in 3D constructs. Bioprinting is again classified as *in vitro* and *in vivo*. A positive impact is created by the method of bioprinting at different stages of wound healing. Bioprinting is preferable as it has better properties of skin constructs, accurate positioning of the bioactive molecules, and improves the speedy construction of skin, thus reducing the recovery time. Skin constructs can be made by extrusion bioprinters and LaBP using cells, collagen, or hydrogels. Dermal bioprinting may be an appropriate solution for effective wound healing, especially in extensive burns and full-thickness skin wounds. Patients would certainly be benefitted from printed skin equivalents that offer shorter healing times and less pain or even improved cosmetic results. Fibroin/gelatin or spidroin (artificial silk spider protein) has shown significant wound healing and scar inhibition (23). The result of the administration of fibroin/gelatin microplastics (MPs), given in table 10.

Furthermore, it was revealed that the re-epithelialization rate improved significantly after both types of MP treatment. Fibrin/gelatin and spidroin MP can promote skin regeneration and avoid fibrosis. It is important to note that in MEFs, neither soluble fibroin nor spidroin induced the expression of pro-inflammatory cytokines. Both fibroin and spidroin, however, had antifibrotic abilities since both proteins caused a decrease in Ctgf and Fgf2 expression. Due to this, only fibroin/gelatin MPs caused temporary inflammatory reactions in fibroblasts and macrophages, although the fact that both MP types had anti-fibrotic capacity *in vitro*. According to studies, fibroin/gelatin and spidroin-MP's ability to promote regeneration may be a result of their ability to suppress dermal fibrosis. Because they act as a scaffold for the formation of cells and tissues, bioengineered MPs are recognized to have a lot of promise. To improve the biocompatibility of fibroin scaffolds, gelatin has been altered. Although microparticles require different mechanisms, fibroin/gelatin and spidroin promote reepithelialization and inhibit scar formation. This has a significant impact on wound healing. Fibroin/gelatin is associated with an inflammatory response, but spidroin is not. These kinds of biomaterials not only downregulate the expression of profibrotic factors, but also prevent dermal fibrosis during wound healing (20).

Table 11: Comparison of the scaffold preparation methods

Methods	Advantages	Disadvantages
Solvent casting	Simple, easy & cost-effective	Protein denaturation and limited mechanical properties
Particulate leaching	less amount of polymer is required	The shape of the pore and the inter-pore openings can't be controlled
Gas foaming method	Harsh, organic solvent-free	Damage to cells and tissues
Phase separation	Easy combination through fabrication	Crucial for the formation of nanofillers
Electrospinning	Excellent antibacterial ability, supporting the adhesion and proliferation of human skin	Cell seeding is an issue. It is a complicated and elaborate process
Rapid prototyping	Easily integrate with the imaging technique	Low resolution, highly expensive equipment
Fiber mesh technique	Mechanical stability allows tissue ingrowth, rapid nutrient diffusion	Structural stability is lacking
Freeze drying	High porosity & interconnectivity	Long processing time (24)

The most recent review states that the polysaccharide and protein components are a must in a scaffold. This reduces the deposition of collagen and acceleration wound healing. The scaffolds are in various forms: Polysaccharide scaffolds, protein scaffolds, oxygen-generating scaffolds, and scaffolds with stem cells. Due to the excellent aqueous solubility and biocompatibility, Bleomycin-loaded dissolving microneedles made up of HA reduced the formation of human dermal hypertrophy. Collagen, i.e., the protein scaffolds, promotes epithelialization and reduces scarring of the wound (14,16,19,21,75). The same result is obtained in oxygen-generating scaffolds, too. Stem cell-seeded scaffolds including hybrid scaffolds, already reviewed above also promote wound healing mainly without scar. Hydrogel scaffolds also play a major role in tissue regeneration, and it can be concluded that the polysaccharide protein scaffolds mimics the skin tissue in terms of function, structure, shape, and mechanical strength, making it an to ideal solution for scarless wound healing (78).

Methods of Preparation of Biomaterials Scaffolds for Scarless Wound Healing

Scaffolds are materials that are engineered to contribute to desirable cellular interaction for the formation of new functional tissues. Polysaccharides and proteins play a major role in the preparation of polymer scaffolds for repair, which act as a template for cell adhesion and migration, forming an equivalent of the extracellular matrix. Different methods of scaffold preparation are shown in table 11.

Chitosan (CS), which is antibacterial, analgesic, and haemostatic, is used as a dressing for healing. Chitosan alginate membrane accelerates wound healing. CS sodium alginate polyethylene glycol films were used for early inflammatory response modulation in the Wister rat model and stimulated collagen synthesis in an ordered fashion which resulting in without wound adhesion formation (79). Hyaluronic acid (HA) acts as a great aid to wound healing. In one study dissolving microneedles, made of HA, loaded with Bleomycin, reduced the formation of human dermal hypertrophic scar. Collagen has poor mechanical strength; hence, it is often combined with other biomaterial scaffolds. Previous studies have proven that this strategy promotes wound healing and also inhibits scar formation as it prevents cell membrane contraction in the wound, promoting the recovery of damaged cells and peripheral nerves (78). Oxygenation of cells plays a great role in enhancing wound healing with the maturation of connective tissues. Oxygen-

ated and biocompatible scaffolds inhibit scarring and promote healing as they continuously generate oxygen. SF, due to its versatility, biocompatibility, adaptable biodegradability, hierarchical structure, and mechanical strength, is suggested as a suitable scaffold as it can be modified to be an effective therapeutic agent, which inhibits scarring (80). Inorganic compounds like peroxides, sodium carbonate (Na_2CO_3), calcium carbonate ($CaCO_3$), perfluorinated compounds, and hydrogen peroxide, can be added to scaffolds to promote oxygenation. They act as oxygen carrier, which helps the wound to heal without scarring, as they provide more than twice the amount of oxygen than natural oxygen-generating scaffolds. Scaffolds with MSCs due to their immunomodulatory and pluripotent nature were also explored as candidates for wound healing. The most commonly used MSCs were bone marrow stromal cells, adipose stem cells, and placenta-derived MSCs. Exosomes, which are produced from MSCs, have shown a positive effect on wound healing without scar (78).

There are different methods involved in the preparation of scaffolds, based on tissue engineering principles. Solvent casting is one of the simplest, easiest, and cost-effective methods that is based on the formation of evaporating solvent to develop a scaffold. It can be prepared in two ways: the first one is to dip the mold in a polymeric solution and then allow it to dry, resulting in a scaffold. Another is the addition of a polymeric solution into the mold, evaporating the solvent, creating a polymeric membrane which is then adhered to the mold. The drawback of this method is the denaturation of protein by the toxic solvent, and this can be overcome by vacuum drying to remove the toxic solvent. In the particulate leaching salt, sugar, and wax are used as porogens. The polymer solution is cast into the mold filled with salt, and when it is dried, leaching of salt crystals occurs, and this leads to the formation of the scaffold. The pore size depends upon the shape, size, and amount of the porogen, but the inter-pore openings cannot be controlled here. The gas foaming process uses high-pressure CO_2 to develop a highly porous scaffold. The amount of gas in the polymer defines the porosity. In this process, the polymer is subjected to 800 psi gas for polymer saturation, destabilizing the CO_2 and causing segregation within the polymer. Pores are caused by volume expansion of the polymer and a decrease in polymer density. The most prominent and widely used method to produce biomaterial scaffolds is electrospinning. This method utilizes the electrostatic force to produce polymeric fibers that range from nanometres to micrometres. It is controlled by a high-intensity electric field between two electrodes, where one is placed in a polymer solution and the other in the collector. A jet of polymer is ejected, which produces fibers by the application of a high electric voltage to the polymer solution, and those nanofibers are deposited on the collector. This method produces scaffolds with structural features suitable for cell growth and tissue organisation and provides an environment for tissue regeneration with physiological functions in the spatial domain. Scaffolds produced by this technique has proven to provide excellent antibacterial ability, supporting adhesion and proliferation of human skin cells (81).

In the fiber-mesh technique, the individual fibers are either woven or interwoven into a 3D pattern of various pore sizes, which is then prepared by polymer deposition on a solvent nonwoven mesh of another polymer through evaporation at subsequent levels. In the fiber bonding technique, the dissolution of polymer in chloroform occurs with the addition of PGA non-woven mesh. The scaffold is formed by bonding PGA polymers to a collagen matrix using threaded collagen stitches. These yield a scaffold of PGA fiber which is then bonded by heat treatment. This PGA mesh provides high porosity and surface area in the scaffold, which provides mechanical stability and allows tissue ingrowth. Melt moulding

Table 12: Accuracy for the different methods of machine learning

Dielectric Data	Permittivity	Permeability	Loss tangent
Accuracy	80.3%	83.6%	90.1%

technique involves Teflon mold filling with gelatine microspheres of specified diameter and PLGA powder, followed by heating, causing the PLGA to adhere together. Freeze drying is another common technique used in scaffold preparation, based on sublimation. Here, high interconnectivity and porosity are achieved by removing solvent from frozen polymer under high vacuum. The pH and freezing rate, control the pore size. Unidirectional controlled solidification leads to a 3D homogeneous pore structure. In this technique, generally small pore size is achieved, but the longer time for processing is the main drawback here (82).

Machine Learning for Minitoring Wound Healing

Many machine learning algorithms have been considered as an alternative to the standard invasive analysis of wound healing. Accuracy of different machine learning methods is shown in tables 12 and 13. The unsupervised technique was used to cluster HSI images of the 3D wound model and monitor the process of wound healing. XHC transforms the hyperspectral cube into matrices with wavelength and hyperspectral features as dimensions. Images from day 0/5 and 10 were used as training samples. The XHC method was compared with the K-means method. XHC converged quicker than K-means (10 minutes for K=7, 3.5 minutes for XHC), and it better covered the features important for wound healing. Haematoxylin and eosin staining were used to analyse the migration and deposition of the fibroblasts in the targeted area. These features, picked up by HIS, resulted in a specific cluster distribution. XHC proved to be a fast and memory-efficient clustering technique to monitor wound healing using spectral images (83). In the dielectric sensor-based machine learning point-of-care tool, they analysed the wounds from healthy skin based on the dielectric constants. The dielectric constants for skin and different wounds obtained from mice were considered as training data. The dielectric constant value was picked up from VNA(vector network analyser). The measured values were compared with those of normal skin, UV burns, puncture wounds, and scratches accounting for mechanical noises that could interfere with the data (27).

The real and imaginary parts of the permittivity was measured to get the loss tangent (ratio of real part to imaginary part). The best results were observed at the range of 1-2 GHz, reducing the requirement for larger bandwidth. Different supervised machine learning models were used to improve upon the results. The models chosen for the task were GMM, SVM, Naïve Bayes, and neural networks. All these models were trained on the dielectric constant values with the motive to differentiate skin from wound (27).

An automated wound healing monitoring system based on wound images taken from a smartphone camera is explained. Around 119 images were captured from an iPhone at a patient's home who was suffering from a chronic wound. Image processing was applied to the images. The image transformation technique

Table 13: Accuracy for different machine learning algorithms

Model	Naïve bayes	GMM	SVM	Neural network
Accuracy	98.4%	98.4%	97.6%	100.00%

was applied to transform the images into the hue-saturation value colour space. This transformation technique was found to be reliable due to its contrasting nature with that of the light background in the original image. Thus, using smartphone photography and relevant image processing, a cost-efficient method for wound healing monitoring was developed. The application of machine learning is not possible here due to the unavailability of large data for training (84).

In the prognostic model for estimating wound healing using GAN, the model was trained on EMR. Time-series networks were developed as these networks require minimal information for training. Temporal information obtained from weekly check-ups of the patients were also added. The model was evaluated by the TSTR method, which created synthetic data that resembles the real data, upon which the model was tested (85). Researchers from another group adopted the same evaluation technique in their GAN model and have proven this to be an efficient evaluation metric. The GAN model achieved an AUC of 0.975, 0.968, and 0.849 for the data from the first, second, and third visits, respectively (26).

Machine learning (ML) for wound classification

Machine learning algorithms can be deployed to classify the wounds based on healing time, thereby aiding in diagnosis and treatment. The ML model was developed to identify the slowly healing wounds. They collected demographic information from patients and collected quantitative information from the wound from 2008 to 2013. The wounds were classified as delayed if the healing time went beyond 15 weeks after showing up in a hospital or relevant centre. Around 40 different types of wounds present in 37 different locations were recorded in the dataset. Random forest, L1 regularised logistic regression, and gradient boosted tree were the various machine learning models selected for this task. Out of these, the gradient boosted tree proved to be the best, and nearly 865 features were selected from the curated information. Out of them, around 381 features did not influence the output. However, 100 features were found to contribute around 95% of the total influence. This means that these features have a high correlation with the output. Some of the characteristic features with high influence are palliative care, depth, wound area, patient age, and measurements taken in the second assessment relative to those of the first assessment. The developed predictive model by Jung MS et.al, showed an area under the regional operating characteristics curve (AUC-ROC) of 0.842 with confidence of 95% and with a Brier reliability score of 0.00018. Thus, the developed model was capable of early identification of chronic wounds, which assists the clinician in better treatment (86). Sang Kyu Cho, et.al developed a machine learning model for chronic wound prediction. This was based on 12 weeks of treatment initiation. They collected 620,356 unique wounds from 532 clinics around 46 states. They have mentioned around 4 different wound classifications based on the evaluation of the clinician. These are zero measurements with complete epithelium covering, flap procedure, graft procedure, and wound margins with zero measurements, approximated by sutures. They categorized the features into three, namely demographic, patient level, and wound level information. The wound area, wound depth, partial thickness, full thickness etc., were manually calculated from the wound. A logistic regression model was used to predict the chronic wound based on the three groups of features. Since there are a lot of features, they added the features in a step fashion and evaluated their contribution to find out the optimum parameters needed for prediction. The AUC and AIC were used for evaluation. Three models were developed based on a stepwise strategy. AUC and AIC of different types of information

Table 14: AUC and AIC for different types of information

Type of information	AUC	AIC
Demographic only	0.556	2,49,549.7
Demographic + patient data	0.605	2,45,548.4
Demographic + patient data + wound data	0.712	2,25,519.3

are shown in table 14. First, with demographic alone, second, with patient alone, and third, the combination of both demographic and patient information. Apart from this, they also developed a classification tree model trained on all three features with a split ratio of 70-30, and the relative variable importance metric was obtained. This metric is obtained by dividing the importance of one variable by that of the highest value of importance.

The location of the wound had the highest relative importance of 1, and wound classification had a relative importance of 0.73. Basic level information was only considered for prediction; information regarding clinical visits and healing progress was not considered for analysis, making this method even more efficient and reliable. Burn classification is another domain where machine learning models are being deployed (25). Similarly, Rowland et.al developed a machine learning model for burn classification based on spatial frequency domain imaging. The entire process was done on porcine models. They artificially induced those wounds using a custom burn tool, and those wounds were allowed to heal for a span of 28 days without adopting any other treatment methods. Around 16 burns were given to the pigs with different types, like full-deep, partial-deep, and superficial. The evaluation of the clinician was considered the ground truth. The SDFI projects sinusoidal waves at different wavelengths to image depth-related optical parameters. Optical phantoms (simulating the optical characteristics of target tissues) at different wavelengths were used for calibration. These were converted to colour images. A cubic SVM was used for the classification. A 5×5 pixel-based ROI was fed to the model. Iterations were considered based on the wavelength of light and spatial frequency. The data from one pig was used for training, and the data from the other pig was used for testing. The first iteration included a subset of the SFDI dataset with 1 spatial frequency and 8 wavelengths. The second iteration includes the entire dataset with 5 spatial frequencies and 8 wavelengths. The third iteration had the same data, but the images were normalised concerning normal skin. The model had to predict the region of unburnt skin, hyper-perfused periphery, no skin graft needed, and skin graft needed. K-means validation (K=10) was used to obtain accuracy, which was around 88.8%. After 28 days, 7 wounds were completely healed. The unhealed wounds were either fully or partially deep or required a skin graft. Table 15 illustrates the metrics for different iterations.

Table 15: Accuracy, precision, and sensitivity for different imaging data

Case	Accuracy	Precision	Sensitivity
Wide field multispectral imaging (best case)	100%	97.6%	100%
Wide field multispectral imaging (worst case)	80%	76.2%	80%
Combined spatial frequency data (best case)	100%	95.2%	100%
Combined spatial frequency data (worst case)	82.5%	89.2%	82.5%
Relative dataset (normal skin)	100%	95.2%	100%
Relative dataset (hyperperfused)	100%	97.6%	100%
Relative dataset (no graft needed)	100%	94.4%	85%

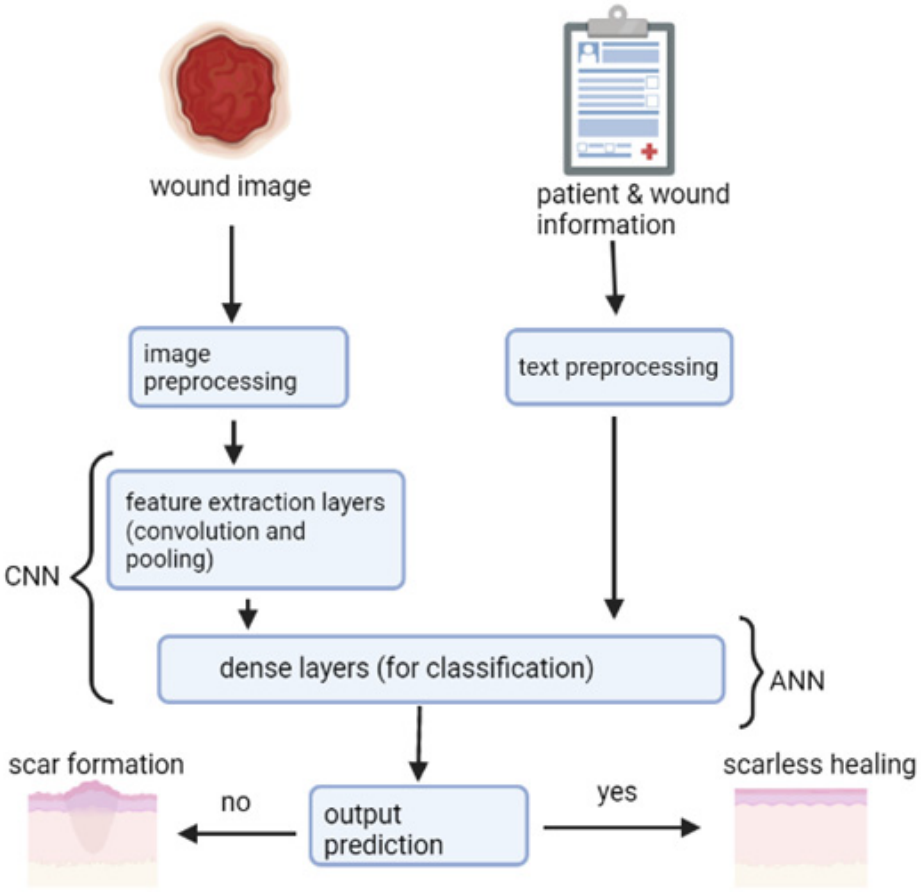

Figure 12. An illustration of the suggested machine learning algorithm

The addition of more illuminance in the spatial frequencies resulted in a better-performing model. This method also predicts the need for skin grafting apart from classifying the wounds. These features come in handy for a clinician, and the model aids them in faster and accurate evaluation of the wounds. Neural networks play a prominent role in the domain of non-invasive wound healing analysis. CNNs were used for wound segmentation (87). Huimin et.al developed a CNN for segmentation of wound and intensity correction with an energy force function capable of detecting edges efficiently, making their algorithm outperform other segmentation algorithms (88). Wang et.al developed a deep convolutional neural network for wound analysis and segmentation. The model could do both healing progress prediction and detection of infection simultaneously. They developed a cost-effective and quick model that took less than 5 seconds to compute the results (33).

Machine learning based opportunities for the development of an appropriate scaffold for scarless wound healing

There is no work done for scarless wound healing to our knowledge. Inspired by the work done in the domain of wound healing, we propose a few machine learning ideas that can be implemented in the domain of scarless wound healing. The aim is to predict/monitor the process of scarless wound healing and to aid the clinician in better diagnosis and treatment (figure 12). The first idea is predicting

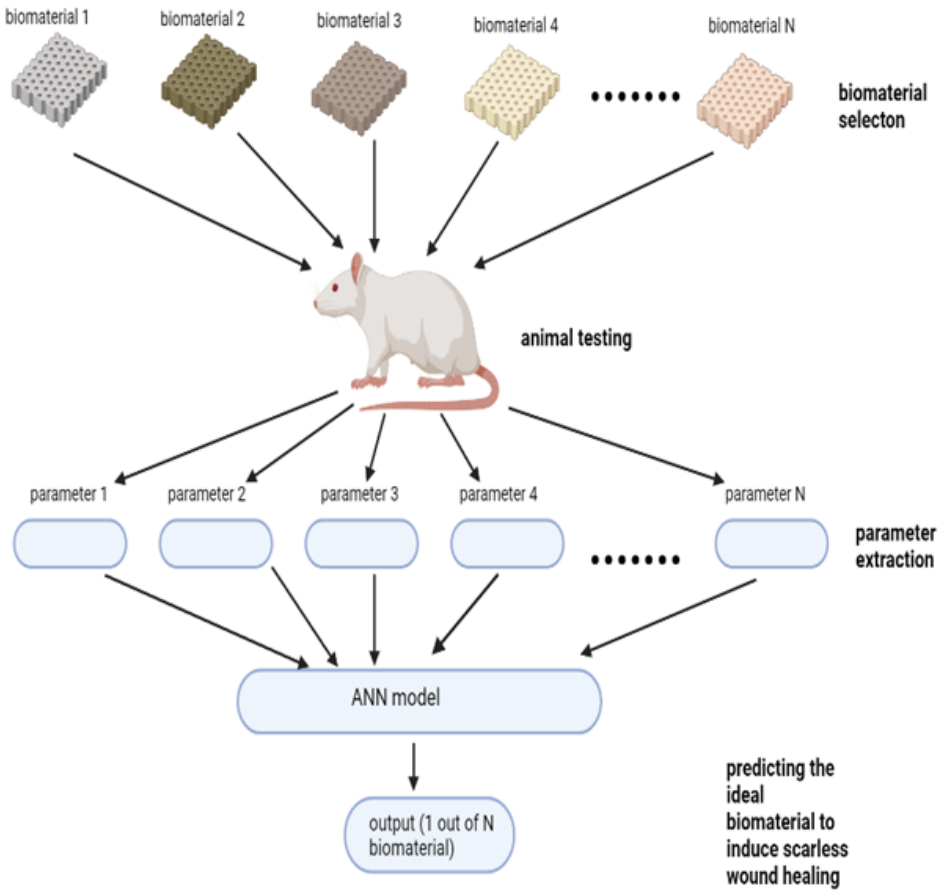

Figure 13. The suggested concept of choosing suitable biomaterial scaffolds for scarless wound healing is represented diagrammatically

whether the given wound will heal without scar formation. This can be done in two ways. The first way is using a deep CNN trained with the images of the wound for predictions. The second way is using an artificial neural network, or any other relevant machine learning model (preferably random forest or naïve bayes) trained with demographic, patient-level, and wound-level data. The combination of both image and text data can also be considered. In this case, we can develop a split-type neural network where one branch of the network processes the image data, and the other branch processes the text data. Both branches converge to produce a single output. This method can be effective due to the combination of data.

The second idea is the monitoring of scarless wound healing using relevant images. This idea is inspired by the different methodologies adopted for monitoring wound healing. Using machine learning or relevant neural networks to develop prognostic models that monitor the healing process. This can be fruitful for the clinician, aiding disease diagnosis and proper treatment. This can be combined with suitable sensors that pick up information from the target site, useful for prediction. An alert system can be developed based on the sensor values and output of the machine learning model, which can be useful for severe and chronic cases.

The third idea is the development of a machine learning model that will predict the ideal biomaterial scaffold that can be incorporated into the human body to achieve scarless wound healing. The data for which the model must train can be

obtained by experimenting with the various existing biomaterial scaffolds on animal models and obtaining certain parameters relevant to scarless wound healing (figure 13). When the values are filled for the same parameters, the model can predict the ideal biomaterial that can best induce scarless wound healing in the patient.

Future Perspectives

Cell-laden scaffolds are being developed to improve cell viability from the development of pre-determined 3D architectures to scaffolds. Incorporation of these cells can result in a desirable geometrical arrangement, along with a huge surface area. Recently, the blending of natural and composite scaffolds has been done for the enhancement of scaffold properties. Natural scaffolds possess the properties of compatibility, and the composite scaffold provides the properties of mechanical strength. Inkjet printing-based fabrication has resulted in the production of scaffolds in a very short time with high accuracy (89). Better visualization and design of the biomaterial scaffold can be achieved by using leading technologies like augmented reality (AR) and virtual reality (VR). Researchers can use these technologies to better design the scaffold based on the user's dimensions and needs.

- To increase cell viability, cell-laden scaffolds are being created with pre-determined 3D designs. The incorporation of these cells can provide a massive surface area and a desired geometrical pattern.
- Recently, composite and natural scaffolds have been blended to improve the qualities of scaffolds. Both the natural and composite scaffolds offer compatibility and mechanical strength features, respectively (90,91).
- Inkjet printing-based fabrication has made it possible to produce scaffolds quickly and accurately [88]. Using cutting-edge technologies like augmented reality (AR) and virtual reality (VR), the biomaterial scaffold may be better visualized and designed.

Conclusion

The likelihood of full tissue regeneration without the burden of long-lasting scarring makes scarless wound healing an attractive frontier in the field of tissue repair. The temporary scaffolds that biomaterials provide act as vital facilitators of this process by promoting cellular responses and encouraging tissue regeneration. The application of machine learning techniques in selecting suitable biomaterials enhances the accuracy and individualization of therapies for scarless wound healing. Machine learning enables the identification of the most suitable biomaterials for each patient's specific needs, leveraging the potential of data analysis to support faster wound healing and improved patient outcomes. Realizing the full potential of scarless wound healing as a game-changing medical strategy will depend on ongoing developments in machine learning applications and biomaterial research.

- Scarless wound healing is an intriguing new frontier in tissue repair because it has the potential to achieve complete tissue regeneration without the burden of permanent scarring.
- Biomaterials serve as temporary scaffolding that serve as essential facilitators of this process by boosting cellular responses and tissue regeneration.
- The accuracy and personalization of treatments for scarless wound healing are improved by the application of machine learning algorithms in the identification of appropriate biomaterials.
- By harnessing the capability of data analysis, machine learning makes it pos-

sible to select the optimal biomaterials matched to each patient's needs, promoting quicker wound healing and improved patient outcomes.

• It will need continued advancements in machine learning applications and biomaterial research to fully realize the potential of scarless wound healing as a paradigm-shifting medical approach.

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REVIEW ARTICLE

The Human Hippocampal Formation: Neuroanatomy and Network

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Abstract

Ever since its discovery, the hippocampal formation has fascinated anatomists and physiologist alike and is one of the most extensively studied structure of the human brain. The hippocampal formation encompasses the hippocampus proper (Ammon's horn/Cornu Ammonis- CA), dentate gyrus, subiculum and entorhinal cortex. It has a circuitry that is widely connected to the rest of the cerebral cortex, and its own internal circuit is rather unique, making it a key centre for episodic memory encoding. Via the fimbria-fornix pathway, the hippocampal formation is connected to the pre-septal area, mammillary body, anterior thalamic nuclei and cingulate gyrus. From the cingulate gyrus, the signals return to the hippocampus forming the Papez circuit. The internal circuit of the hippocampal formation is mainly an excitatory network, with the entorhinal cortex acting as its gateway. Signals from the entorhinal cortex project to the dentate gyrus, are transmitted to CA3 neurons and finally reach CA1 neurons. From the CA1, output signals project back to the entorhinal cortex, via the subiculum. Key features of this circuit that are essential for episodic memory encoding and its long-term storage are a rich recurrent collateral network in CA3 and post-synaptic modification of signal via long-term potentiation. Multiple sensory inputs pertaining to an event, such as sensory, emotional, and cognitive information, are funnelled into the hippocampus via the perirhinal/parahippocampal cortices and the entorhinal cortex. The input signals converge on different CA3 neurons, and owing to its recurrent collateral system, CA3 region is able to associate each component of an ongoing event/experience with the other. This associatively encoded memory forms a memory trace. The memory trace is later shifted to the cerebral cortex for storage and retrieval. The remarkable feature of this system is that although a collated memory trace is formed, each individual component still remains separate and is not lost. Hence, when any one component (cue) is visualised or experienced, it is enough to bring back the whole memory. Once the cue is perceived by the hippocampus, the network is able to recall all the neurons that were activated during the formation of the memory trace and re-activate the set of neurons to retrieve the memory. Episodic memory also has spatiotemporal components, which are perceived by various specialized cells in the hippocampal formation and cortex and are implicated in spatial navigation. Thus, the hippocampal formation enables us to store and remember life experiences and orient ourselves in the environment, thus influencing memory, learning and behaviour. This review provides an overview of the anatomy and connectivity of the hippocampal formation, along with a brief commentary on its role in episodic memory encoding.

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Introduction

Hippocampus is a paired grey matter structure located in each mesial temporal lobe and forms part of the limbic system. Phylogenetically, it is one of the oldest parts of the brain and is highly conserved across all the mammalian species, both structurally and functionally. It is involved in memory processing, learning,

behaviour and spatial navigation. With its unique anatomy and fascinating physiology, it has captured the interest of neuroscientists for centuries. The term hip-

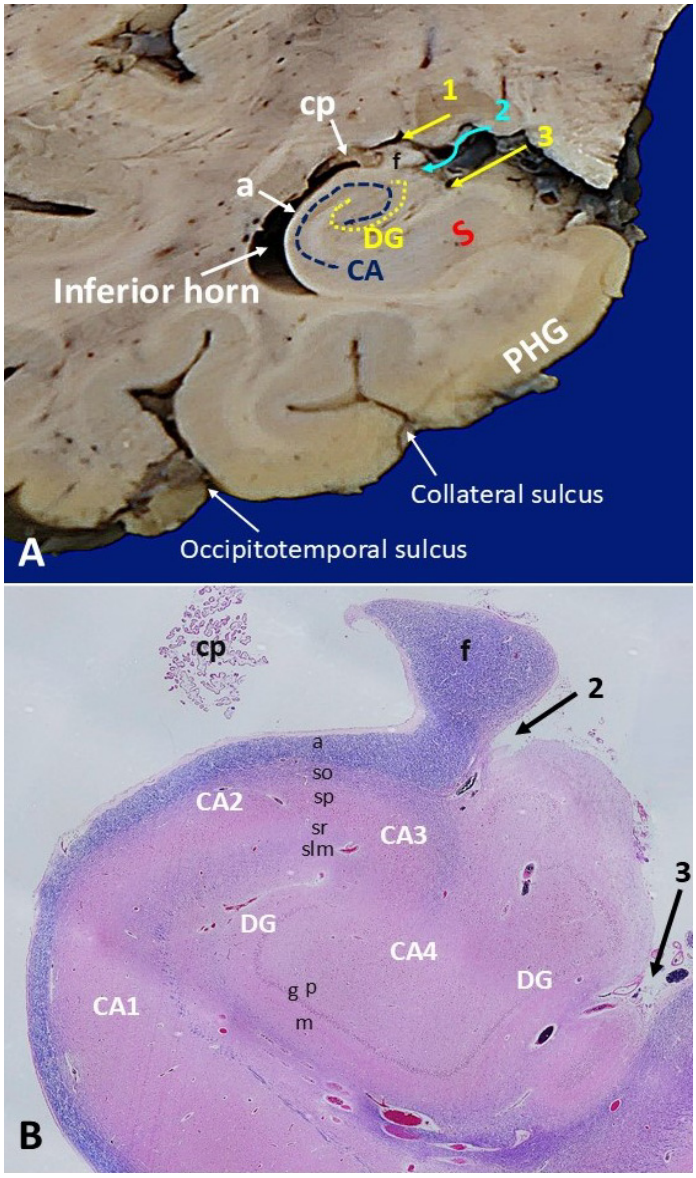

Figure 1: Coronal section passing through the body of the hippocampus. A: Gross anatomy with the overlaid dashed lines representing dentate gyrus (DG) and Cornu Ammonis (CA). The Ammon's horn or CA is continuous with the subiculum which further continues as the parahippocampal gyrus (PHG) which at this level of section, corresponds to the parahippocampal cortex. There are three major fissures that are related to the hippocampal formation: the choroidal fissure (1), fimbriodentate fissure (2) and hippocampal fissure (3). B: Microscopic anatomy on sections stained with myelin stain (Luxol fast blue). The typical C-shaped interlocked anatomy of DG and CA is evident. The white matter sheath (stained blue) covering the hippocampus is the alveus (a) which converges to form the fornix (f). The layers of dentate gyrus (m- molecular layer, g- granular layer and p- polymorphic layer) and the Ammon's horn (a- alveus, so- stratum oriens, sp- stratum pyramidalis, sr- stratum radiatum and slm- stratum lacunosum-moleculare) are marked. [cp: choroid plexus]

pocampus was first coined by the anatomist Arantius in 1587, who likened it to the seahorse. Later, De Garengeot, among others, named the curved structure of hippocampus as *cornu ammonis* or Ammon's horn after the Egyptian god Amun Kneph whose symbol is a ram (1).

The grossly visible anatomical structure in the floor of the inferior horn of the lateral ventricle is the hippocampus. However, a more appropriate term would be the hippocampal formation. Generally speaking, the hippocampal formation includes hippocampus proper (Ammon's horn/Cornu ammonis, CA), dentate gyrus, subiculum and entorhinal cortex. In the literature, both the terms have been used interchangeably and 'hippocampus' could represent the hippocampal formation or hippocampus proper, depending on the context of usage.

Neuroanatomy: Gross Anatomy

The hippocampus, as an elongated structure along the floor of the inferior horn of the lateral ventricle, is formed by two interlocked grey matter structures: the Ammon's horn and the dentate gyrus. It extends from the amygdala anteriorly to the splenium of corpus callosum posteriorly. It resembles a seahorse in the axial plane and has three parts: the head, body and tail. The anterior portion or the head is broad and marked by a number of groves, resembling a paw and hence, called '*pes hippocampi*'. On coronal sections, its posterior portion (the body and the tail) appear as a C-shaped, rolled-in structure and hence termed Ammon's horn. Posteriorly, the hippocampus progressively tapers and forms the vestigial *gyrus fasciolaris* and *inducium gresium*. The latter is a very thin grey matter layer that runs along the dorsal aspect of corpus callosum and terminates in the paraseptal region (2).

The ventricular surface of the hippocampus is covered by a white matter sheath called the alveus which is formed by the efferent pathway axons emanating mainly from the pyramidal neurons in the Ammon's horn and the subiculum. Medially, the alveus converges to form the fimbria, which runs posteriorly and curves upwards. At the splenium of the corpus callosum, it continues as the crus, body and columns of the fornix, which run dorsal to the thalamus, separated by the choroidal fissure. The two crura of the fornix are joined together by the hippocampal commissure at its posterior aspect. Figure 1A shows the coronal section of the brain at the level of the body of the hippocampus with its anatomical relations to temporal lobe structures.

Neuroanatomy: Histology

The Ammon's horn and the dentate gyrus are archicortices, meaning they are more primitive than the neocortex and have a three-layered cortical architecture. Dentate gyrus is a V-shaped structure and consists of three layers: the molecular layer, granular layer and polymorphic layer. The polymorphic layer is continuous with the hilus (CA4 sector, see below). The granular layer consists of compact layers of granule neurons (2). The limb of DG between the CA3 and CA1, separated by the hippocampal fissure, is the suprapyramidal blade and the other is the infrapyramidal blade. Ammon's horn can be resolved into five layers: the alveus, stratum oriens, stratum pyramidalis, stratum radiatum and stratum lacunosum-moleculare (Figure 1B). Stratum pyramidalis is formed by the excitatory pyramidal neurons, the bases of which are directed towards the alveus. The axons arising from the base of the neurons form the alveus and fimbria. The basal dendrites ramify in the stratum oriens and receive commissural fibres from identical areas of the contralateral hippocampus. The apical dendrites ramify in stratum

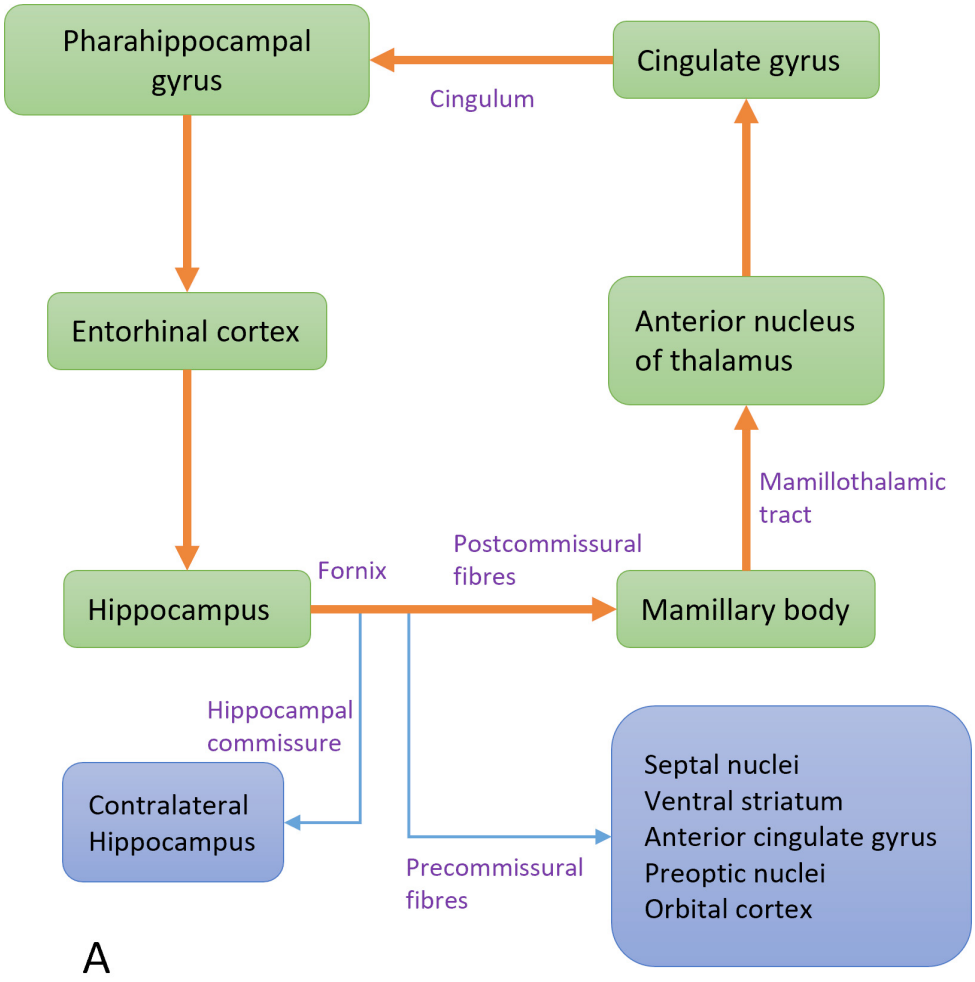

Figure 2: Diagrams depicting the hippocampal networks. A: Pathways connecting hippocampus with other structures of the brain. Papez circuit is represented in green and orange

radiatum and receive commissural fibres from non-identical areas of the contralateral hippocampus, from recurrent collateral from other CA3 neurons, inputs from entorhinal cortex and mossy fibres from dentate gyrus (2). The cornu ammonis can be subdivided into four regions or sectors: CA1, CA2, CA3, and CA4 (Figure 1B) (3). The CA4 sector is located within the two limbs of the dentate gyrus and many do not consider it as a part of cornu ammonis, but rather as the dentate gyrus hilus (4). CA1 is continuous with subiculum following which there is entorhinal cortex or parahippocampal cortex (the former at the anterior and the latter at the posterior level of hippocampus).

Neurophysiology: Network and Connections

The apparently simplistic three-layered architecture of the hippocampus hides behind a very complex network and unique physiology. The hippocampal formation is one of the few brain structures that receives a variety of highly processed sensory information and its internal circuitry is capable of comparing and collating these inputs.

The main connecting pathways (Figure 2A) associated with the hippocampal

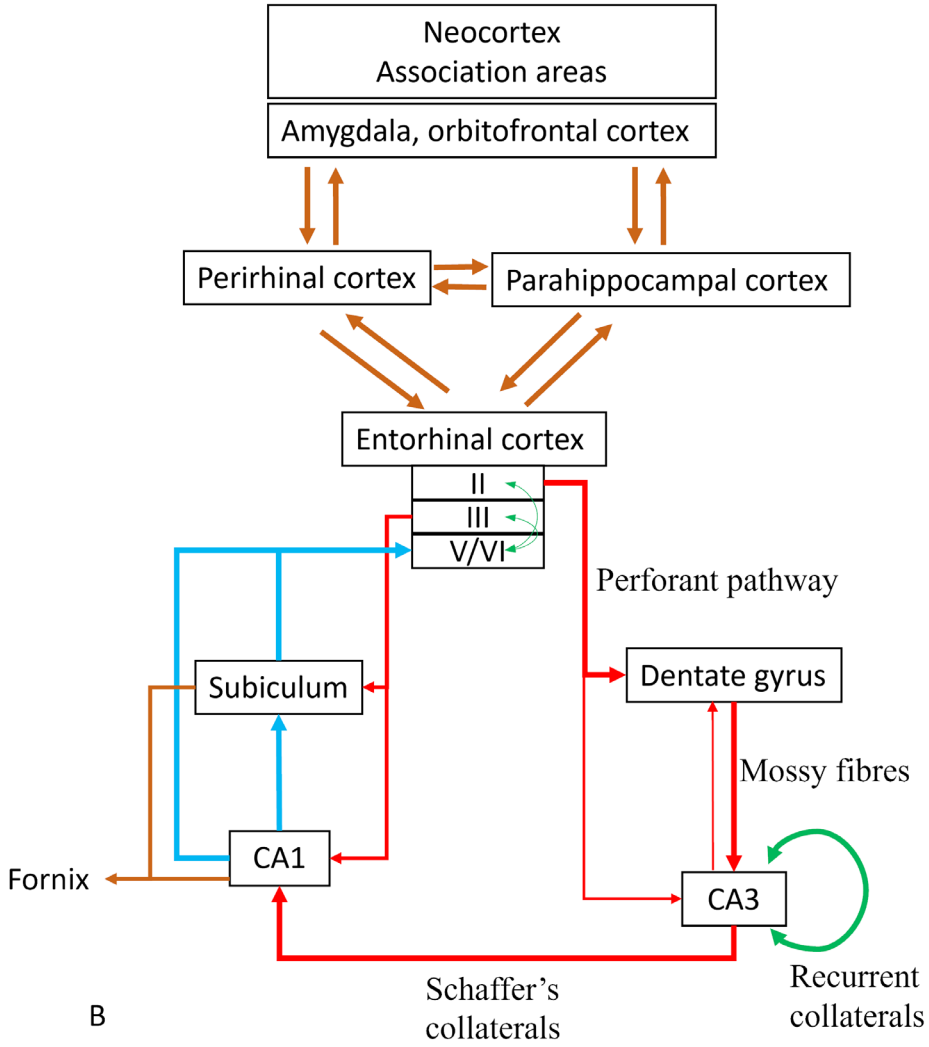

Figure 2: Diagrams depicting the hippocampal networks.B: Intrahippocampal circuit. Various unimodal and polymodal sensory inputs are relayed to the perirhinal and parahippocampal cortices and reach the entorhinal cortex. Perforant pathway fibres from the entorhinal cortex project mainly to the dentate gyrus. From the dentate gyrus, signals are transmitted to CA3 neurons via the Mossy fibres. The signals are integrated or associated with one another in the CA3 via the recurrent collateral network. The integrated signals are transmitted to the CA1 neurons via the Schaffer's collaterals. CA1 forms the major output of the hippocampus and the output signals are project back to the entorhinal cortex, via the subiculum. From the former, the hippocampal output is backprojected into the neocortex

formation are:

1. Angular bundle, including the perforant pathway: carrying the fibres between entorhinal cortex and dentate gyrus and Ammon's horn.

2 Fimbria-fornix pathway, including Papez circuit: connecting hippocampal formation to the thalamus, septal nuclei, hypothalamus and brainstem. The fibres in the columns of the fornix mainly terminate in the mammillary body via the postcommissural fibres. And the smaller precommissural fibres of the fornix terminate in the septal nuclei, ventral striatum-nucleus accumbens, cin-

gulate gyrus, olfactory nucleus, medial frontal cortex and gyrus rectus (4).
3. Commissural pathway: connecting the two hippocampi. However, in humans there is hardly any interaction between the two hippocampi via the commissures.

Internal circuit: As opposed to the neocortex, traditionally, hippocampal formation has been described to have a predominantly unidirectional 'trisynaptic' intrahippocampal circuit without feedback circuits. The afferent signals travel from entorhinal cortex à dentate gyrus à CA3 à CA1 and efferents from CA1 go back to entorhinal cortex via the subiculum. However, modern techniques have revealed that it is not so simplistic with extensive interconnections within and between different components of the hippocampal formation including feedback mechanisms (5,6). In addition, connections between two components are not uniform throughout their structure in terms of the type and volume of input and output signals. The connectivity and the information processed vary along the transverse and longitudinal axes of any given component. The connections are also segregated based on the layers of the cortex from which they originate (laminar distribution of the projections). Consequently there is massive interconnectivity and parallel processing of various signals (5,7,8).

Figure 2B depicts the internal circuit of the hippocampal formation with only the main connections being represented (8–10). Few points that need highlighting are:

- The entorhinal cortex acts as a gateway to the intrahippocampal network as signals to and from the neocortex must pass through it.
- From the entorhinal cortex, signals to the dentate gyrus and CA3 neurons originate from layer 2, while those to CA1 and subiculum from layer 3. The efferents from the CA1 neurons, via subiculum, terminate in its deep layers.
- The neurons in the deep layers of the entorhinal cortex synapse with the superficial neurons.
- CA3 has a rich 'recurrent collateral pathway' where axons of CA3 cells synapse with the dendrites of the same and other CA3 neurons along the longitudinal axis of the hippocampus.
- Signals from CA3 can also directly terminate in the entorhinal cortex, bypassing the CA1.
- CA3 also provides feedback projection to the dentate gyrus.
- Various unimodal and polymodal inputs are directed towards the hippocampal formation from different cortices and the hippocampal output is reciprocally back projected into the same cortices.

Functions: Memory, Learning and Spatial Navigation

The hippocampal formation is involved in memory, learning and spatial navigation which are all inter-related functionsn (11,12). Though it's critical role in long term memory formation, particularly the episodic memory is known since decades, recent studies have also shown that the hippocampal formation also has a role in short term memory (13).

Episodic memory: i.e., memory of an event, results due to the interplay between the higher order association cortices (perceptual regions) and the hippocampus over time (14). An event consists of a set of items that occur together, such as the object, place and time: the 'what/who', 'where' and 'when'. Episodic memory is created by 'association' and has an 'unstructured format'. Different components of an event, which are perceived by different cortical areas, are 'associated' with

each other as a single memory. And yet, the individual components themselves are kept discrete so that any one component can trigger the whole memory when needed, i.e an 'unstructured format'. Hippocampal network lies at the centre of this process by providing a spatiotemporal framework for encoding memory in a flexible manner (7,10). It not only enables formation and storage of new memory, but also the updation of memory with new inputs and retrieval/recall. Once this associative memory is formed, its representation is shifted from hippocampus to different cortical areas as long-term memory (8,15). The physiological processes unique to hippocampus that facilitate memory and learning include associative synaptic modification- long-term potentiation and recurrent connectivity (16).

The object (the 'what') information from the temporal and occipital association areas is transmitted to the perirhinal cortex, from which it is further relayed to the lateral entorhinal cortex. The spatiotemporal information (the where and when) from the striate cortex, auditory association area, posterior parietal cortex and retrosplenial cortex converge on the parahippocampal cortex and are relayed to the medial entorhinal cortex (8,10,16). The perirhinal and parahippocampal and entorhinal cortices also receive emotional/reward-related information from the orbitofrontal cortex, amygdala and temporo-polar area (8,10,16). From the lateral and medial entorhinal cortex, the varied unimodal and polymodal information is transmitted further to the hippocampus via the perforant pathway.

The object and the spatiotemporal information are brought together by the recurrent collateral pathway in CA3 (16). This auto associative network enables each pattern to be associated with itself and with others. Also, the network facilitates a distributed way of memory where the different components of an event are distributed over different neurons which can be associated or collated to form a single memory construct or engram. From the CA3, the associated memory is transmitted to CA1 from which it is back projected to the neocortex (from where the input signals had arisen) for storage. The object information and spatial information will be stored separately in their appropriate cortical areas. For proper neocortical representation and storage, the back projection pathway from CA3 to neocortex will interact with the incoming information, resulting in associative learning (9).

Though association network is classically described in CA3, information association also happens in the more proximal areas of the circuit. The perirhinal, para-hippocampal and entorhinal cortices do not merely act as a conduit for signals. There are networks within and between these cortices that enable some degree of association between the different types of inputs. Polymodal information pass sequentially through the perirhinal/parahippocampal cortices, entorhional cortex and finally hippocampus. As we move towards the hippocampus, the association of the information increases in complexity (8). CA3, however has the highest associative ability and is able to combine continuous spatial with discrete object representations and to recall the complete representation from either a spatial or object cue (16).

The collection of neurons in the hippocampal formation and neocortex that are activated by an event are bound together to form the episodic memory trace or engram. The hippocampal component acts as a pointer to the neocortex. The pointer when activated will reactivate the neocortical traces for the recall of the complete memory (16,17).

Spatial memory and navigation: Cognitive maps are produced by sequences of transient neuronal activation that keep track of and order spatial and non-spatial information (continuously varying) in an allocentric or egocentric reference

frame. Various contents/components of the environment are stored in cognitive grid or map aiding navigation.

In rodents, the hippocampal formation contains specialized cells such as the place cells, grid cells, head direction cells and boundary cells that help the animal make a spatial grid or map and recall a place or a route to reach a place. Place cells are present in the CA1 and different place cells get activated when the animal is present in a particular place. Grid cells in the entorhinal cortex, presubiculum and parasubiculum provide the spatial representation. Head direction cells in various areas (presubiculum, postsubiculum, anterodorsal thalamus, entorhinal cortex, retrosplenial cortex, mammillary bodies and thalamus) respond to the animal's head relative to fixed landmarks, contributing to the spatial processing (11).

In primates, including humans, the spatial information is stored via the 'spatial view cells'. They are activated by either looking at or by simply visualising a place/space without 'directly being at a place' (16). The information that is stored is about the 'spatial view' and not about the 'in the place'. Hence, humans can visualize a place even though we may not have visited the place physically. In addition, head direction and whole body motion neurons form part of a system for remembering the direction and distance travelled (18).

Computation model: Based on computational models, the dentate gyrus acts as a competitive network. It is postulated to perform pattern separation in which each component of an event is kept independent. This minimises the interference of one event on another (9,10,16). The recurrent collateral system in CA3 acts as a 'continuous attractor network' which allows pattern completion, error correction and generalisation of memory retrieval cues. It enables rapid associations between any spatial, object and reward information (7,10,16). The mossy ûber inputs to CA3 is critical for encoding, whereas the direct perforant pathway inputs from the entorhinal cortex onto the CA3 are more important during the retrieval phase. CA1 is presumed to collate the CA3 information, compared it with the current perceptual input and then form a single representation of the event (the individual components are no longer distinct) which is transmitted back to the neocortex. These associatively learned back projections are important for retrieval of the whole memory (9,16,17).

Summary

The role of hippocampal formation in long term memory formation, especially episodic memory lies in its ability to bind varied object-related and space-related sensory and reward/emotional inputs to form a single memory trace. It communicates practically with every neocortical and association areas via the perirhinal, parahippocampal and entorhinal cortices and the object and spatial signals enter the hippocampus via their dedicated pathway. As the signals move towards the hippocampal system, level of integration of the signals increases and CA3 with its collateral system, enables the ultimate association or integration of signals. Traditionally, it was thought that the signals are transmitted unidirectionally from cortex to hippo and back to cortex without interaction or feedback mechanisms. But, it is evident that there exists multiple interconnections between the different areas of the hippocampal formation including feedback systems. Additionally, there is diversity in the rostrocaudal and mediolateral extent of an area, in terms of the type of signals it handles and its connectivity to other areas. With this complex network, hippocampal formation sits at the apex of episodic memory trace formation and recall. The process is very dynamic with the output signals from hippocampus continually interacting with the input signals, resulting in continuous

updation of the memory trace as it forms. As rightly described by Cossart et al., hippocampal networks form cognitive maps, stored as sequences of related experienced events and visited places that can be mentally travelled in space and time (19).

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REVIEW ARTICLE

Lint- free Technology in Wound Dressings: Technology Landscape, Challenges and Opportunities

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Abstract

Wound dressings play a pivotal role in modern healthcare, facilitating the wound healing process, preventing infections, and enhancing patient comfort. With recent progress in medical technology and an increasing focus on patient outcomes, wound dressing technology has been evolving rapidly. In this context, lint-free dressings have emerged as a pragmatic solution. The adoption of lint-free technology in the development of wound dressings is now being recognised as one of the key requirements for effective dressing, especially in deep and chronic wounds, to prevent further damage or infection by shedding lint onto the wound surface. Several fabrication technologies have been adopted for the development of advanced wound dressings in the form of felts, fibers, foams, and adhesives. This article delves into the most recent trends and product breakthroughs in lint-free wound dressings highlighting their need in the realm of chronic and burn wounds and offering valuable insights into the current market dynamics. Furthermore, it addresses the challenges and opportunities for further enhancements in this field.

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Introduction

A wound is defined as a flaw or interruption in the integrity of the skin or mucosa's epidermis as a result of thermal or physical injury or the presence of a medical condition. Wounds are categorised as acute or chronic wounds based on the length and kind of the healing process [1]. Depending on the depth, size, and severity of the damage, an acute wound is a wound that typically heals fully within a predictable timeframe. Mechanical injuries (abrasions, tears, penetrating wounds, or surgical wounds), radiation, electricity, corrosive chemicals, or thermal sources are the main causes of acute wounds. On the other hand, when skin wounds do not heal quickly and could possibly recur, they become chronic wounds. These wounds are unable to heal due to persistent wound pathology, vascular disease, repeated trauma or prolonged pressure, concurrent medical conditions, such as diabetes mellitus, venous insufficiency, or the presence of a malignancy, or coincident wound complications like infection or ischemia. Wound classification helps healthcare professionals determine the best course of treatment, including wound care, infection prevention, and potential surgical interventions. The choice of classification often depends on the specific context and purpose, whether in a clinical setting, research, or forensic analysis.

Wound care and management can vary depending on the location, size and type of the wound. Proper wound assessment, cleaning, and infection prevention are essential steps in the treatment process. In some cases, wounds may require medical attention or surgical intervention to ensure proper healing and prevent complications.

A range of wound healing dressing materials are available to promote healing; nevertheless, proper healing depends on the material selection based on the type of lesion. Additionally, unlike wound bandages that are designed to hold wound dressings in place, dressings must actually come into contact with the wound to be effective. One crucial function of the dressing for acute wounds is to keep the wound exposed to growth factors and cytokines that promote healing, as these are less readily available when the lesion is uncovered.

Additionally, dressings shield the wound from damage, enable matrix materials to stay in contact with it, lessen infection, and enhance the electrical gradient of the wound. The ability to maintain a moist environment and prevent wound desiccation while absorbing wound drainage and preventing maceration, promote epidermal migration, permit gas exchange, trigger angiogenesis and the production of connective tissue, provide protection against bacterial infection, maintain an adequate tissue temperature to enhance blood flow, maintain electrical gradient, and be non-adherent to facilitate removal and debriding action are all characteristics of the ideal wound dressing. In this in-depth analysis, we provide a summary of the effect of lint-free technologies in the development of wound dressings. We also provide an extensive review of the market potential and patent survey in this realm. The challenges in the future development of lint-free dressings are also highlighted in this review.

Factors Affecting Wound Healing Process

Several factors can influence the complex and dynamic process of wound healing. These elements may aid or hinder the healing process [2-4]. Some of the main elements that can influence how well a wound heals include:

1. Type of Wound: The type of wound has a big impact on how quickly it heals. In general, wounds are categorized as either acute or chronic. Compared to chronic wounds, such as pressure ulcers or diabetic ulcers, acute wounds, including surgical incisions or small cuts, typically heal more swiftly and reliably.
2. Size and Depth of the Wound: Larger and deeper wounds often heal more slowly. A deeper wound may involve damage to underlying tissues like muscles, which can further delay the healing process. A larger wound requires the body to produce more tissue in order to seal it.
3. Wound location: The location of the wound can significantly impact its healing rate. In comparison to wounds in locations with inadequate blood supply, such as the feet, those on the face typically heal more quickly.
4. Blood Flow: Healthy blood flow is necessary for wound healing. Blood accelerates the healing process by delivering essential nutrients and oxygen to the wound site. Healing can be hindered by diseases such as vascular disease, which reduces blood flow.
5. Infection: Infections can significantly impair wound healing. Inflammation, tissue damage, and delayed recovery can result from bacterial, viral, or fungal infections. Careful wound management and infection prevention are essential.
6. Chronic Diseases: The body's capacity to heal wounds can be impacted by

underlying medical conditions such as diabetes, autoimmune diseases, and chronic illnesses. Healing is more difficult due to these disorders' frequent immune system and blood flow impairments.

7. Age: As we get older, wound healing tends to take longer. Due to decreased immunological function, impaired collagen formation, and other age-related factors, recovery may take longer for older individuals.
8. Nutrition: Proper nutrition is essential for the healing of wounds. For tissue healing, proteins, vitamins -particularly vitamin C and vitamin A - and minerals, such as zinc, are crucial. The healing process might be hampered by malnutrition.
9. Smoking and Substance Abuse: Smoking and some drugs, such as alcohol and illegal narcotics, can impede the healing of wounds. The immune system can be weakened by substance addiction, while blood flow and oxygen delivery to tissues are reduced by smoking.
10. Medication: Some medications, such as corticosteroids and immunosuppressants, may prevent wounds from fully healing. It's important to discuss any side effects with a doctor.
11. Wound Care: Proper wound care is necessary for maximum healing. While failing to keep the area clean, moist, and covered can encourage healing, neglecting wound care can result in complications.
12. Chronic stress can impair the body's healing ability by weakening the immune system and exacerbating inflammation. Stress management is essential for both overall health and wound healing.
13. Obesity: Carrying around too much weight can strain the body's resources and impede circulation, potentially delaying wound healing.
14. Other considerations include Radiation therapy, chemotherapy, and genetic factors, all of which can impact wound healing.

It is important to note that individual elements and their interactions might differ significantly. Therefore, in particular situations, a thorough evaluation by a healthcare professional is crucial for controlling and optimising wound healing. Underlying medical issues, infection prevention, nutritional support, and advanced wound care strategies may all be included in treatment programs.

Wound Dressings

Wound care is a crucial aspect of healthcare, and the development of advanced bioactive wound dressings has revolutionised the field. These dressings are designed to accelerate the healing process, minimise infection risk, and enhance patient comfort. There is a plethora of wound dressings that have been used over the years, with new advancements in this segment emerging to meet the demands of the medical fraternity and the global healthcare market. Mainly, these dressings are classified as traditional and bioactive or advanced wound care dressings owing to the period in which they have been developed and the unique advantages that they offer, catering to the needs of specific types of wounds.

Lint–free Absorbent Technologies in Wound Dressings

Lint-free absorbent dressings have gained significant attention in the field of wound care due to their role in minimising the risk of contamination, promoting optimal wound healing, and reducing the potential for nosocomial infections. Wound dressings are fundamental in modern healthcare, facilitating the healing process and protecting wounds from contamination. Looking into the number of articles published through searching keywords 'Lint free and absorbent wound

dressings' indexed in PubMed, there has been a linear increase in the number of publications from 1980 to 2023, as shown in figure 1. This highlights the importance and potential of these dressings, as well as the commercial need for them, as evidenced by the research on the development of such dressings. Lint-free dressings, as the name suggests, offer an advantage by significantly reducing the risk of lint particle shedding, which can be a potential source of wound contamination.

Types of lint-free dressings

Lint-free dressings come in various forms, including woven and non-woven fabrics, silicone-coated dressings, and hydrocolloids. Each type has distinct properties and is chosen based on the specific wound characteristics and clinical requirements.

Woven and non-woven fabrics

Lint-free woven and non-woven fabrics are often made from synthetic materials like polyester or polyethylene. These materials are engineered to minimise lint shedding, making them suitable for wound dressings. They provide an effective barrier against microbial infiltration and are available in various sizes and shapes to fit different wound types. Moreover, these dressings can be impregnated and treated to become smart dressings, providing an added advantage [5,6].

Silicone-coated and silicon foam dressings

Silicone-coated dressings are designed to adhere to the wound surface without causing trauma upon removal[7-9]. Their non-adherent properties help prevent tissue damage, reduce pain, and minimize the risk of lint contamination. These dressings are especially useful in chronic wound care. These dressings are often provided as foam dressings. Van den Kerckhove et al provides an extensive overview of the treatment and prevention of hypertrophic (burn related) scars using silicone based dressings in their review article [10].

Hydrocolloid dressings

Hydrocolloid dressings combine the benefits of a lint-free surface with moisture management capabilities. They generate a moist environment that promotes wound healing and are effective in managing moderate to heavily exuding wounds. Thomas (2009) highlighted the importance of hydrocolloid dressings in

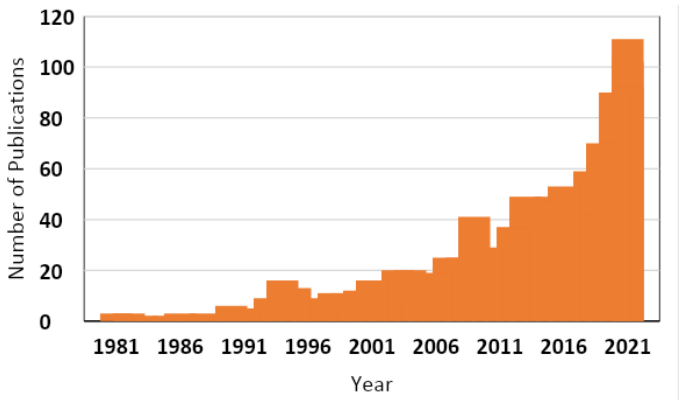

Figure 1: List of articles published through searching the terms 'Lint-free and absorbent wound dressings' indexed in PubMed from 1980 to 2023

wound management practice in his review article [11]. The impermeable nature of hydrocolloids provides a protective covering to the wound, permitting washing or showering while helping to prevent the spread of pathogenic microorganisms. In yet another review article, Keogh et al. (2018) explored the potential effects of hydrocolloid wound dressings on healing pressure ulcers in individuals in any care setting [12]. Dealey (1993) provides a detailed review on the actions of hydrocolloid dressings and the type of wounds for which they are most suitable [13]. The range of hydrocolloid products is reviewed, along with their individual advantages and disadvantages.

Lint-free dressings and wound healing

Lint-free dressings contribute to wound healing in several ways. The absence of lint particles reduces the risk of contamination, which is vital in preventing infection. Infections can delay wound healing and lead to complications. Lint-free dressings also help maintain a clean and sterile environment around the wound, which is crucial for optimal healing.

Infection prevention

In addition to wound healing, lint-free dressings play a pivotal role in infection prevention. Contaminants like lint particles can introduce foreign bodies and pathogens into the wound, increasing the risk of nosocomial infections. The lint-free nature of these dressings minimises this risk, making them a valuable choice in critical care and surgical settings.

Patient comfort

Patient comfort is an essential consideration in wound care. Lint-free dressings, by being non-adherent and reducing the risk of lint-related irritation, help enhance patient comfort. Patients experience less pain and trauma during dressing changes, leading to improved overall satisfaction and compliance with wound care regimens.

Lint-free dressings have demonstrated their utility in wound care through their ability to minimize contamination, facilitate wound healing, and prevent infections. Their non-adherent properties and moisture management capabilities make them versatile tools in the armamentarium of healthcare providers. These types of dressings provide a base platform for the development of advanced bioactive dressings. As future research continues to refine and expand our understanding of these dressings, their role in wound care is likely to grow, further improving patient outcomes.

Market Survey & Technology Tracking

Technology landscaping of non-lint bandages enables us to systematically identify, analyse, and prioritize the technologies employed in this field. This comprehensive approach helps us gain a better understanding of the current landscape and facilitates the prediction of more favourable outcomes. While dressings and bandages are indispensable medical products readily available in the market, the technology involved in rendering them lint-free, thereby preventing contamination and ensuring a sterile environment, is frequently overlooked.

Companies employ a diverse range of methods and techniques to produce lint-free dressings, contributing to the quality and safety of these essential medical supplies. This includes a) material selection, b) Advanced manufacturing process, c) lint control system, and d) surface treatment strategies

Material selection

Non-woven fabrics and microfiber materials are commonly employed due to their minimal lint-shedding properties in applications such as wound dressings. Examples of suitable materials include polyurethane and regenerated cellulose. Biopolymers such as gelatin, chitosan, and alginate have also been explored for this purpose [14]. To enhance the efficiency of these dressings, absorbent layers made from materials such as alginate, silver sulfadiazine, hydrocolloid, chlorhexidine, hydrogel, paraffin, and vaseline® are often incorporated. This versatile technology, with its focus on material selection and design, contributes significantly to the advancement of wound care and medical applications. When selecting biomedical polymers for the creation of lint-free dressings, several essential requirements must be met. These requirements encompass non-toxicity, the absence of allergic responses, compatibility with sterilisation processes, satisfactory mechanical properties, elasticity, durability, and biocompatibility. A representation of commonly selected strategies in lint-free bandages is given in figure 2.

Advanced manufacturing processes

Numerous manufacturing processes are suitable for producing lint-free wound dressings. One such method, the melt-blown process, represents a nonwoven manufacturing system that offers direct conversion of polymers into continuous filaments, subsequently transforming these filaments into randomly laid nonwoven fabrics. Notably, the melt blowing process has achieved the remarkable feat of producing microfibers with an average diameter as small as one micron under commercial settings. This achievement has broadened the range of materials that can be utilized for lint-free wound dressings [15,16].

The second methodology is the electrospinning technique, which is another valuable method for creating lint-free materials. This process involves the conversion of polymers like polyurethane, PCL (Polycaprolactone), PVA (polyvinyl alcohol), and chitosan into nonwoven fibers. These electrospun fibers, characterized by their fine and uniform structure, serve as a foundation for the development of lint-free wound dressings. The versatility of these manufacturing processes, combined with their ability to produce ultrafine fibers, significantly enhances the range of materials available for the creation of high-quality, lint-free wound

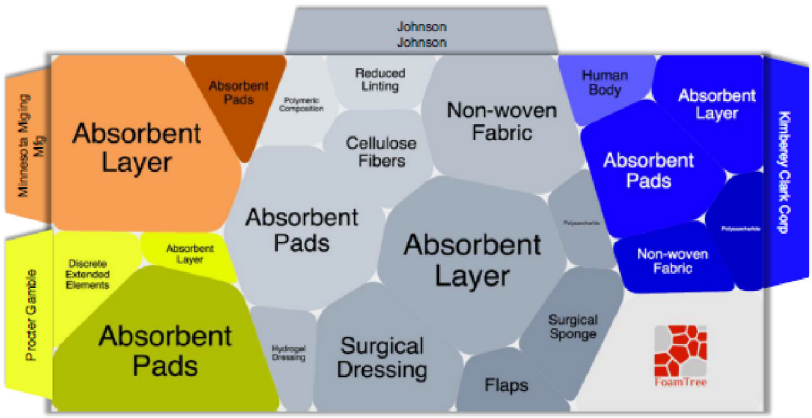

Figure 2: A schematic representation of commonly selected strategies in lint-free bandages

dressings, thereby contributing to advancements in wound care technology.

Spun-bond process is yet another technology used in the production of lint-free bandages, offering an efficient one-step manufacturing process. In this method, a thermoplastic polymer, like polyester, nylon, polypropylene, or polyethylene, is melted and extruded through a spinneret. The extruded fibers are then rapidly cooled, and they are laid onto a moving conveyor belt to create a continuous web. The fibers get bonded together as the web cools. This process results in the direct formation of lint-free fabrics suitable for use in bandages, ensuring high-quality, non-linting wound care materials [17].

Needle punch process is another technology employed in the production of wound dressings. In this process needles are used to mechanically entangle the fibers, creating a strong, stable fabric through repeated piercing. This nonwoven material can be further shaped and bonded as needed, providing customizable wound dressings with attributes like absorbency, breathability, and durability. Several literature reports stating the use of needle punch technology are available [18-20].

3D printing technology, known for creating intricate three-dimensional structures by layering diverse materials, has revolutionised the development of lint-free wound dressings. This innovation enables the production of skin tissue and constructs resembling natural skin, supporting on-demand therapies and complex pharmaceutical forms. The 3D printing process involves using computer-aided design (CAD) software to create a product, which is then printed layer by layer. Its customizability is a key feature, allowing for precise fabrication of wound dressings. The trends and opportunities of 3D-printed wound dressings have been extensively reviewed [21-23].

Surface treatments

Surface treatment is an indispensable technique for enhancing the lint-free characteristics of wound dressings. This multifaceted approach involves processes such as surface smoothing, fibre sealing and bonding, electrostatic force control, coating applications, integration of anti-lint additives, and the utilisation of heat setting. These combined treatments effectively mitigate the presence of loose fibers, thus averting lint contamination and upholding the wound dressings' integrity and cleanliness. This is of paramount importance in the realm of effective wound care and diverse medical applications. Among these techniques, coating methods play a pivotal role in strengthening the lint-free properties of wound dressings. By applying a thin layer of material to the dressing's surface, they serve

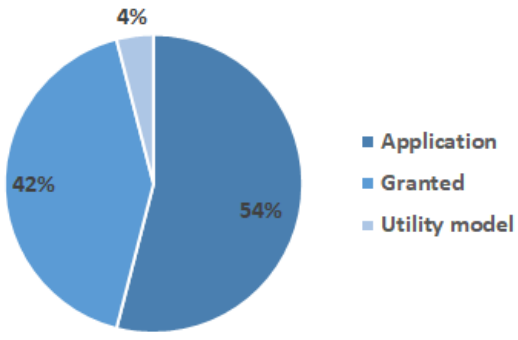

Figure 3: A Pie-chart representation of patent filing trend in the domain of lint-free bandages

as a crucial strategy for lint reduction. These coatings serve as a protective barrier, adept at encapsulating loose fibers and preventing their dispersal into the wound or its surroundings. Additionally, coatings can contribute to a smoother surface texture, securely binding any stray fibers, thus minimizing the risks of lint contamination. In some cases, anti-lint additives are incorporated into these coatings, further enhancing their lint-resistant qualities. The choice of coating material can be customized to meet specific requirements, including transparency, breathability, adhesion, and durability, aligning perfectly with the dressing's intended application [24,25].

Patent Landscape

A comprehensive analysis of the patent landscape within the realm of lint-free medical bandages underscores the notion that the technology for producing such bandages may still be in its nascent or developmental stages. Figure 3 gives a representation of patent filing trend in the domain of lint-free bandages. This observation is particularly intriguing when we consider the broader context of the global market for medical tapes and bandages, which was estimated to be worth USD 7.0 billion in 2016 and is anticipated to exhibit a compound annual growth rate (CAGR) of 5.10% through 2025.

Examining the patent data in this segment, we find that approximately 54% of the applications are still pending, 42% have been granted, and 4% fall under the category of utility models. However, this percentage of patent filings appears relatively modest when contrasted with the substantial market size. Several factors may contribute to this phenomenon. Firstly, it is plausible that the technology has not yet witnessed a ground-breaking innovation or invention, which could be holding back extensive patent activity. Alternatively, there might be multiple alternative approaches or materials available for addressing the issue of lint in bandages, discouraging significant investments in this specific area

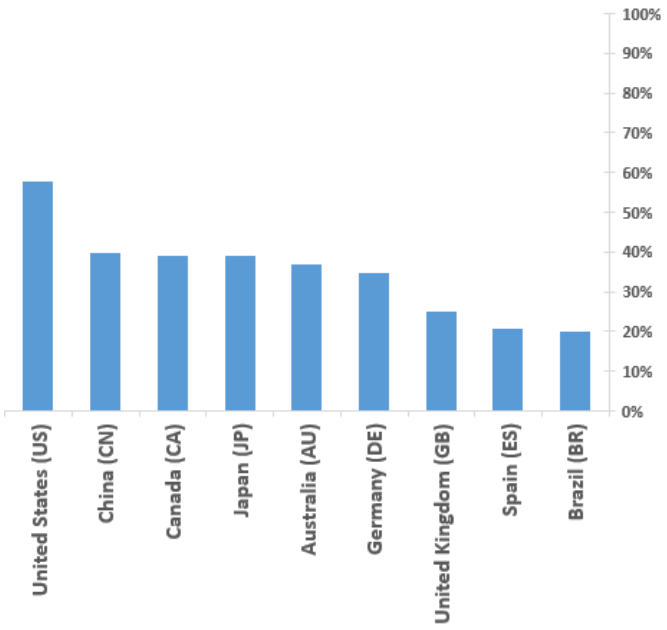

Figure 4: A country-wise analysis of the patent landscape for lint-free bandages

Another possibility is that the patents filed for lint-free technologies may have applications beyond just the medical industry. Consequently, companies could be filing patents without imposing any limitations on their use, potentially diluting the focus on lint-free bandages within the patent landscape.

Further exploration of the patent survey reveals a noteworthy trend. The primary emphasis of companies engaged in the development of lint-free bandages centres around exploring diverse compositions that can be applied to the final product or experimenting with variations in the layered structure of these bandages. This observation suggests that innovation in this field predominantly revolves around enhancing the functionalities and applications of bandages rather than exclusively concentrating on their lint-free characteristics.

The country-wise analysis of the patent landscape for lint-free bandages reveals that the United States is taking the lead in terms of percentage protection, with over 50% of the patents originating from this region (figure 4). However, despite Japan being a significant player in the field of patent protection, it appears that substantial innovation in the technology domain is somewhat lacking. And India, on the other hand, presents a significant gap in terms of innovation in the lint-free bandage technology domain. Market reports shed light on the major players actively engaged in this technology sector. Prominent names include 3M, Medtronic (Covidien), Derma Sciences Inc, Johnson & Johnson, Smith & Nephew PLC, B. Braun Melsungen AG, Medline Industries Inc., Paul Hartmann AG, Cardinal Health Inc, and Molnlycke Healthcare Inc. The stakeholders in this domain from India are listed in table 1, and the top key players across the globe are given in figure 5.

One noteworthy observation from the patent landscape is that only a select few top players in the market are actively filing patents related to lint-free bandages. This suggests the likelihood of a broader application of the underlying technology. It's plausible that these key players are relying on companies specializing in lint-free technologies, such as those in the textile industry, rather than making direct investments in the processes. This strategic approach could explain the comparatively lower number of patents within the medical category, as the innovation and development of lint-free bandages are intertwined with other fields and industries.

When analyzing patents related to lint-free technology, it becomes apparent that not all of these patents pertain to medical applications (figure 6). In fact, the

Table 1: The stake holders from India in the domain of lint-free bandages

	List of Companies
1	Komal Health Care Pvt. Ltd.
2	Pradeep Surgical Dressings Pvt. Ltd.
3	Aditya Meditex Private Limited
4	Jajoo Surgicals Private Limited
5	Ansuya Surgicals Limited
6	ACME Surgicals
7	Medicaux Healthcare
8	Goldwin Medicare
9	K.S.Surgicals Pvt.Ltd
10	Datt Mediproducts
11	Sutures India
12	Precision Coatings Pvt. Ltd
13	Meghdoot Pharma

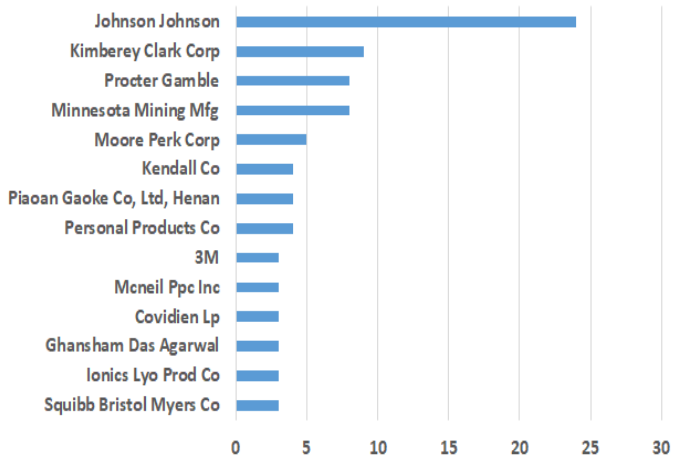

Figure 5: The top key players across the globe in the domain of lint-free bandages

majority of patent claims in this field emphasize the composition as a pivotal element. The key innovators in lint-free technology primarily concentrate on perfecting the absorbent layer to achieve a lint-free outcome. In contrast, the specific type of dressing is not frequently highlighted in the patent claims, with only a limited number of applications mentioning dressing types. It's worth noting that there are patents that do address the medical applications of lint-free technology, particularly in the domains of trauma (n=15), burn treatment (n=17), and ulcer management (n=7).

Another trend that emerges is that the majority of dressings fall under the category of occlusive dressings (figure 7). Occlusive dressings are specialised medical dressings used primarily in first-aid situations. These dressings are designed to create an air- and water-tight seal over a wound or injury, typically achieved through the use of a waxy coating. This seal not only provides protection but also minimises the risk of infection. However, it's important to note that occlusive

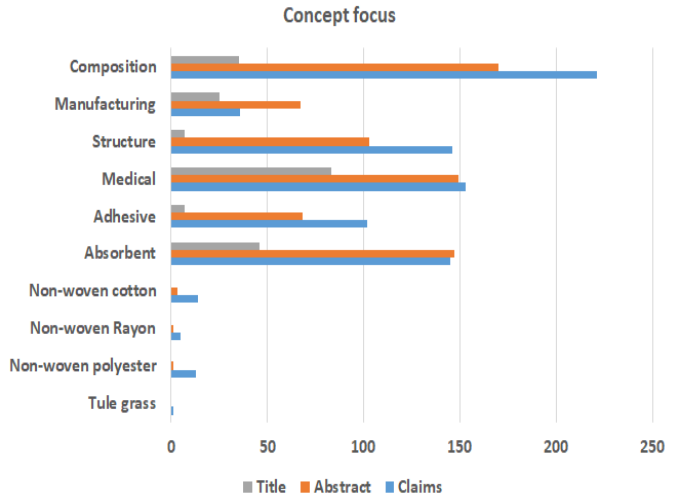

Figure 6: The concept focus when analysing patents related to lint-free technology

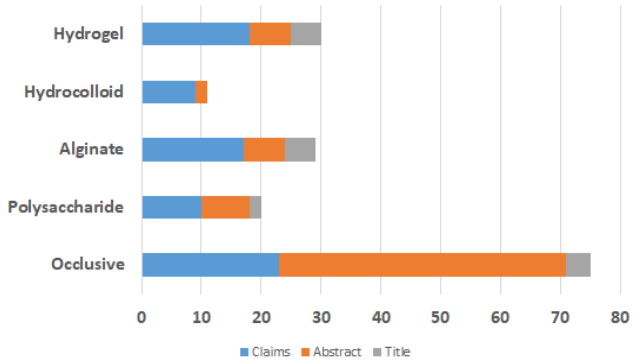

Figure 7: Patent analysis on the type of dressings

dressings generally lack the absorbent properties commonly found in traditional gauze pads. Despite the prevalence of occlusive dressings, there is a growing recognition that in broader wound dressing applications, lint-free technology incorporating an absorbent layer can offer distinct advantages. These dressings, which combine lint-free technology with an absorbent layer, have the potential to provide superior wound care solutions. Such dressings are particularly well-suited for wounds where absorption of exudate (fluid discharge from the wound) is essential for effective healing.

Several patent claims in this field describe dressings that incorporate an absorbent layer with materials such as polysaccharides, alginates, hydrocolloids, and hydrogels. These absorbent materials enhance the dressings' capacity to manage exudate while maintaining a lint-free environment, offering a balance between effective wound care and lint prevention.

When considering the features as a criterion in these patents, a clear pattern emerges where the majority of claims emphasise the soft texture of the dressing as a key attribute. Following this, patents frequently highlight features such as washability, durability, and affordability as important aspects of their innovations. Notably, while 35% of the patents address cost-related issues, it's important to clarify that the primary focus of these patents is not solely centered on reducing costs. Instead, the emphasis is on enhancing the overall quality and functionality of the dressing while ensuring it remains affordable for a wide range of users. This suggests that patent holders are striving to strike a balance between cost-effectiveness and performance excellence (figure 8).

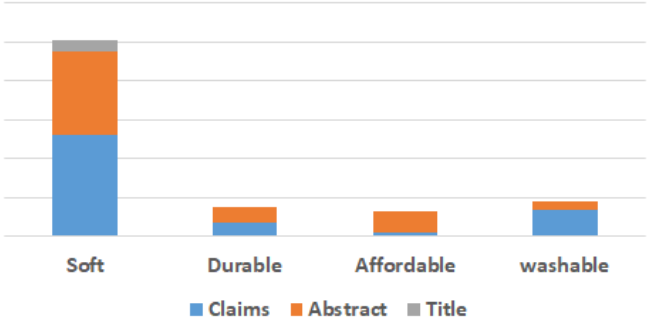

Figure 8: Patent analysis on the Features of lint-free dressings highlighted

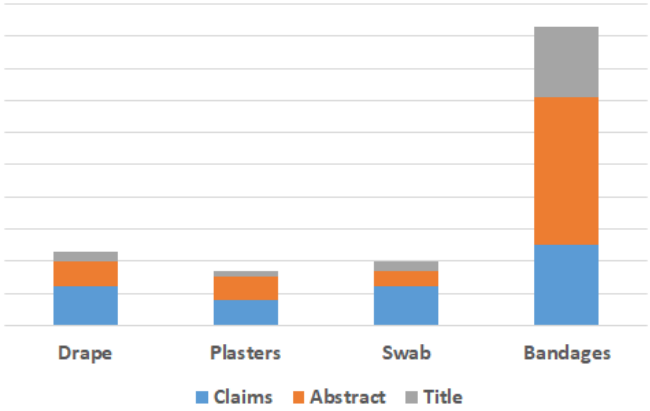

Figure 9: List of the most common product types covered by these patents

As mentioned previously, the most common product types covered by these patents are primarily bandages, followed by drapes, swabs, and plasters. This distribution reflects the industry's focus on developing improved dressing solutions for various medical applications, with bandages being the most prevalent due to their widespread use in wound care.

Challenges in Lint-free Technology and Opportunities for Improvement

Although the field of lint-free technology in wound dressing holds promise for improving patient outcomes, several challenges hinder its widespread acceptance. Major challenges associated with the adoption of lint-free bandages, along with indicative solutions, include:

a) Occlusive Barrier Problem: Occlusive dressings, which aim to create an airtight and waterproof seal over wounds, may pose challenges related to moisture regulation and gas exchange at the wound site. This issue could be addressed by incorporating semi-permeable layers into dressings, allowing controlled airflow while maintaining an occlusive seal.

b) Poor Air Permeability of the Wound: Inadequate air permeability in dressings may hinder the exchange of oxygen and moisture between the wound and the environment, potentially impacting the healing process. Solutions may involve using advanced materials or design elements to enhance air permeability without compromising the integrity of the dressing.

c) High Cost: Cost considerations can limit the accessibility of advanced wound dressings, especially for healthcare systems with budget constraints. Focusing on optimising production processes, materials, or formulations may reduce manufacturing costs without compromising quality.

d) Waterproofing of Wound Surface: Balancing the maintenance of a waterproof barrier with optimal wound care can be challenging, as excessive waterproofing may trap fluids and hinder healing. Innovative designs allowing for selective waterproofing while promoting adequate fluid management could be proposed.

e) Gel Blocking when Superabsorbent Polymer (SAP) Particles Swell: Superabsorbent polymers used in dressings to absorb bodily fluids can form gel-like barriers when they swell. Incorporating advanced materials to prevent gel blocking can ensure consistent absorption capacity.

f) Hardness Problem: The hardness or rigidity of certain dressings can affect patient comfort and conformity to wound contours. Innovations enhancing the flexibility and softness of dressings without compromising their structural integrity can address this issue.

g) Poor Handling/Mechanical Strength/Fitness: Dressings should be easy to handle, maintain their structural integrity, and conform to the wound area without causing discomfort. Modifying the design, material selection, and structural reinforcement can address these challenges.

h) Poor Biocompatibility: Dressings lacking biocompatibility may potentially lead to allergic reactions or irritations. The use of biocompatible materials and coatings can enhance the safety and comfort of the dressing for patients.

i) Relying on Natural Clotting for Non-Blood Fluids: Dressings that rely solely on clotting mechanisms may not effectively manage non-blood fluids, such as exudate. Innovations involving novel materials or structures to enhance the management of various wound fluids can improve overall performance.

j) Poor Liquid Absorbing Capability: Dressings with limited capacity to absorb and manage wound exudate would compromise their function. Innovations involving enhanced absorbent materials or multi-layered structures designed to improve liquid absorption capabilities can overcome this challenge.

Addressing the challenges associated with lint-free technology in wound dressings has led to the development of various patented solutions. Table 2 compiles the patents with indicative solutions for the proposed challenges. These innovations aim to strike a balance between creating effective occlusive barriers, improving biocompatibility, enhancing absorbency, and optimizing cost-effectiveness, ultimately advancing the field of wound care.

Conclusion

The technology landscape of lint-free wound dressings is continually evolving, driven by the ever-changing demands of modern healthcare. The introduction of innovations such as smart dressings, nanotechnology, biodegradable materials, and 3D printing into the lint-free technology sector is poised to propel the industry forward. Moreover, factors such as a growing ageing population, the rise in

Table 2: Lists of patents with indicative solutions for the proposed challenges

Challenges proposed	Patents with indicative solutions
Hardness problem	US3678933A US20060105028A1
Poor handling/mechanical strength/fitness	WO2016135038A1 WO9011719A1 CN101107020A
Poor biocompatibility	DE102006020498A1 WO9011719A1
Rely on Natural Clotting - leaking non-blood fluids	EP2586467B1
Poor liquid absorbing capability	CN2436131Y GB640800A EP344913B1

surgical procedures, and an intensified focus on infection control contribute to the expanding wound dressing market. For healthcare providers and manufacturers alike, staying informed about these trends is not only essential but also pivotal in delivering optimal patient care and maintaining a competitive edge in this dynamic sector. As wound care technology continues to advance, it holds the promise of substantially improving patient outcomes, reducing complications, and enhancing overall quality of life.

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COMMENTARY

Statistical Thinking in Health Science Research: Beyond the Software

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Introduction

'Could you please help me apply some statistics to my research?' Upon hearing such a request, statisticians may smile, but their minds are likely occupied with the question of what the researcher is truly seeking. It is indeed essential to understand the multifaceted meaning of the term Statistics before requesting help to "*apply some Statistics*" in your research.

As a scientific discipline, the term Statistics refers to the science of collecting, organising, analysing and drawing conclusions based on the evaluations of numerical data. However, the term is also used to describe numerical facts or figures. For instance, the infant mortality rate in India was reported as 30 per 1,000 live births for the year 2019. [1] The numerical fact '30 per 1,000 live births' describes infant mortality statistics in 2019 in India.

Let us now return to the various ways in which the term *"apply statistics"* is understood within the context of research. A researcher's specific statistical needs often depend on the stage of the research process. For instance, statistical methods may be required during the planning phase to design the study, develop research instruments, structure the sampling strategy, calculate an appropriate sample size, and manage data collection. Once the data has been collected, additional statistical techniques are typically employed to prepare it for analysis—this includes data cleaning, organisation, and, when necessary, the transformation of variables.

At this stage, researchers need to clearly understand the nature of the *variables* that comprise their statistical data, as this knowledge is fundamental to the effective application of statistical methods. These variables represent the features of the subjects or items measured in the study and form the foundation for meaningful data analysis. Applying statistics during the analysis phase involves selecting appropriate techniques to describe these variables, examine relationships among them, and assess uncertainty when generalising findings in alignment with the research questions. Most importantly, researchers must be able to derive meaningful insights and conclusions from the data—moving beyond the mechanical application of statistical procedures.

Therefore, it is crucial for researchers to identify and communicate their specific needs when consulting with statistical experts. Such communications help make the consultation more efficient and reduce the chance of confusion or misunderstandings when dealing with complex issues.

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Some fundamental concepts that the researcher must know while applying statistics

Why variation matters?

I once received an interesting question from a researcher:
"Which methods will be suitable for analysing data collected from a single individual?"

It was challenging to explain that statistical methods are intended for analysing sets of observations. Its core purpose is to address variability in data and summarise the overall behaviour of measured attributes within a broader context. Statistical analysis becomes irrelevant when there is no variability, especially when data comes from only one sample unit. In contrast, when measurements of an attribute are gathered from several individuals (sample units), statistical methods help describe that attribute and summarise how it behaves across the group.

However, the genuine curiosity behind the researcher's question impressed me, especially when he elaborated his query using a practical example. He described that a patient's blood pressure is monitored regularly during a clinical procedure, and variations in the readings are observed to ensure the patient's well-being. One might even calculate the average blood pressure over time to confirm that everything is under control. Isn't that statistics? Yes, it is! Basic descriptive statistical methods are used here to detect patterns in the data. However, these methods rely on multiple observations from the same patient over time and are used to assess that individual's clinical condition.

If the researcher were to collect similar blood pressure readings from a group of individuals who underwent a similar procedure and wanted to evaluate how blood pressure behaves across the group during the process, broader statistical techniques would be necessary. As noted earlier, statistics would then help describe the overall patterns and variation across the patients, allowing the researcher to draw meaningful conclusions and potentially make inferences.

Seeing the spread: understanding distribution

Measurements of an attribute often differ from one individual to another—hence, we refer to them as *variables*. The distribution of a variable describes how the values from a set of observations are spread out. For example, figure 1 illustrates the distribution of plasma glucose levels based on a state-level survey conducted in Kerala in 2016, which included 12,012 individuals. [2] The histogram shows that most participants had plasma glucose values clustered around 100 mg/dL. However, there were individuals with elevated levels, some exceeding the normal level of 126 mg/dL, and a few with values greater than 200 mg/dL.

The histogram shows the distribution of a numerical measurement, whereas the bar chart shows the distribution of a variable measured categorically. For example, figure 2 illustrates the distribution of the "number of NCD risk factors" reported by the participants. It shows that the highest frequency was observed in participants with two risk factors present, followed by three, and more than 400 participants reported having five or more NCD risk factors.

The population - sample construct and the generalizability of findings

Another concept of paramount importance in statistics is the population-sample construct. Researchers make inferences about observed *random phenomena* related to their study objectives based on a limited sample. More simply, they conduct

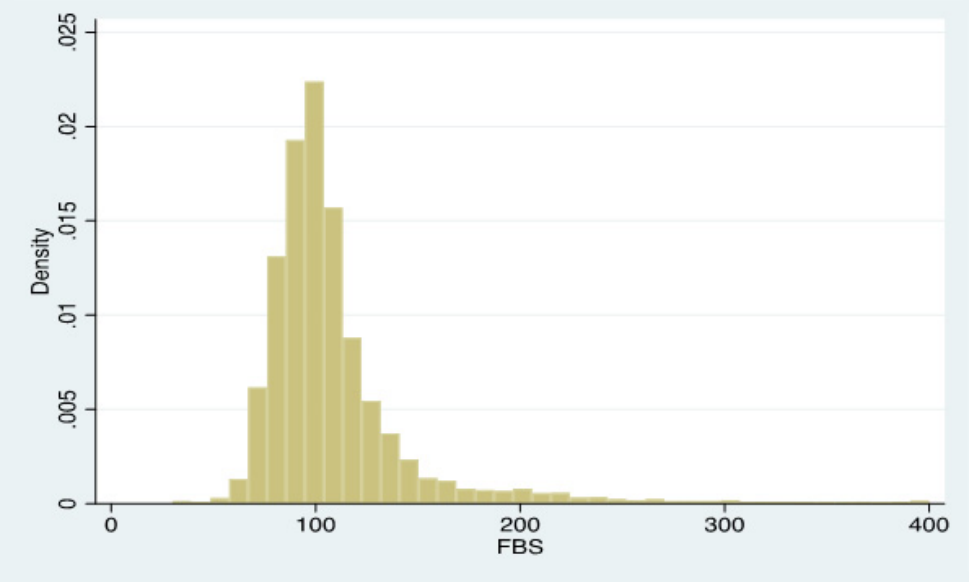

Figure 1: Histogram showing the distribution of Fasting Blood Sugar values (mg/dL) in a state-level survey of 12012 participants

their studies on a relatively small number of units that represent a larger group, called the target population, and generalise the observations from the sample to this broader population using statistical evidence and reasoning.

The key point emphasised here is that researchers use a representative sample from the target population for practical reasons. However, they must also address

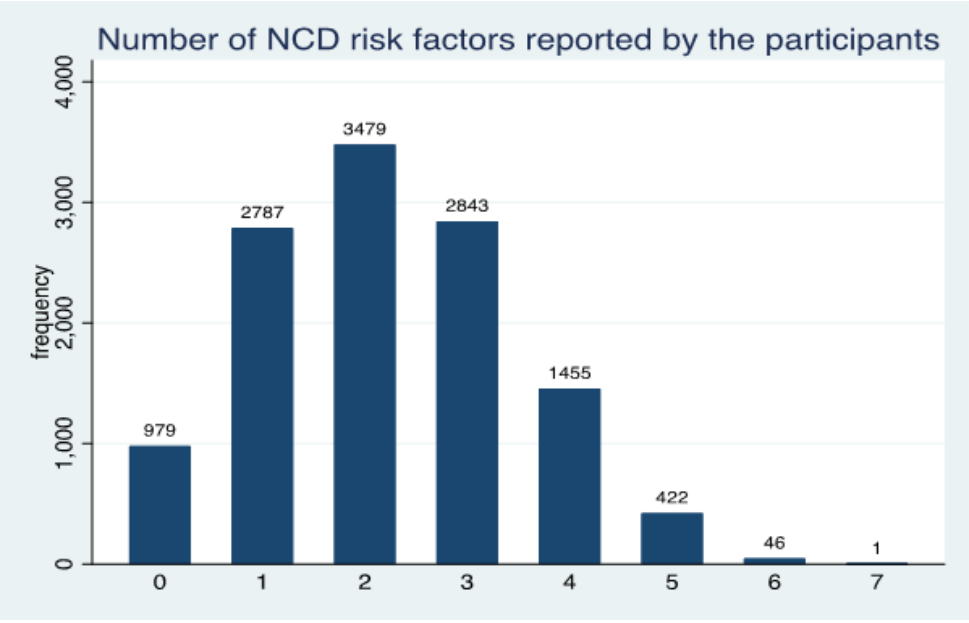

Figure 2: Bar chart showing the distribution of the number of NCD risk factors reported by the study participants in a state-level survey of 12012 participants

a crucial question: ”How reliable are your findings when applied to the target population**?”** The results obtained from the sample need to be generalised to the broader study population, along with an assessment of the statistical evidence supporting that generalisation. This process enables the research community to evaluate the significance of the findings and draw valid, evidence-based conclusions.

Descriptive and inferential statistical methods

Statistics help you understand and describe your data according to your research objectives. The techniques used for this purpose are generally referred to as descriptive statistics. In addition, statistics allows you to evaluate the evidence derived from your sample data to generalise findings to the broader population and draw valid conclusions. The methods used for this process are generally known as inferential or analytical statistics.

To illustrate these concepts, let us consider the fasting blood sugar (FBS) data from the state-wide survey. The objective of the survey was to study the prevalence of noncommunicable disease (NCD) risk factors among the adult population of Kerala. Due to practical limitations with time and resources, it was not feasible to survey the entire population. Therefore, a sample survey was designed to select a representative group from across the state. Following a well-defined sampling strategy, 12,012 individuals were selected from all 14 districts of Kerala [2].

One of the measured NCD risk factors was fasting blood sugar (FBS). The distribution of the observed FBS values is shown in figure 1. Measures of central tendency, such as the mean or median, can be used to describe the average FBS level among participants, serving as representative values for the group. Additionally, including measures of variation (such as standard deviation or range) provides deeper insights into the spread of FBS values in the sample. The FBS values were also classified into three categories, with the frequency and percentage of participants in each group reported. This is known as a frequency distribution, a descriptive statistical method used to summarise and present categorical data (table 1).

Although the sample size for the survey was 12,012, not all individuals had recorded FBS values; approximately 410 values were missing for various reasons. The arithmetic mean of the 11,602 valid observations was 109.3 mg/dL, with an average deviation from the mean (standard deviation) of 39.4 mg/dL. It is worth noting that some researchers mistakenly assign a value of “0” to missing observations. However, this practice leads to biased estimates. When calculating the mean, including zeros artificially inflates the total number of observations (the denominator) without changing the total sum (the numerator) since zero adds no value. For example, suppose the total number of observations is taken as 12,012 instead of the actual 11,602. In that case, the calculated average will be lower than the actual average, misrepresenting the central tendency of the data. Therefore,

Table 1: Descriptive values for the variable FBS

Fasting Blood Sugar (N=11,602)	Mean (SD)	Median (Range)
	109.3 (39.4)	100 (30-400)
FBS category	**Frequency**	**Percentage**
<100	5,684	48.99
100-125	3,932	33.89
>125	1,986	17.12

Table 2: The 95% Confidence Interval for the estimated mean and proportion

FBS	Estimates	95% CI for the estimate
Mean FBS	109.3	108.5-109.9
Proportion of individuals with FBS >=126	17.1%	16.4% - 17.8%

properly handling missing data is crucial for accurate and meaningful statistical analysis.

Let us return to the estimated average FBS value of 109.3 mg/dL from the sample. Can we conclude that the average FBS value in the entire population of Kerala is 109.3? Since we do not have the actual population mean, this sample estimate is our best possible guess. However, some uncertainty always exists; different samples drawn from the same population may yield slightly different averages. Here is where inferential statistics becomes essential. We can estimate a range with a certain surety, a confidence interval, within which the true population mean is likely to lie. This range accounts for the variability between different sample estimates within the population.

Based on our data, the 95% confidence interval for the mean FBS value is 108.55 to 109.98 mg/dL (table 2). This means we are 95% confident that the actual average FBS in the population lies within this interval. The relatively narrow width of the interval, combined with the sample estimate of 109.3 mg/dL, indicates a high level of precision, suggesting that our sample provides a reliable estimate of the true population mean.

The FBS data show that 17.1% of study participants had fasting blood sugar (FBS) values of 126 mg/dL or higher (table 1). The reliability of this sample estimate can be evaluated by calculating a confidence interval for proportions. Although the observed sample proportion is 17.1%, we can infer with 95% confidence that the true proportion of individuals with FBS e”126 mg/dL in the population lies between 16.4% and 17.8% (table 2).

One key point to note here is the large sample size, leading to the narrow width of the confidence intervals. Larger samples tend to produce estimates closer to the true population values, thereby improving the generalizability of the results. However, data quality becomes especially crucial in large-scale studies. Indeed, a large sample has no value in generating reliable estimates if the data quality is compromised.

While confidence intervals are one method of statistical inference, statistical tests are another essential tool used to assess significance, particularly when researchers aim to evaluate relationships or associations observed in sample data. In many cases, both methods are applicable and complementary. However, for generalizing findings with solid statistical evidence, ensuring adequate sample size is a crucial factor. As a result, sample size estimation receives greater attention when preparing the research proposal. Appropriate sample size estimation beforehand would help the researcher balance the scientific, economic and ethical concerns of including participants while doing the research.

Statistical tests and significance - beyond just the p-value

Which statistical test should I use in my Data? Before you ask this question, it is crucial to understand that descriptive statistics is the gateway to the statistical in-

ference methods applied to your data. The researchers often overlook the summary tables or graphs derived from the data, and many fail to recognise the importance of appropriately applying descriptive statistical methods. A data expert can answer your question correctly only with a clear understanding of your research questions, the nature of the variables, and the descriptive results generated from your data.

Another typical question among researchers regarding the type of statistical test is whether they can apply alternate statistical test to obtain a significant p-value, as the applied test did not show statistical significance. The point again emphasised in this context is that exploring alternative statistical tests requires understanding the study objectives and distribution of variables. One should consider whether it is simply because of not getting a significant p-value or is it for a more genuine reason, or whether there is any significance behind the nonsignificant finding meriting further exploration

Here, we should consider diverse issues. First, the significance level or the cut-off for the p-value to decide the statistical significance is an arbitrary value. By convention, we take a cut-off of 0.05 to determine the significance. The actual p-value you get from the statistical test itself will help make a proper interpretation, rather than desperately concluding that your finding has no relevance as your p-value is greater than the conventional cut-off. Suppose the researcher observed some clinically relevant results without statistical significance while considering the conventional cut-off. One reason for the nonsignificant results may be an inadequate sample size to detect the effect significantly. A post-hoc statistical power calculation and justifying the limitation may be an option in this case to support the finding.

When doing research, relationships may not always exist; sometimes, the nonsignificant finding is suggestive of no statistical evidence for an association using your sample data. Researchers should admit this and discuss the possible reasons for such observations from their data, especially when similar studies reported a positive finding but they could not find that in their research. Considering other statistical tests to get significant differences may not be an option when the results are nonsignificant after applying a correct statistical method and when there is no clinical relevance for the observed difference or effect. However, exploring the data in other possible ways to observe patterns, relations, or hidden facts may add to the findings in certain situations. Fabrication or falsification of your data to get statistical significance in this context is undoubtedly unscientific and unethical. It is a serious concern negatively affecting the integrity of the research and the researcher.

Another reason for getting a nonsignificant result may be the inappropriateness of the applied statistical test in that context. For example, when you use an independent t-test in paired data, the p-value will be larger than what you get from a paired t-test if the data are positively correlated, which may result in a nonsignificant result when a significant difference exists between pre- and post-values. If the data are correlated, the variance of the mean difference will be different from that of independent samples, contributing to the test statistic value and resulting in a different p-value. [3] Thus, an independent t-test will fail to detect the significant difference in paired data. Also, sometimes the criteria for a parametric test may need to be revised, and an alternative nonparametric option should be considered if required.

Conclusion

Research is confined to different tasks, and it is essential to include the time re-

quired for statistical analysis when planning the time frame of your research. A focused investigation of data pertaining to the specific objectives is advisable for the researchers to complete their analysis appropriately within the desired time frame. Unfortunately, in certain situations, statistical software is misunderstood as an automated toolbox that magically gives a statistical output when supplied with data. It is often overlooked that statistics is a science by itself, and data analysis is a time-consuming process that requires intellectual, scientific, and logical thinking. The statistical packages only help the researcher to apply the tool to obtain the results. The quality of the research output depends on the quality of the input data, the suitability of the methods used, and the proper presentation and interpretation of the obtained results.

It is worth spending some quality time with your statistical advisor in the initial stages of the study to discuss the aim of your research, study design, and sampling. Such discussions will enable you to plan your analysis well and help you save time and effort, making final data analysis convenient and faster. Hence, seeking advice from a statistician or someone knowledgeable in statistics is a welcoming approach to ensure the study is conducted rigorously and correctly. Statistics has a vital role in scientific research. Transparent and robust statistical analysis strengthens the credibility of research findings and ensures reproducibility. It will contribute to ethical research practices by promoting transparency, accuracy, and accountability in data collection, analysis, and reporting.

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