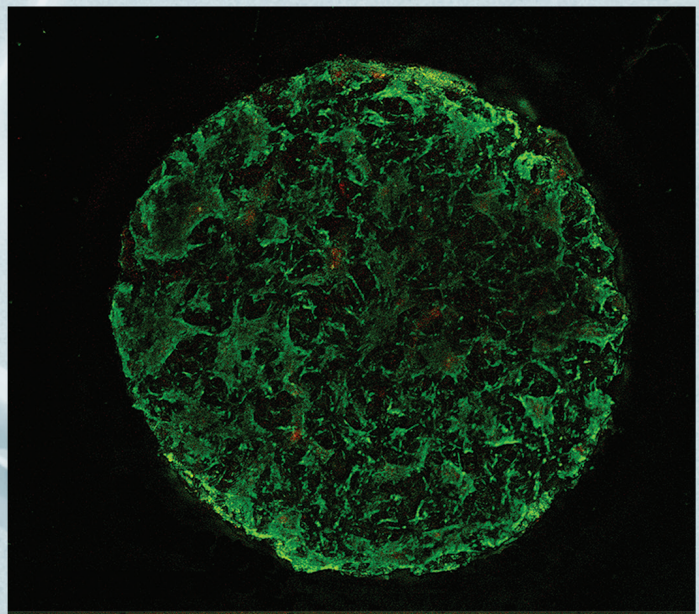
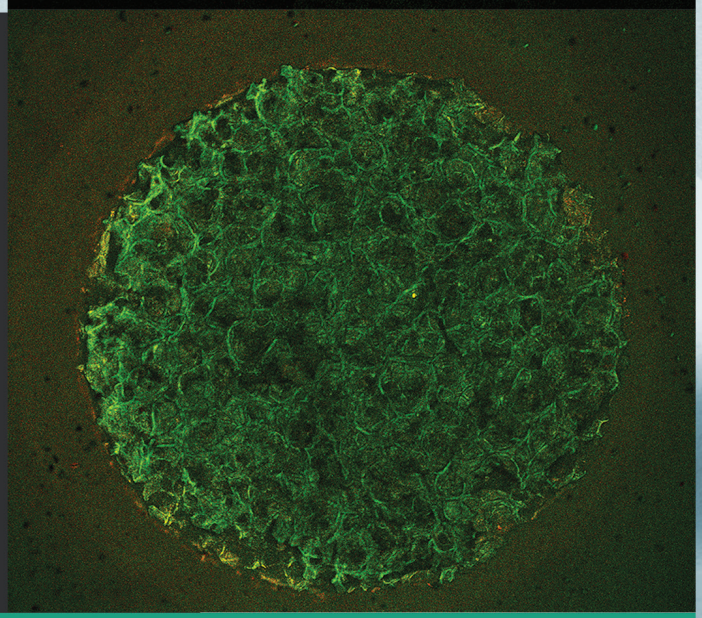




OPINIONS IN

Medical Sciences, Technology and Health

- Robotic Neuro Surgery
- Burn Wound Dressing
- Melatonin Detection

October 15, 2024

Volume 2. Issue 1&2

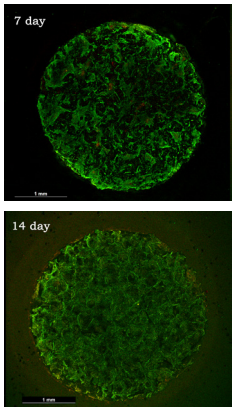
OPINIONS IN Medical Sciences, Technology and Health

Cover Photos

Gelatin vinyl acetate 3D porous scaffold system promoting stem cell adhesion and proliferation

Lynda V Thomas, Division of Tissue Engineerng and regenerative Technologies, BIoMedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

The cover image features Live-Dead imaging of rat adipose-derived mesenchymal stem cells cultured on freeze-dried porous Gelatin-vinyl acetate copolymer scaffolds (GeVAc). These rat adipose derived mesenchymal stem cells were cultured and retrieved at 7 days and 14 days post-culture. For staining the constructs were incubated with calcein AM (4 Mm) to stain the live cells and Ethidium bromide (2 Mm) to stain the dead cells. The constructs were visualised using the Leica Mica Confocal microscope. The image of the 7-day construct shows the mesenchymal stem cells populating the outer surface of the scaffold with minimal cell spreading into the intracellular spaces within the scaffold. The constructs on 14th day showed uniform spreading of cells throughout the scaffold with penetration and growth of cells well into the scaffold's interior with minimal cell death. This study shows that the Gelatin-vinyl acetate copolymer scaffold supports stem cell growth and proliferation and would be an ideal scaffold for tissue engineering applications.



Cover Design and Logo Credit
Aishwarya Nandkumar

Scope

Technology has been breaking boundaries in medical sciences. We here at SC-TIMST were the torchbearers of innovations in medical sciences in India by the amalgamation of 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 neuro-logical 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 commercialisationand public health.

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OMSTH

Contents:

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Editorial

Maya Nandkumar A

The emergence of Artificial intelligence has had far-reaching effects on medicine and healthcare delivery. What was once thought impossible has suddenly become possible.

Globally, Healthcare systems have to grapple with many challenges to achieve the 'four pillars of health for all, which are to improve population health, improve the patient's experience of care, enhance the caregiver experience and reduce the rising cost of care. These pillars serve as the guiding principles for healthcare delivery and are crucial for understanding the context of AI's role in healthcare.

The application of technology and artificial intelligence (AI) is not just a solution to many of these supply-and-demand challenges in healthcare but a promise of improved patient care. It's a reassuring sign for the future of medicine.

The convergence of multimodal data from the omics platform, innovations in mobile technology and the Internet of Things (IoT), and increasing computing power were all forerunners of AI's impact on medicine. AI can diagnose diseases, develop personalised treatment plans, and assist clinicians with decision-making.

US FDA has realised that the future of health care lies in harnessing the great potential of AI and ML (machine learning). It has taken leadership in this arena and approved AI and ML-enabled devices. The devices in this list have met the FDA's applicable premarket requirements, including a focused review of the devices' overall safety and effectiveness. A few of them are: ARVIS® Shoulder from Insight Medical Systems, Inc. for Neurology, SmartChest from Milvue for Radiology, Cardiac CT Function Software Application from Circle Cardiovascular Imaging for Radiology, SIS System from Surgical Information Sciences, Inc. for Radiology, Roche Digital Pathology Dx (VENTANA DP 200) from Ventana Medical Systems, Inc. for Pathology and its applications in tissue engineering, regenerative technologies and robotics in surgery are opening up a wide range of opportunities for implementing personalised medicine and ultimately reducing the undesirable side effects and delivery of health care in its truest sense.

This combined two issues of Opinion in Medical Sciences Technology and Health (OMSTH) focus on some forerunner technologies where AI and ML can make remarkable contributions for maximising benefit of technologies and ironing out the constraints.

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We have a review on robotic neurosurgery. Robotic surgery, in general, is an innovation that extends the surgeons' hands and eyes, enabling them to perform complex surgical maneuvers manoeuvres with precision and flexibility, even in inaccessible regions of the human body regions. Neurosurgery has greatly benefited from this.

As per WHO estimates, there are 180000 deaths every year due to burns. India has a very high incidence of burn injuries, about 6-7 lakhs, and this is the second largest class of accident injuries. The burn injuries are today a tremendous public health concern, with death rate higher than that for malaria and tuberculosis and impacting the life of not only the patient but also the family. Moreover, it is also a primary economic concern. Here, we have a review on collagen, a significant component of healthy skin sourced from marine organisms, which could pave the way for better wound healing and minimal scarring. We also have a translation experience review on a biopolymeric scaffold for burn wound care, which is all set to reach the clinics.

There is a review on the importance of melatonin and the need for detection, specifically in cases of sleep disorders, and future perspectives. AI and ML would greatly benefit these areas of human health. Nanomaterials and nanotechnology have captured the imagination of the medical fraternity with their potential for diagnostics, imaging, and therapeutic applications. Harnessing nanomaterials for various theragnostic applications will become possible in combination with machine learning and artificial intelligence when designing suitable diagnostic devices.

In one of our future issues, we will focus on Artificial Intelligence and Machine Learning.

Robotic Neurosurgery: State-of-art and Future Perspectives

Lokesh Vellore Dasarathan, Sam Scaria, George C Vilanilam*, Gowtham Matam, Ganesh Divakar, Bhushan Akhade, Akhilesh Gouda, Krishnakumar Keshavapisharady

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Abstract

The journey from the current 'driverless cars' to a future predicted world of 'surgeonless surgery' may be long and arduous. As the automation levels of surgical procedures gradually expand, an entirely human surgeon-devoid surgical operation may be a distant yet real possibility. In this narrative review, we explore the fascinating beginnings of robotic surgery and its growth into almost all surgical subspecialties. Precision, indefatigability, ease of access, remote access, fewer adverse events and technical edges make robotic surgery an advantageous prospect and a healthy competitor for traditional surgical techniques. With humble origins in the late 1990s, robotic surgery has evolved rapidly and adapted to several unmet needs of conventional surgical procedures. The Da Vinci robotic system has found applications that have almost become the standard of care in several surgical specialities like gastrointestinal surgery, head and neck surgery, gynaecological surgery, and urology. In neurosurgery, robotics has evolved to assist deep brain biopsies, placement of invasive EEG electrodes, deep brain stimulation, placement of burr holes/twist drill craniotomy for surgical access to the cranium and other applications. The advent of surgical robotics also raises several ethical challenges and medicolegal dilemmas. We explore the broad applications of surgical robotics, its limits and fallacies and also provide a futuristic perspective on the future robotisation potential of routine surgical operations. The robotic explosion is bound to transform surgical outcomes and reduce adverse events, thereby revolutionising traditional surgical techniques and disease cures.

Citation: L V Dasarathan et al. (2024) Robotic Neurosurgery: State-of-art and Future Perspectives, Opn. Med. Sci. Technol. Health, 2024; 2(1&2): e23002

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Introduction

"Men have become the tools of their tools."
Henry David Thoreau

The robotic revolution promises a better quality of human life, and surgical outcomes are not far behind. Enhancing surgical efficiency and elevating procedural outcomes has been the hallmark of all surgical research and training efforts[1,2]. The advent of robotics in surgical sciences has further sharpened the scalpel of surgical efficiency. Better outcomes with lesser human effort have been the selling point of surgical robotisation[3,4]. Automation and indefatigability, miniaturised access, precision, autonomy, reduced adversity, remote access in operator-hostile terrain, etc., are exciting incentives that make robotic surgery an evolving force. The automation potential and levels of human surgeon involvement in surgical robotics are ever changing, with newer frontiers of autonomy being established over time.[1,5]

The Surgical art amidst the Robotic Revolution - History and evolution

A reprogrammable, multifunctional manipulator designed to move material, parts, tools, or specialised devices through various programmed motions to perform various tasks is termed a robot[1,6]. Thus robots are the intelligent connection of perception to action. Karel Capek, a Czech playwright, coined "robota", a Czech word meaning "servant" or "worker". Issac Asimov, in 1942, gave shape to this term in his science fiction works. General Motors used robots for the first time in the industry in the 1960s, a technology they acquired from the inventor George Devol[3].

The Neuromate (approved in 1999) to perform stereotactic brain biopsy was the earliest surgical robot. Next came the Robodoc used in hip prosthesis replacement and AC Robot in knee operations [6]. The da Vinci Surgical System revolutionised robotic surgery and several subspecialties like gastrointestinal surgery, head and neck surgery, gynaecological surgery, urology, etc, in 2000.

Robotics in Surgical Specialities

Automation is the critical edge provided by surgical robots and refers to actions beyond conscious human thought. Dexterity for delicate skilled mechanical tasks is another advantage of robots. The miniature robotic arm can reach less accessible locations in the human body, providing an access advantage. The precision offered is also superior to that of the human surgeon's efforts. Repetitive tasks can be performed tremor-free and without fatigue. In 1983, the first Surgical robot in Orthopaedics came into use. Later on, in 1995, the da Vinci Surgical System (Intuitive Surgical, Sunnyvale, California) revolutionised robotic surgery. It was the first commercial operative surgical robot for general laparoscopic surgery used as early as 1999. Its hallmarks included reliability, intuitive control, 6 degrees of freedom dexterity, grip control, and stereoscopic visualisation. Several surgery specialities like urology, orthopaedics, gynaecology and head/neck surgery have been early adopters of robotic surgery (Fig 3).

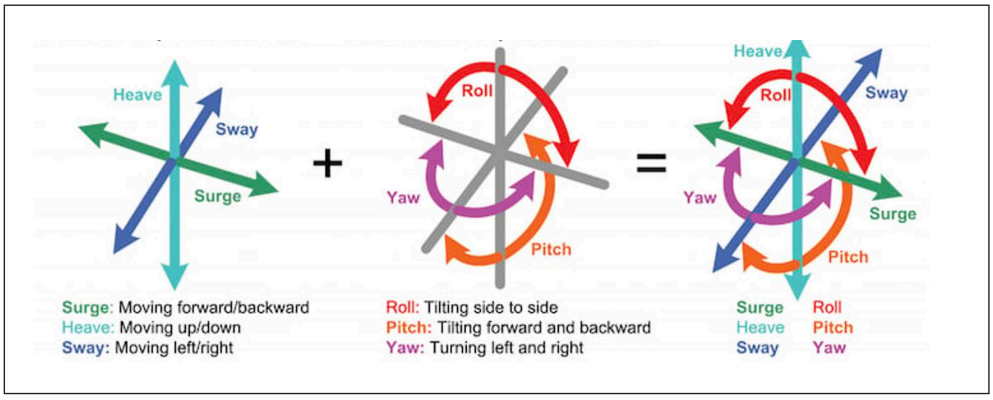

Figure 1 : The degrees of freedom(DOF) for robotic arm motions
A. Translational, B. Rotational, C. Combination movements

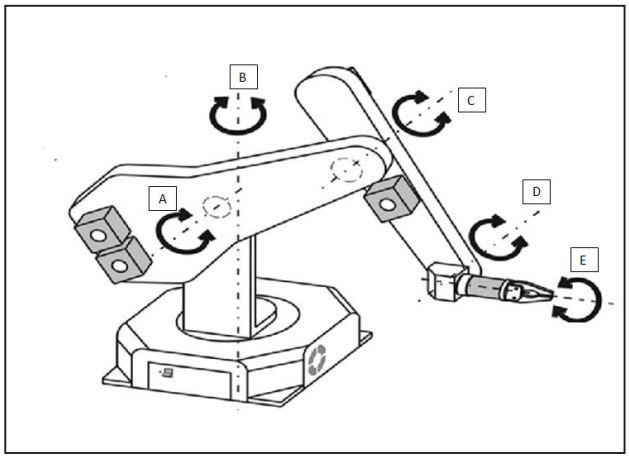

Figure 2 : The Robotic Joints and their degrees of freedom (DOF)
A. Shoulder rotation, B.Waist rotation, C.Elbow rotation, D.Wrist rotation, E. Hand grip rotation

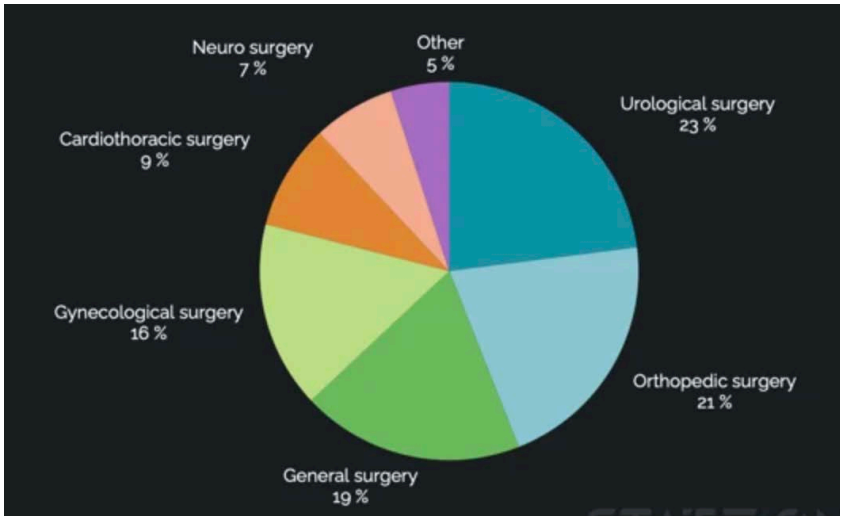

Figure 3 : Current status of Neurosurgical robot usage across Surgical specialities

Neurosurgical robots

Neurosurgery deals with the delicate nervous system and has less room for error and adverse events. So, the adoption of robotic surgery in brain and spine operations has been gradual yet promising. The robotic neurosurgery spectrum has expanded to include skull base endoscopy, depth electrode placement for chronic invasive EEG monitoring, deep brain stimulation, LASER ablations of intrinsic brain lesions, skull base and spinal tumour resections[7-10]. Each of these needs a different level of robotic autonomy, and fully automated procedures have not yet become a reality. Tremor-free surgical dexterity, precision, indefatigability, stereoscopic vision, enhanced visual magnification, miniaturised access in small workspaces and more degrees of freedom for surgical instruments are the edges offered by surgical robotisation (Fig 4-8).

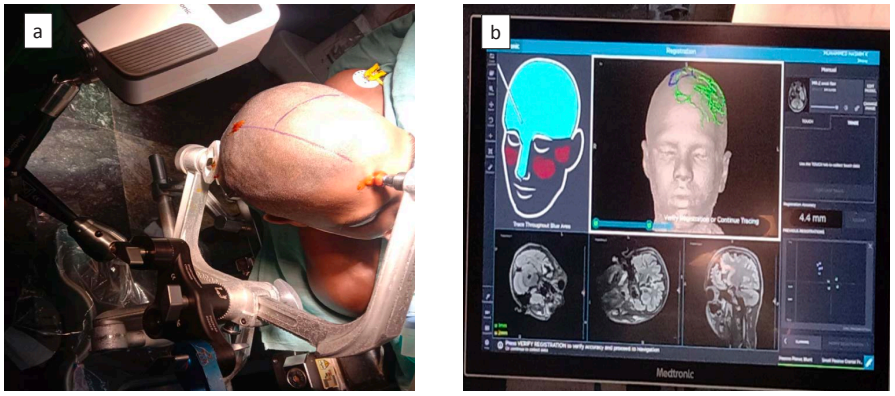

Figure 4 : Robotics in Neurosurgery –For placement of invasive EEG electrodes A. Patient's position and robotic sensor placement, B. Robotic Planning station

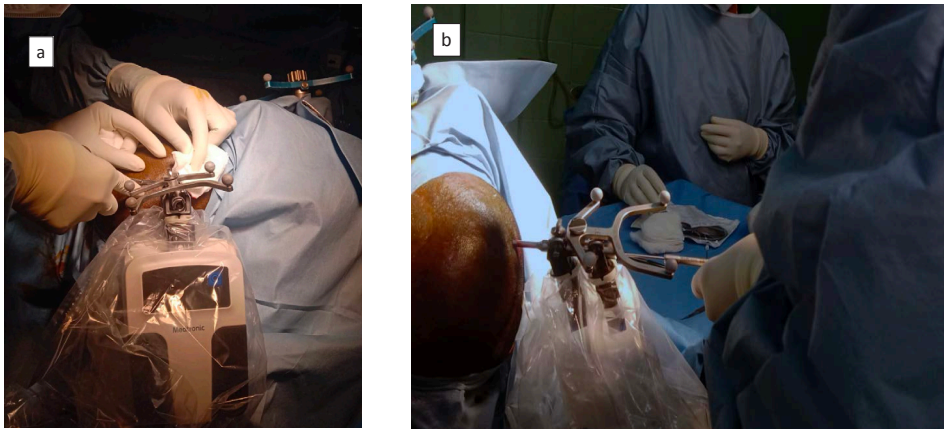

Figure 5 : Robotics in Neurosurgery –Invasive EEG electrodes
A. Cranial navigation and robotics to place invasive EEG electrodes, B. Insertion of invasive EEG depth electrodes

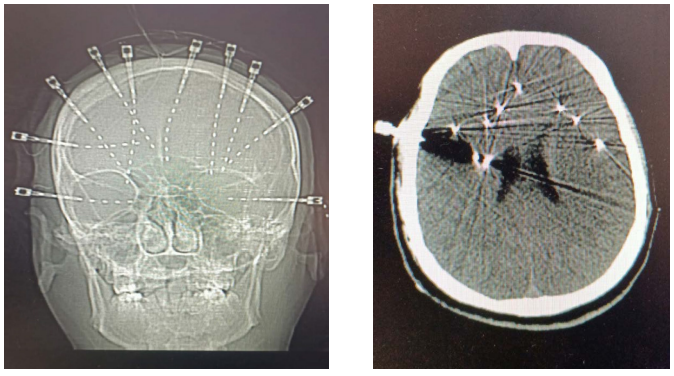

Figure 6 : CT scan post procedure – Invasive EEG robotic assisted electrode placement for MRI negative epilepsy

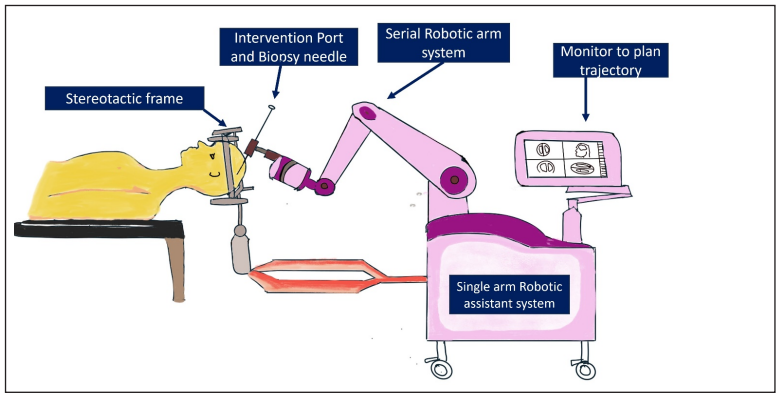

Figure 7 : Schematic diagram showing single arm serial robotic system with inbuilt stereotactic frame (e.g. ROSA – Robotic surgical assistant)

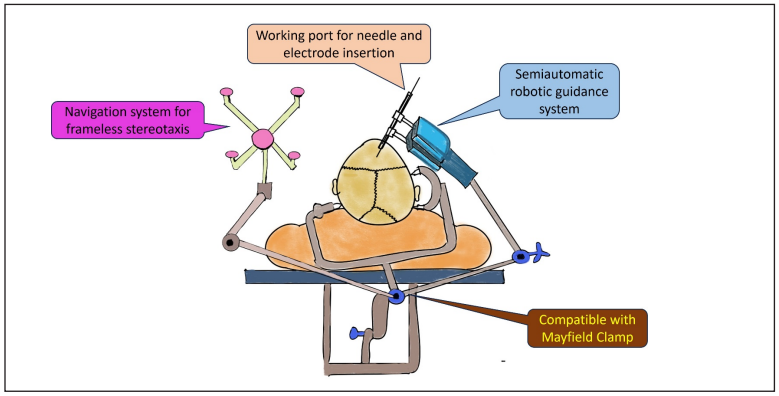

Figure 8 : Schematic diagram showing a semiautomated robotic system with Frameless stereotaxis using navigation systems. Fixed with Mayfield clamp (e.g. Stealth Autoguide, Medtronic)

The complex intricacies of craniospinal surgery and the narrow safety margin make neurosurgery a more difficult prospect for general adoption of robotic skill[11,12]. The human surgical force and its capacity for overrides and corrective checks make the possibility of complete robotic surgical autonomy a tough prospect at present. However, as the complex neurosurgical operation has several sub-tasks, it could be possible to robotise specific steps of the multifaceted operation with certain advantages[9].

Current autonomy status of neurosurgical robots

Robotic systems could be active or passive (master-slave). The active system provides robots with much more autonomy, with the surgeon intervening only when needed. The intermediate form of these two systems is the semi-active system in which the robot offers some assistance to the surgeon(e.g. the Neuromate)[13,14].

The three models include,

1) The supervisory controlled system in which the robot performs actions based on a surgical plan, which is made using computerised axial tomography (CT) scans or magnetic resonance imaging (MRI).
2) The Tele-surgery system uses a real-time haptic system interface where the robot replicates the surgeon's movement from the interface.

3) A shared control system is a symbiotic system where the surgeon has complete control and the robot assists in hand manipulation of the instrument (Table 1).

Table 1 : Levels of Neurosurgical Autonomy in robotisation

Level 0	Level 1	Level 2	Level 3	Level 4	Level 5
No autonomy	Robot assistance	Partial (Task) autonomy	Conditional autonomy	High autonomy	Full autonomy
Everything manually done by the surgeon	Machine assists with a fixed task in one way	Can operate but each system has to be activated by the surgeon	Can operate itself but constant surgeon attention needed	Can operate under specific conditions Surgeon attention not needed,override available	No human surgeon attention or intervention needed
Example Surgeon on a console in the operation room controls the entire surgery done by robotic arms	Example Robotic trajectory planning for placement of depth electrodes	Example Robotic burr hole placement	Example Robot senses surgeon's movement and adjusts position accordingly	Example Futuristic prospect- Fully automated surgical procedure with surgeon available on site for overrides and checks	Example Futuristic prospect- Fully automated surgical procedure with no human presence on site

Uniqueness and Spectrum of Neurosurgical Robots

Neurosurgical operations are far more complex than the mechanical tasks that constitute each surgical procedure. Besides the mechanical movements, there are several intangible and irreproducible aspects that make automated mechanization almost impossible to achieve. The surgeon's experience, anatomical knowledge, terrain familiarity, mayday situational corrections and adaptability to variations, are far more complex for a machine learning force to achieve. Besides this, surgical creativity and ingenuity make neurosurgical operations too complex for autonomous inanimate machines to learn and replicate.

Several levels of autonomous robots have been used in neurosurgery, and the technology and its outcomes continue to evolve. Evolving ethical and medicolegal concerns are also ill-defined and challenging[19-23]. Roboethics, that deals with ethical technological developments preserving human interests and safety, is also a growing concern[24-28].

The da Vinci®/Intuitive Surgical Inc., Sunnyvale, CA and the Zeus®/Computer Motion

Inc., Goleta, CA), Minerva system, the PathFinder robot (Prosurgics/Armstrong Healthcare Ltd. Coleraine, UK) NeuRobot, NeuroArm (University of Calgary, Calgary, Alberta, Canada), Renaissance (MAZOR Robotics, SurgiScope system (Intelligent Surgical Instruments and Systems/ISIS-, Grenoble, France), SpineAssist (Mazor Robotics Ltd., Caesarea, Israel), Neuromate® (Renishaw), ROSA ONE® Brain(Zimmermet), etc are the broad spectrum of neurosurgical robots with varying degrees of autonomy[8,9,11,12] (Table 2).

Table 2 : Neurosurgical robots

Robotic system	Design	Registration system	DOF
PUMA, Unimation, 1985[1]	Industrial serial robotic arm	Frame-based CT	6
Minerva, Lausanne,1993[3]	CT-mounted serial arm	Frame-based CT	5
Neuromate, Renishaw,1997[5]	Mobile serial robotic arm	Frame/frameless CT/MRI	5
Zeiss MKM, Carl Zeiss,2003[6]	Serial surgical microscope	Frame/frameless CT/MRI	6
NeuroMaster, Beijing,2003 [7]	Serial robotic arm	Frameless CT/MRI	5
SurgiScope, ISIS,2003[8]	Ceiling-mounted parallel manipulator	Frameless MRI	7
PathFinder, Prosurgics, 2006[9]	Mobile serial robotic arm	Frameless CT	6
ROSA, Zimmer Biomet, 2012[10]	Serial robotic arm	Frame/frameless CT/MRI	6
Renaissance, Mazor Robotics, 2015[11]	Skull-mounted parallel robot	Frameless CT/MRI	6
iSYS1, Medizintechnik GmbH, 2016[12]	Skull- mounted parallel robot	Frameless CT/MRI	4
Stealth Autoguide (Medtronic),2021[13]	Mayfield clamp-mounted, serial robotic arm	Frameless CT/MRI	4
CorPath GRX Robotic System,2020[14]	Translational and rotational endovascular catheter movements	For coronary Endovascular procedures, still not FDA approved for intracranial use	-
Neuroarm,Calgary,2008[15]	Telesurgery, MRI compatible	Prototype built, commercially not available	7
Da Vinci robotic system, California,2000[16]	Telesurgery	In cadaveric stage	7

Robotic Neurosurgical Interventions-Current Standards of Care in Neurosurgical Subspecialties

This robotic system has been used for transoral odontoidectomy, intrauterine repair of myelomeningocele, and spinal schwannoma [9,11]

The advantages of robotic surgery include high-definition 3-D stereoscopic

visualisation, better control with increased accuracy and reduced tremor, and a high range of instrument motion even in constrained spaces such as deep, narrow corridors. These advantages may help us solve the obstacles frequently encountered during keyhole skull base surgery. The narrow corridor of robotic keyhole surgery provides 7 degrees of freedom and 90 degrees of articulation, which are superior to the 4 degrees of freedom and 0 degrees of articulation of standard keyhole instruments.

Robotic assistances are also incorporated in neuro-endovascular procedures, such as translational and rotational movements of the catheters. CorPath GRX Robotic System (Corindus Inc, Massachusetts) is one of its kind and has been approved for use in cardiac and peripheral interventions. The current robotic systems utilised in endovascular surgery fall into the surgeon-driven mechanism. They consist of a mechanical robot on the patient side and a radiation-shielded operator control station. It allows the operator to control catheters and guidewires via sensors and joysticks. Robotic systems have advantages in endovascular surgeries, such as less human radiation exposure, better performance navigating through complex vascular anatomy, and improved stability of the catheter tip, which reduces the number of movements required.

Although the application of robots in neurovascular intervention is still taking baby steps, encouraging progress has been achieved in integrating this technology into clinical settings.

The CorPath GRX is also widely used in endovascular neurosurgery for procedures such as endovascular coiling and has a strong potential for optimising stroke thrombectomy.

The FDA-approved Magellan Robotic System (Hansen Medical, Auris Health, Redwood, CA, USA) has also shown promise in neurovascular procedures, especially in treating carotid artery stenosis (CAS)[29-31].

Robotics in spine surgery

The field of spinal surgery always needs to be precise and errors are not allowed especially with instrumentation. The freehand technique of pedicle screw placement has long been utilized since its inception, but there is a potential for inaccuracy depending on the surgeon's experience and skill. Malpositioned pedicle screws may have significant clinical and medicolegal implications. It ranges from minor nerve root irritation, inadequate fixation, dural injury and CSF leak, perforation of the viscera, inadvertent injury to major vessels or spinal cord damage. The traditional open method of fixation carries a lot of radiation exposure to health workers and the patient. The most tested and currently available robotic device is, the Mazor robot. It is a miniature bone-mounted robot that has 6 degrees of freedom. A preoperative CT is used to plan trajectories, and intraoperative fluoroscopy is used to register the images. The robot then guides the surgeon to the appropriate trajectory.

The ROSA robot includes a mobile floor-fixed base attached to a robotic arm with 6 degrees of freedom. A second mobile base has a navigation camera mounted to it. The ROSA is an image-guided device using an iliac pin as a reference point. Either intraoperative fluoroscopy or intraoperative CT can be used for planning. Currently, both robots only have applications in placement of screws for spinal instrumentation.

Surgical subtasks and their automation potential

Narrow surgical spaces in neurosurgery create challenges relating to instrument

crowding and uninhibited movement. Robotic arms have overcome the issue of triangulation and movement, but crowding and workspace restrictions still add to the technical challenges. Critical functional neural tissue with a narrow scope for error and disruptive forces make the current spectrum of robotic neurosurgical applications very limited to burr hole placements, depth electrode placements, endoscopy, biopsy and a few others.[8.12]

Degrees of freedom (DoF) allow a robot arm to move anywhere in the surgical workspace(Fig 1). The da Vinci Surgical System, used primarily in other surgical specialities, has four arms, each with seven DoF, controlled by two working arms and a three-dimensional (3-D) stereoscopic view of the field that surgeons can use in a telesurgical manner (Figs. 1 and 2).

Kinematic edge of neurosurgical robotics

Robotic-assisted telesurgery offers an edge in comfort, accuracy, stamina, and dexterity. Motion amplification and filtering can be included in robotic-assisted minimally invasive surgery. As micro-neurosurgery involves accurate small incisions, deep vascular dissections and resections, motion filtering removes hand tremors from the surgeon by accurate programming, allowing the surgeon to make smaller resections with larger applied motions. Safety and outcomes are thus greatly improved.

Meaningful human control of surgical robot autonomy.

Meaningful human control (MHC) refers to the state where the limitations and safety risks posed by autonomous robotic surgery are overcome by overrides and corrections by the human surgeon. Complex surgical subtasks with high stakes attached would be the last to be completely automated and would retain an element of meaningful human control.[9-12]

Suturing is the most significant surgical task to be automated due to the difficulty of carrying it out using telerobotic systems without force feedback. Hence, a considerable amount of research work is currently devoted to autonomous suturing. A single camera is combined with an elliptical position measurement algorithm to find the needle, while simple markers are used to find the suturing points[6,8,9].

Limitations in Robotic Competencies

Surgical creativity, innovation, adaptability to pathological variations, and corrective responses to adverse situations are all competencies that are a challenging prospect for a robotic force[5,8,9]. The absence of adequate force feedback mechanisms makes robotic neurosurgery a problematic challenge. Tactile, proprioceptive and haptic mechanisms are essential for robotic neurosurgery innovations. Tactile feedback may be provided by effectors/robotic arms using thermal, vibratory, mechanical, light, and biochemical sensors so that a characteristic signature for each tissue penetrated is recognised in real-time by the probe and transmitted to the mainframe computer, which, in turn, assists the surgeon to shape the appropriate response. Future developments in force feed mechanisms with haptic interfaces may realise the full potential of robotics in neurosurgery.

Future directions

Integrating newer technologies with surgical robotics, such as informational

technology, neuronavigation, telemedicine, nanotechnology, micromachines, microelectromechanical systems, and sophisticated computational networking, will expand robotic neurosurgical frontiers.

Conclusion

Autonomous surgical robots could replace human surgical efforts, offering significant additional advantages. Precision, indefatigability, miniatured access, and overall better outcomes with surgical robots may redefine standards of surgical care and enhance disease-cure outcomes.

Abbreviations/Acronyms: LASER Light amplification by stimulated emission of radiation CT computerised axial tomography MRI magnetic resonance imaging DoF-Degree of freedom MHC-Meaningful human control

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Oceanic Healing: Harnessing Marine-Derived Collagen for Advanced Burn Wound Therapy and Skin Tissue Engineering

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Abstract

Burn injuries represent a significant challenge in medical treatment, often resulting in extensive damage to skin tissue and requiring sophisticated approaches for effective regeneration. In recent years, skin tissue engineering has emerged as a promising avenue for addressing this challenge, offering innovative solutions to promote wound healing and skin regeneration. Among the various biomaterials investigated, marine collagen has garnered attention for its unique properties conducive to tissue repair and regeneration. This article highlights the advanced treatment strategies in burn management incorporating marine collagen as a key component in tissue engineering. Marine collagen, derived from sustainable marine sources, exhibits excellent biocompatibility, biodegradability, and low immunogenicity, making it an ideal candidate for skin regeneration.

Moreover, marine collagen resembles human collagen, facilitating seamless integration into the damaged tissue and promoting natural healing processes. Therefore, using sophisticated tissue engineering techniques, such as scaffold-based approaches and cell therapy, marine collagen can be made into a scaffold or matrix to promote cell proliferation, differentiation, and tissue remodelling. Furthermore, incorporating bioactive molecules and growth factors enhances its regenerative potential, accelerating wound closure and minimising scar formation. In conclusion, utilising marine collagen in advanced burn management holds great promise for improving the outcomes in skin tissue engineering by promoting efficient wound healing and facilitating the regeneration of functional skin tissue.

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Introduction

In India, tackling the management of burn wounds presents a formidable challenge, with an estimated 265,000 annual burn-related fatalities and a staggering 7 million individuals suffering from burn injuries each year (Burns, National Health Portal of India -2019). Burns stand as a pressing public health concern, precipitating injuries to skin, tissues, and organs, and emerge as prominent contributors to both morbidity and mortality rates. Within the realm of burn management, complete recovery is obstructed due to diminished self-renewal capacity of the injured skin, heightened susceptibility to infections, and the desiccation of the wound environment. Effective regeneration of damaged tissues and organs is a pivotal pursuit in contemporary medicine. Addressing these formidable medical challenges necessitates the exploration of efficacious remedies for burn treatment, wherein Tissue Engineering emerges as a promising avenue within the realm of Regenerative Medicine. This review provides an overview of the normal structure and functions of skin and the impact of burns on skin integrity. It also highlights current strategies in tissue engineering aimed at managing burn injuries.

Skin Structure and Functions

The skin, the body's largest organ, consists of multiple layers of cells and tissues. Its architecture comprises epidermal cells and connective tissue, functioning synergistically to carry out essential tasks. This multi-layered structure comprises the epidermis, dermis, and hypodermis (Fig.1).

Epidermis:

The outermost skin layer is made up of a stratified, squamous epithelium consisting of keratinocytes and dendritic cells. Together with appendages such as hair, nails, and derivative structures like pilosebaceous follicles, sweat glands, and pores, they form the physical components of the skin, ensuring a waterproof barrier and maintaining skin integrity. The cells present in the epidermal layer are keratinocytes, corneocytes, basal cells, melanocytes, Langerhans cells and Merkel cells. The Epidermis is further subdivided into five layers including; Stratum corneum, Stratum lucidum, Stratum granulosum, Stratum spinosum and Stratum basale.

Dermis:

This thick layer, situated beneath the epidermal layer, is characterised as a robust, fibrous connective tissue. It forms an intricate network primarily composed of two proteins, elastin and collagen fibres. It also contains various structures such as hair follicles, sweat glands, a vascular network, epidermis-derived appendages, fibroblasts, mast cells, and macrophages. Serving multiple functions, this cellular layer contributes to the skin's pliability, elasticity, and tensile strength, safeguarding against mechanical injuries while regulating thermal conditions and sensory stimuli. Cells present in the dermal layer are Fibroblasts, macrophages and mast cells. The dermis is subdivided into two layers, the papillary dermis and reticular dermis.

Hypodermis:

Below the dermis lies this subcutaneous layer, comprised of adipose and connective tissue with the underlying bone and muscle sheath. Enriched with vascularised areolar tissue, this abundant adipose tissue functions to store fat and provides insulation for the body.

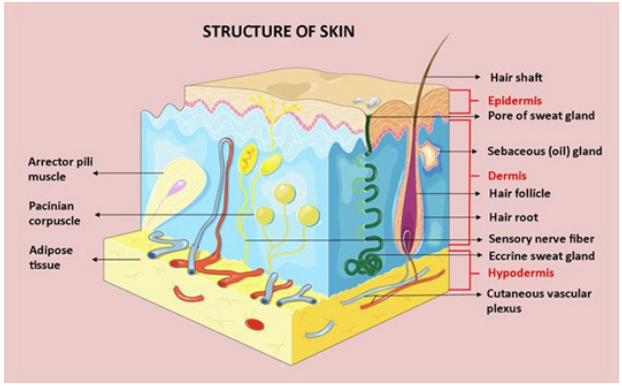

Figure 1: Structure of human skin

Skin wound and classification

The wound is an injury caused by disruption or a break in the skin structure. It is possible to have open wounds with exposed body tissue and broken skin or closed wounds with damaged tissue under intact skin. Wounds are classified based on several factors according to their site, degree of tissue damage or loss, type of injury, aetiology, wound depth, healing time, the integrity of the skin, morphological characteristics and degree of contamination and severity of the wound. Depending on the healing time, the wound is mainly classified as acute or chronic; based on the degree of tissue loss, general wounds may be classified as superficial, partial, and full thickness. According to the level of contamination, a wound can be classified as a clean wound, contaminated wound, infected wound, colonised wound, etc. Usually different types of wounds are generally described using different terminologies, such as surgical incisions, burns, lacerations, ulcers, and abrasions. They can generally be included in the category of acute or chronic wounds (Andreassi et al., 2005).

- **Acute and chronic wounds**

An acute wound is an injury or damage to the skin, and it is healed by the predictable and expected rate of progress through the phases of normal healing and leads to the complete closure of the wound. It takes few weeks to heal the wound completely. It includes rapid recovery, normal immunological response and exhibits rare colonization in the case of infections which may occasionally occur. Some examples include abrasions, animal bites, scratches, superficial burns, and surgical wounds.

In contrast, chronic or non-healing wounds are slow, with uncoordinated healing processes and show no predictable development towards healing within several months. They have persistent inflammation, impaired angiogenesis and primary infection with prolonged and disordered healing. It has an abnormal immunological response, with common colonization occurring during the time of the healing process. Diabetes-related foot ulcers, venous ulcers, pressure ulcers, vascular lower limb ulcers, arterial ulcers, and infected wounds are examples of chronic wounds (McGuckin et al., 2003).

Burn wounds: Burns are one of the most common household injuries characterized by severe skin damage and caused by heat, overexposure to the sun or other radiation, chemicals, and electrical contact, and most burn injuries happen by accidents (Fig. 2). Most burns are in the category of thermal burns by scalding hot liquids or hot solids or flames. Most flame burns result in partial or total destruction of skin and tissue cells. Of these, 55% are caused by scalds, 40% by electrical burns, and 5% by chemical burns (Evers et al., 2010).

Many factors determine the severity of burn injuries; the major one is the degree or depth of the burn, and the others are the percentage of the burned skin area, the age of the injured persons, location, type, etc. According to the degree of severity and penetration of the skin's surface, burns are classified as first-degree, second-degree, and third-degree burns.

First-degree burns:

Commonly termed superficial burns, they cause minimal skin damage. They mainly affect the outermost layer of skin, the epidermis. They may cause redness with pain,

and the main characteristics are erythema (pink to red), without blisters, skin blanches, and tingling; pain responds well to cooling and lasts about 48 hours, healing in 3-7 days (Granger et al., 2009).

Second-degree burns:

Superficial partial thickness or second-degree burns affect both the epidermis and dermis. They may cause swelling with red, white, or splotchy skin, oedema, large blisters, and severe pain with red-based broken epidermis. They can also cause scarring and require prolonged healing over 2-3 weeks. In this condition, a graft may be needed to repair and restore the entire structure of the skin (Habif et al., 2017).

Third-degree burns:

Severe skin damage reaches the fat layer beneath the skin; the burnt area appears black/brown or white. Almost all tissues are destroyed in the affected area, with disruption of nerves leading to numbness and oedema. Scarring and contractures are present in this condition; tissues are open, and fat is exposed. There is little to no pain, and it takes weeks to months to heal the wound in an unfinished/incomplete manner. Hence, it is termed as full-thickness burn (Venter et al., 2015).

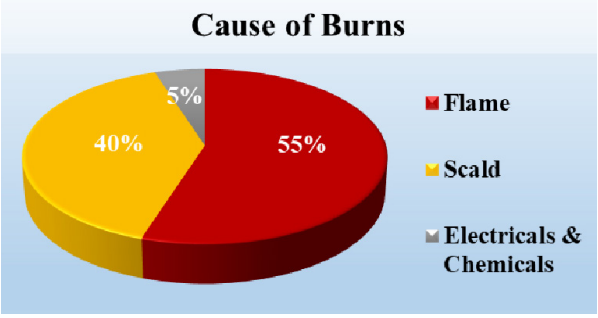

Figure 2: Causes of burn injury

Burn wound management

The management of burn wounds is a major challenge around the world. Emergency treatment and medical care are very important in wound management, especially in reducing the risk of severity and infection. The current trend is a very holistic approach where the ideal conditions of natural healing are promoted so that there is timely healing and restoration of skin function, leading to the maintenance of the quality of life. In such a scenario, the choice of wound care and selection of wound dressing for burn injury is very crucial, and it is based only on the size and depth of the burn or depends on the degree of burnt area. In the case of local wound care management, cleansing and debridement are routine procedures for treating burn wounds, and wound dressings are usually changed regularly, generally incorporating topical antimicrobial agents. Hence, the optimal dressing or agent may help prevent or control infection or enhance wound healing (Wasiak et al., 2013; Norman et al., 2017). Immediate topical application of medical agents can help reduce rapid colonisation of bacteria in the wound site by protecting the wound surface, maintaining moisture, and stimulating burn healing. As part of local treatment, cleansing and debridement are routine procedures for treating burn wounds, and wound dressings are usually changed regularly, generally incorporating topical antimicrobial agents. Hence, the optimal dressing or agent helps to prevent or control infection or enhance wound healing. Regarding chronic burn wound

management, the burn victim being resuscitated and stabilised is the first step in restoring anatomy, preserving function, and rehabilitating the patient with temporary burn wound coverage (Tenehaus et al., 2018).

- **Burn wound dressings- current status**

Different types of wound care agents and dressings are currently available to enhance healing efficacy and reduce infections. The major classes are synthetic and biologic wound dressings.

Local burn wound care : Synthetic dressings are the major solution for the management of local burn wound care. Different types of gauze and non-adherent films, combined with/without topical antimicrobial agents, are used as wound dressings in the current scenario to manage local burn wounds. Films, foams, alginates, silicones, hydrocolloids, and hydrogels are commonly used solutions for burn management.

Antimicrobial agents : The most commonly used topical antimicrobial agents are silver-containing agents such as silver sulfadiazine, nanocrystalline silver and silver nitrate. Silver ions-containing agents have broad spectrum of antibacterial activity and anti-inflammatory properties. Hence, it helps to reduce infection and inflammation (Chaloupka et al., 2010). Bismuth-impregnated petroleum gauze is usually used for minor superficial partial-thickness burns and is applied as a single layer over the burn site. After epithelisation, the dressing will separate from the wound and help to decrease the pain (Malpass et al., 2003). Mafenide acetate is used for patients with dense bacterial proliferation in burn wounds. It may benefit from this ointment, a potent carbonic anhydrase inhibitor and an excellent alternative to Silver sulfadiazine (SSD) (Ahuja et al., 2009). Chlorhexidine is another topical agent mainly used for superficial partial-thickness burns and is a long-lasting antimicrobial skin cleanser. Others like honey, Povidone-iodine and Dakin's solution have broad-spectrum antimicrobial activity and healing efficacy.

Temporary Burn Wound Coverage : Skin substitutes were a major discovery which assisted in addressing burn wound management to ensure maximal benefit to the patient so that there would be minimum pain and restoration of skin functionality. Skin substitutes are of two major types: acellular and cellular. In the acellular category in the class of biologic grafts, semi-biologic skin substitutes are biobrane and biobrane-like grafts. These major class of biological graft materials used as temporary coverage of burn wounds help to induce accelerated healing of burns and other wounds, reducing scar contracture and pain. The other type of skin substitutes are allografts, xenografts, and others (human amnion). These skin substitutes are considered dermal templates to improve wound healing with permanent wound coverage solutions. Different types of skin substitutes are biologic, synthetic, or biosynthetic materials that provide temporary or permanent solutions for burn treatments. Using semi-biologic skin substitutes can reduce the repeated changing of wound dressing and enhance the healing efficiency (Ahuja et al., 2009). Biobrane and Biobrane-like grafts are another type where a large surface area of burn can be covered, such as for hands, feet, and joint burnt areas. Biobrane is made up of the combination of a thin semipermeable silicone membrane with a layer of type 1 porcine collagen on a woven nylon fabric having a mesh-like appearance (Lang et al., 2005; Whitaker et al., 2008). Some examples of Biobrane dressings are Transcyte (neonatal fibroblast incorporated into Biobrane), Suprathel (resorbable caprolactone-based materials), and Omiderm (hydrophilized polyurethane membrane) etc.

Cascade of burn wound healing mechanism

Burn wound management is a complex cascade with related events of haemostasis, inflammation, cell proliferation, and matrix remodelling. Chronic burn wounds can be classified based on the characteristics of the burnt area, like pain, colour, capillary refill etc. Superficial partial-thickness (SPT)burn is a painful burn injury with a reddish colour. It affects the epidermal layer and papillary dermis layer. Deep partial-thickness (DPT)burn affects the reticular dermis layer. Finally, full-thickness (FT)burn is affects epidermis, dermis and even the subcutaneous adipose tissues, bones and muscles and also the nerves (Stone II et al., 2018).

Phases of burn wound healing

Haemostasis: This is the first cellular response of the burnt area through red blood cells and platelets to initiate the coagulation process, endothelial and fibroblast cell migration, macrophage activation, and provisional matrix production.

Inflammation: The second response is inflammation through neutrophils, basophils, eosinophils, dendritic cells, Langerhans cells, natural killer cells, T and B cells. Macrophages and mast cells initiate homeostasis, vasodilation, innate immune response etc.

Cell proliferation: Active cell proliferation cascade occurs through stem cells, hematopoietic cells, endothelial progenitor cells, embryonic-like stem cells, keratinocytes, melanocytes, endothelial cells, fibroblasts, and adipocytes. These cascades promote inflammation coagulation, vasculogenesis, and generation of epithelial cells (keratinocytes and melanocytes), hair follicles, and sweat glands. It also supports the re-epithelialization of burnt tissues.

Matrix remodelling: This process involves re-vascularization, re-epithelialization and collagen re-organization of the damaged burnt area. (Stone II et al., 2018)

Wound healing signaling-carrier molecules

Burn wound healing is generally regulated through the following steps like haemostasis, inflammation, cell proliferation, and matrix remodeling, which involves complex biochemical and cellular mechanisms (Boateng et al., 2008). During the healing process, the activation of the inflammation process, proliferation, angiogenesis, cell differentiation, and migration occurs through different molecular signalling and carrier molecules. Major signalling molecules involved in the activation process are RAS/MAP Kinase, PI3Kinase pathway, JAK-STAT signalling, JNK and AKT, SMAD etc., with different receptors including FGF, EGF, TGF-β, JAK-STAT. They augment cellular activities through a regulated cascade of inhibitor or activator events.

Properties of ideal wound dressing

An ideal wound dressing, as emphasized by research (Domb et al., 2014; Rezvani et al., 2019), should possess specific characteristics. It should effectively minimize drainage and fluid loss while ensuring the wounded area remains adequately moist. Furthermore, the dressing should facilitate the exchange of gases such as oxygen, carbon dioxide, and water vapor at the wound site. Additionally, it ought to mitigate bacterial colonization and other microbial flora to safeguard against severe infections. The dressing's functionality

should extend to reducing wound surface progression and necrosis while maintaining an optimal moisture level. Moreover, it must be biocompatible, biodegradable, non-toxic, and cost-effective. In addition to these attributes, an ideal wound dressing should alleviate pain and minimize scar formation. Lastly, it should offer mechanical support and be easily replaceable or interchangeable when needed.

Tissue Engineering

Tissue Engineering is a new arena of Regenerative Medicine. This field explores different technologies to improve or replace biological parts using the combination of cells, biomaterials and biological augmenting molecules. This multidisciplinary field aims to configure a functional platform to adhere, restore, retain and recover the damaged areas into a complete functional structure. A new part or a whole organ can be constructed through tissue-engineered strategies that isolate cells, influence growth factors, and place cells on or within matrices. Finding a platform for the proper growth of functional tissues or organs as biological substitutes is necessary.

The triad of Tissue engineering constitutes cells, scaffolds and growth-stimulating signalling molecules. A scaffold is the tissue engineering platform or structural support that provides proper attachment of cells for further development. It helps in the subsequent adherence of cells and enhances the appropriate interaction and communication of cells to form a new structure (Chan et al., 2008). Skin scaffolds are usually made of polymeric biomaterials - either natural or synthetic, which support cellular activities and simultaneously act as delivery vehicles for cells and designed drugs in tissue regeneration. Cell adhesion and proliferation on a scaffold depends upon the properties of the specified scaffold, such as size, shape, nature of the material, thickness, biochemical-physicochemical properties, surface morphology, porous nature, degradation rate, water adsorption capacity and mechanical strength etc. (Sanz-Herrera et al., 2009; Foong CY. et al., 2017). All these parameters influence the proper healing and successful regeneration of tissues on scaffolds. Growth signalling molecules like growth factors and other active signalling molecules play a significant role in the complete development of cells to form tissues on scaffolds. It enhances the developmental cascade, instructs the cellular rearrangement and stimulates cell proliferation in a well-ordered manner. Typically, tissue repair is a cascade of cellular and molecular events controlled by an extensive choice of cytokines, proteins, and growth factors. Accordingly, the wide range of synthetic growth factors is considered as remedial molecules for the repair and regeneration of tissues. Given this use, it is necessary to produce growth factors on a large scale in the therapeutic industry. But their use is limited by the high cost of production. Interestingly, plant-derived molecules or phytochemicals used in traditional Ayurvedic medicine are alternate solutions for the expensive synthetic growth factors in the current global scenario (Sivamani et al., 2012; Joseph et al., 2018).

• Skin Tissue Engineering

The field of skin tissue engineering specifies the remodelling and restoration of skin structure through the modification and regeneration of skin layers such as the dermis, epidermis and skin appendages - hair, nail, and glands. Skin remodelling encompasses various strategies, including developing skin substitutes, creating in vivo skin models, and the fabrication of biomaterials, among other techniques. Skin is the largest organ of the body, comprising about 15% of the total adult body weight. The primary role of the skin is to act as a barrier against the environment. It restricts the invading organisms,

regulates the homeostasis of the body through blood vessels and sweat glands, regulates the dehydration mechanisms and permits the sensation of touch, heat, and cold. In the current scenario, complete restoration of damaged skin is unattainable due to the lack of proper natural enhancements in the development of sebaceous glands, hair follicles, sweat glands and skin layers (Somuncu et al., 2018). Fortunately, over the last couple of years, numerous researchers have been exploring and progressing in developing an appropriate skin substitute. As a result, by exploiting various biomaterials, either of natural or artificial origin, advanced technologies or approaches have been evolving and expanding the field of tissue engineering.

Natural biomaterials as scaffolds

Scaffolds support cell growth and tissue regeneration. They are highly porous materials that help the migration of cells and guide the growth of cells. Different synthetic or natural biomaterials act as scaffolds for tissue regeneration. Over the last few decades, varieties of synthetically and naturally derived biomaterial scaffolds have been established in the field of tissue engineering research. Naturally derived biomaterials are generally categorized into two groups: a) Protein-based natural biomaterials and b) Polysaccharide-based natural biomaterials (Barua et al., 2018).

- **Protein-based natural biomaterials**

Silk, keratin, collagen, fibrin, gelatin, elastin etc. are grouped under protein-based natural biomaterials. Proteins' structural composition, intrinsic strength and elastic properties are due to their composition and combination of multiple tandem repeats of short amino acid sequences (Abascal et al., 2018). This consists of proteins and amino acids to enhance the growth of tissues and provide structural support.

- **Polysaccharide-based natural biomaterials**

Chitosan, alginate, hyaluronan, cellulose, dextran, pullulan, agarose, chondroitin sulfate, etc., are classified under the polysaccharide-based natural biomaterials for potential tissue development in the field of tissue engineering. These find wide applications in different aspects of tissue engineering because of their non-toxic nature, biocompatibility, biodegradability, and cytocompatibility without eliciting an immune response (Bharadwaz et al., 2020). These polysaccharides can easily be derived from sources such as animals, plants, and microorganisms and hence possess better biocompatibility and bioactivity than their synthetic polymer counterpart. These natural biomaterials offer additional advantages like an abundance of natural sources, ease of isolation, chances of structural modifications etc (Tekale et al., 2022).

Marine-Derived collagen biomaterials for burn wounds

Collagen is the main structural protein that appeared as an elongated form of fibril composed of three amino acids connected to form a triple helix structure, commonly called a collagen helix (Fig. 4). Collagen is an insoluble fibrous protein widely distributed in the extracellular matrix of humans and other vertebrate species. It contains 30% of total proteins, a major member of natural polymers and mostly in connective tissues. Collagen helix is made up of two identical chains (α1) and an additional chain that differs slightly in its chemical composition (α2). Combination of amino acid sequence glycine-proline-X and glycine-X-hydroxyproline, where X is any amino acid other than glycine, proline or hydroxyproline with a repeating pattern of sequence is the existing common motif of

collagen structure (Brodsky et al., 2005) (Fig.3). There are 28 different types of collagen found in vertebrates, each consisting of at least 46 different polypeptide chains named as type I to XXVIII. Collagenous domains are found in many other proteins as well. Type I collagen is the most abundant collagen in the human body, especially present in tendons, skin, artery walls, cornea, fibrocartilage, bones and teeth. It is actively involved in tissue repair and healing and is also observed in scar tissue after the healing process.

Marine-derived collagen is a kind of biodegradable, biocompatible protein material sourced mainly from scales, fins, bones and skin of fish. A few fish species used for the purpose are listed in Table 1. Collagen as a biomaterial is widely used in tissue engineering applications due to its low antigenicity and high content of essential and non-essential amino acids that maintain skin elasticity. Researchers have been exploring the concept of collagen as biomaterials because of its availability, cost-effectiveness, and low extraction cost with its favourable physicochemical properties. The major applications of collagen are in the construction of artificial skin substitutes used to manage severe burns and wounds (Singh et al., 2011; Gould et al., 2016). Simultaneously, collagen is also used in reconstructive neuro-plasticity, oral, peripheral nerve regeneration(Han et al., 2014), tendon surgeries and wound healing (Yan et al., 2010), extrahepatic bile duct regeneration (Li et al., 2012) and myocardial repair (Gao et al., 2011).

Our research group extracted collagen from the skin of Red Snapper (Lutjanus argentimaculatus) and characterised it using various analytical techniques such as UV-visible spectroscopy, X-ray Diffraction analysis, and Fourier Transform Infrared Spectroscopy to confirm its purity (Sundar G. et al., 2022), compared with commercial collagen as control. Additionally, the cytocompatibility, cell viability, and wound-healing efficacy of the extracted collagen were evaluated. Finally, this collagen was utilised to fabricate a collagen electrospun scaffold incorporated with phyto-nanoparticles for burn wound healing applications (Fig.4)

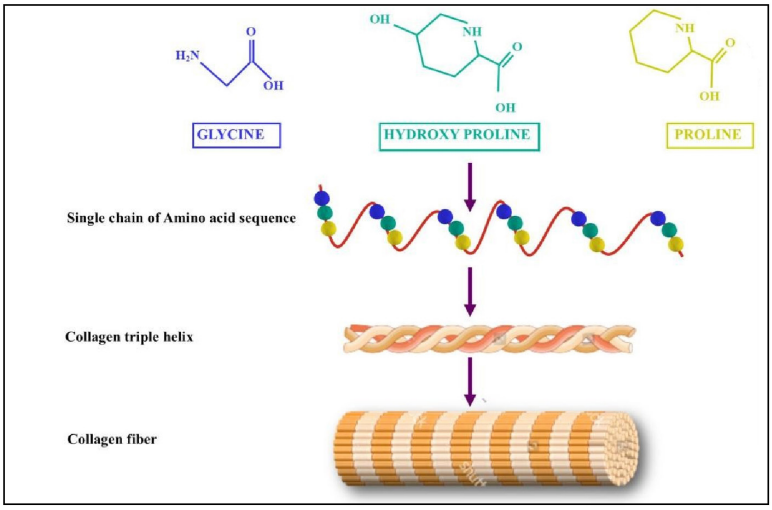

Figure 3: Schematic representation of collagen structure

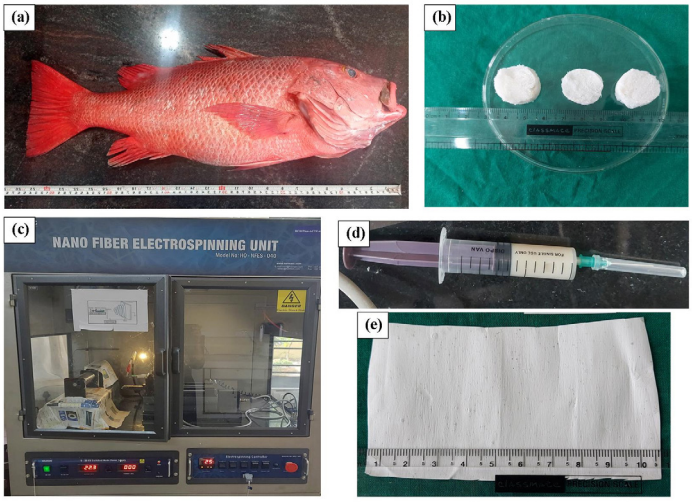

Figure 4: Fish Collagen- (a) Red Snapper (Lutjanus argentimaculatus) fish, (b) Extracted fish collagen sponge, (c) Electrospinning unit for the fabrication of collagen scaffolds, (d) Collagen solution in syringe for electrospinning, (e) Product - phyto-nano particles incorporated collagen electrospun scaffold for burn wounds.

Table 1.1: Selected examples of collagen extracted fish species.

Sl.No	Fish Species	Reference
	Nile Tilapia (Oreochromis niloticus)	Huang et al., 2016
	Bamboo shark (Chiloscyllium punctatum) and Blacktip shark (Carcharhinus limbatus)	Kittiphattanabawon et al., 2010
	Striped catfish (Pangasinodon hypophthalamus)	Singh et al., 2011
	Baltic cod (Godus morhua)	Skierka et al., 2007
	Catfish (Clarias gariepinus)	Momoh et al., 2013; Akunne et al., 2016
	Snakehead Murrel (Channa striatus)	Pasha et al., 2015
	Rainbow Trout (Oncorhynchus mykiss)	Schmid et al., 2013
	Common Carp (Cyprinus carpio)	Schmidt et al., 2013
	Finletted mackerel scad (Magalaspis cordyla)	Sampath Kumar et al., 2012
	Bluefin Trevally (Caranx melampygus)	Rethinam et al., 2016
	Gray mullet (Acipenser gueldenstaedtii)	Cherim M et al., 2017
	Jellyfish (Rhizostoma pulmo	Ahmed Z et al., 2021
	Sea Urchin	Melotti L et al., 2021

- **Red snapper (Lutjanus argentimaculatus)**

Red snapper (Lutjanus argentimaculatus), also known as red mangrove snapper, Stuart evader, purple sea perch, red bream, red perch, red reef bream etc. (Fig.4a), belongs to the Animalia kingdom, Chordata phylum, Actinopterygii class, Perciformes order, Lutjanidae family, Lutjanus genus, and is L. argentimaculatus. This marine fish is commonly distributed in Indo-West Pacific Ocean from African coast to Samoa and the Line Islands and from the Ryukyus in the north to Australia in the south. Marine red snapper is a great choice for extracting collagen protein because it is an excellent source of marine collagen, and the extraction yield is very high too. (Jongjareonrak et al., 2005; Zaelani et al., 2019). There is potential for red snapper skin to serve as an alternative source of collagen that could increase the value of fishery and food waste. Red snapper fish became the choice for the study for several reasons - Firstly, its widespread availability made it a practical choice. Secondly, the fish's high collagen content was particularly interesting to our research. Thirdly, its impressive lifespan, with a maximum observed age of 21, added an intriguing dimension to our investigation. Moreover, the species' ability to reproduce multiple times during the summer spawning season further contributed to its suitability for the study.

Conclusion

In conclusion, the utilisation of marine-derived collagen biomaterials hold significant promise for tissue engineering applications, particularly in the advancement of therapy for burn wounds. The unique properties of marine collagen, such as high biocompatibility, excellent wound healing properties, and abundance, make it an attractive candidate for developing innovative wound dressings and scaffolds. Through its ability to mimic the natural extracellular matrix of human skin, marine-derived collagen facilitates cell adhesion, proliferation, and tissue regeneration, promoting accelerated wound-healing processes. Furthermore, its sustainable sourcing from marine resources aligns with the growing emphasis on environment-friendly and renewable biomaterials. As research in this field continues to evolve, marine-derived collagen biomaterials are poised to play a pivotal role in revolutionising the treatment of burn wounds, offering new avenues for improving patient outcomes and quality of life in the health care system.

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Fabrication of Foam-based Porous Biopolymeric Scaffold for Burn Care: From Lab to Clinic

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Abstract:

The present review discusses how the Biopolymeric scaffold developed at IITD-AIIMS, New Delhi, was designed and developed to meet the requirements of second-degree burn wound care. The scaffold was fabricated and assessed in vitro and in vivo, both in rat and swine models. Additionally, its biocompatibility studies were conducted as per ISO standards. This study led to the transfer of technology to the Pharmaceutical Industry in India. The present review also gives a brief overlay of wound healing.

Wound healing:

The first line of defence in the human body is the skin, which helps to protect the skin from the environment and regulate temperature. The skin has two layers - the outer epidermis and the inner dermis. These layers help with vitamin D synthesis, sensory perception, dehydration, and immune protection[1]. The outer epidermal layer has keratinocytes that aid in the proliferation of the basal layer and differentiate into the cornfield epidermal layer. The skin's epidermis is highly regenerative, with stem cells that help maintain homeostasis and assist in wound healing[2].

According to the Wound Healing Society, a wound disrupts the skin's typical anatomical structure and function. A fully healed wound, typically after a minor injury, returns to its standard anatomical structure, function, and appearance within a reasonable period [3]. Depending on the type of tissue damage, wounds can be classified as epidermal, deep dermal, or full thickness. The healing process involves hemostasis, inflammation, proliferation, and ECM remodeling[4].

The wound healing process involves forming new cells secreted by extracellular matrix proteins, such as fibroblasts and keratinocytes, growth factors, inflammatory proteins, and collagen synthesis. While minor wounds usually proceed with the normal wound-healing process, damage to the dermal layer can take longer to heal, leading to risks such as infection, inflammation, and scar formation[5].

Burn wounds and their types:

Burn wounds are a significant public health issue and one of the most traumatic injuries that can lead to death, disability, and disfigurement. According to the World Health Organization, over 300,000 deaths occur every year worldwide due to burn injuries[6]. In India, an estimated 7 million burn incidents are reported annually. The annual cost of wound care treatment for deep burn wounds, which can result in scar formation, has reached $20 billion. Burn injuries cause excessive water loss from the body due to exudation and evaporation, and they can impair multiple vital functions of the skin. These

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injuries occur when the skin encounters heat, chemicals, or electricity, causing the rupture of skin layers. Burn wounds are classified into three types based on the extent of damage to the skin layers. First-degree burns, also known as superficial burns, cause redness and swelling of the skin and mainly affect the epidermal layers of the skin. Second-degree burns involve the dermal layers of the skin, where fibrinous exudate may develop over the wound, and large amounts of skin can be lost. Third-degree burns are the most severe and are full-thickness burns affecting the epidermis, dermis and underlying tissues, including nerve and blood vessels, so that all sensation in this area is lost and needs immediate surgical intervention [7,8].

Available wound dressings and Skin substitutes:

Various types of wound dressings are available in the market, including films, hydrocolloids, alginate, hydrofibre, hydrogels, foam-based, and antimicrobial dressings. These dressings serve different purposes and are used for treating partial to full-thickness burns and other wound conditions[9,10]. For instance, film dressings like Tegaderm™ are polyurethane-based coated as an adhesive to protect wounds from microorganisms. Hydrocolloid dressings, on the other hand, are semipermeable polyurethane films incorporated with sodium carboxymethyl cellulose particles and are suitable as primary wound dressing. Alginate wound dressings are called secondary wound dressings composed of brown seaweed and are helpful in the absorption of exudates from the wound[11,12]. Collagen-based wound dressings like Kollagen-D and NeuSkin ™ are helpful for second-degree burn wound healing, but the main disadvantage is their immunogenicity response toward the patient. Among these, hydrogel-based scaffolds are pivotal in engineering soft tissues because hydrogel texture acts as natural tissue with akin mechanical properties and chemical

composition[13,14].

The wound healing process is a complex process that involves various cell types, from extracellular and intracellular signals. Wound dressings keep the wound moist, which helps activate cells to heal the wound through the repair mechanism rather than the regeneration of skin tissue[15,16]. Skin substitutes are pivotal in treating patients with inadequate skin available to cover the wounded surface and help heal wounds. Skin substitutes are categorised into acellular or cellular products. Acellular products are derived from the human cadaveric dermis by removing cellular products and affixed with a collagen or fibronectin scaffold[6]. Cellular-based skin substitutes are composed of living cells like fibroblasts and keratinocytes within a scaffold, and the cells can be autologous or allogeneic[17–19]. The basic requirements of a tissue-engineered skin substitute should be rapid availability, autologous, reliable wound adherence, minimum donor site mobility, clinical manageability, and improved scar quality, and it should be economically affordable. Commercial tissue-engineered skin substitutes are available in the market based on cellular or cell-containing matrices, and acellular skin substitutes like Integra™ have been developed[19]. However, the main disadvantages of these matrices are their high costs, immunoreactive reaction to patients sensitive to collagen, and limited availability.

Role of natural bioactive components in wound healing

The term “bioactive” is a combination of “bio-” and “-active,” which refer to life and dynamic energy, respectively. A bioactive substance is a substance that affects or triggers a

reaction in living tissue. These substances can be synthetic or from natural sources such as plants, animals, or microorganisms[20]

Bioactive components such as flavonoids, growth factors, glycosaminoglycans, nanoparticles, and stem cells play a significant role in burn wound healing. Flavonoids are polyphenolic components and plant secondary metabolites found in fruits, vegetables, and beverages such as tea, coffee, beer, wine, and fruit drinks[21]. They have been reported to have antiviral, anti-allergic, anti-inflammatory, antitumor, and antioxidant activities that are of considerable interest for their potential beneficial effects on human health[22,23.]

Growth factors (GFs) are biological stimuli that promote stem cell proliferation and differentiation. They play a critical role in cell-matrix and cell-cell interactions during all stages of wound repair. Many growth factors, such as fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and granulocyte-macrophage colony-stimulating factor (GM-CSF), have been shown to have a beneficial effect on the healing process, both in animal models and in patients suffering from different types of wound healing disorders[24–26].

Glycosaminoglycan (GAGs) extracellular matrix (ECM) macromolecules such as hyaluronic acid, chondroitin sulfate, dermatan sulfate, and heparan sulfate are critical players in wound healing applications[27]. GAGs monitor several aspects of tissue repair, including activating inflammatory cells to enhance immune response and provide a structural framework for fibroblasts and epithelial regeneration. Hyaluronic acid helps initiate wound healing by acting in the angiogenic process and regulating the ECM and its metabolism[28]. Chondroitin sulfate and dermatan sulfate activate fibroblast and epithelial regeneration and increase the proliferation of keratinocytes and migration, respectively. Heparan sulfate improves cell adhesion and accelerates the wound-healing progress

29,30.

Translational Research was conducted in the Centre for Biomedical Engineering Lab at IITD and AIIMS.

We first initiated the research on electrospun scaffolds; however, due to their limited mass production, we moved to the design and fabrication of foam-based scaffolds. The knowledge obtained from the electrospun scaffolds was used to develop biopolymeric scaffolds for clinical settings.

Electrospun scaffolds:

Using bioactive molecules and glycosaminoglycans (GAGs) to fortify scaffolds effectively creates new tissue-engineered biomaterials. In this study, we evaluated the impact of electrospun nanofibrous composite scaffolds (made of cationic gelatin, hyaluronan, and chondroitin sulfate) containing sericin, along with the contact co-culture of human mesenchymal stem cells (hMSCs) and keratinocytes on the differentiation of hMSCs towards the epithelial lineage. The cationic gelatin was created by grafting quaternary ammonium salts to the backbone of gelatin using a novel synthesis process. Release kinetics studies showed that the primary mechanism of GAGs and sericin/gelatin release was Fickian diffusion[30–32].

To evaluate the biocompatibility of the electrospun scaffold, we conducted LDH and

DNA quantification assays on human foreskin fibroblast, human keratinocyte, and hMSC. All three cell types showed significant proliferation (approximately 4-6-fold) when cultured on the electrospun scaffold containing sericin. After five days of contact co-culture, we observed that an electrospun scaffold containing sericin promoted epithelial differentiation of hMSC in terms of several protein markers (keratin 14, ΔNp63α, and Pan-cytokeratin) and gene expression of some dermal proteins (keratin 14, ΔNp63α).

These findings will help advance the development of skin tissue engineering scaffolds by improving our understanding of the skin regeneration and wound healing process. However, producing nanofibrous-based scaffolds on a large scale for translational studies is challenging. Hydrogel-based scaffolds may be a more practical option to address this since they are easier to process and more cost-effective. An ideal wound healing scaffold setting for clinical evaluation should possess effective healing properties, an extracellular matrix that mimics nature, mechanical and thermal resistance, biodegradability, biocompatibility, cytocompatibility, and manufacturing technology expertise[33]. The following foam-based scaffold was designed and developed, which could be used in 2nd-degree burn wounds.

Foam-based scaffold – In-vitro and In-vivo study:

Based on the earlier research carried out in our laboratory at IITD and AIIMS, New Delhi, on electrospun and hydrogel scaffolds with bioactive ingredients and nanoparticles, we moved to develop a foam-based scaffold made from biopolymers of gelatin, hyaluronic acid, and chondroitin sulfate (G-HA-CS scaffold) in appropriate proportion, which could treat 2nd-degree burn wounds without the need for donor skin autograft. The scaffold was fabricated by dissolving 10% gelatin under a magnetic stirrer at 45 ±2 °C. To this solution, dissolved chondroitin sulfate and hyaluronic acid in appropriate proportion were added and stirred for ten to fifteen minutes. The final mixed solution was poured on the sterile petri plate and crosslinked in a specific manner, using EDC 10 mM for 15 min at 4 ± 1 °C, to get two-layered structures, which would degrade in a controlled manner and allow the epithelisation without scar formation. After cross-linking, hydrogel plates were lyophilised and sterilised using gamma radiation at 2.5 Mrad for further experiments. The details of fabrication, as well as physical and chemical characterisation, are provided in reference [34].

The scaffold was tested for effectiveness before and after sterilisation using various methods, including scanning electron microscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, and thermal gravimetric analysis. In vitro studies showed that the scaffold promoted cellular proliferation. Additionally. as biocompatibility assessment is essential for any medical device, the scaffold was tested to ensure it meets regulatory requirements. Once these requirements are met, the scaffold can be used in clinical trials. The biopolymeric scaffold was tested using Good Laboratory Practice and OECD guidelines and applications. Various parameters like skin sensitisation test, acute systemic toxic test, implantation study, intracutaneous reactivity test, in vitro cytotoxicity test, and bacterial reverse mutation test were evaluated and found safe for burn wounds[35].

The scaffold was compared to Integra™ in rat and swine models of second-degree burn wounds and showed remarkable healing potential. The wound contraction rate, reduction of IL-6, TNFαand C3, and expression of healing markers all demonstrated

significant skin regeneration. The scaffold also promoted angiogenesis and skin regeneration by modulating CD-45, cyclooxygenase-2, and MMP-2. The histological and MT findings further supported the results. Mechanistically, we observed modulation of CD-45, vimentin, N-cadherin, MMP-2, and COX-2 in the treatment groups, indicating their modulation as one of the essential contributors to the observed effective healing response.

The efficacy of the G-HA-CS scaffold has tremendous potential for clinical translation owing to its successful exhibition of remarkable therapeutic efficacy in the rat and swine second-degree burn model with biocompatibility performance[34].

Recently, pharma companies have shown interest in and have taken the technology. We have received the CDSCO approval to initiate the pilot clinical studies. The developed foam-based scaffold will help burn patients at a much more reasonable price. It will serve as a "Make in India" device comparable to Integra, which is unaffordable for the Indian population[36,37].

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Need for Melatonin Detection: Advancements in Methods, Challenges and Future Directions

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Abstract

Melatonin, an indole amine primarily synthesised by the pineal gland, is integral to regulating circadian rhythms, maintaining sleep, and various physiological parameters. In recent years, melatonin has gained importance due to its pleiotropic roles such as blood pressure regulation, vasomotor effects, immune functions, detoxification of free radicals, control of tumour growth, direct and indirect anti-apoptotic effects, neural development during pregnancy, ageing, and so on. Measuring melatonin levels is essential, especially in rising circadian rhythm sleep disorders, cancers and other neurodegenerative diseases. In the current fast-changing technological advancements, cost-effective, rapid, sensitive detection techniques for melatonin assay are required, which may be readily available and easy to use even at home. This review highlights the various prevalent detection methods of melatonin, including the enzyme-linked immunosorbent assay (ELISA) and recent advancements, such as the development of lateral flow immunoassay (LFA) strips and aptamer-based colorimetric assays emphasising their potential for non-invasive, rapid, and accurate measurement of melatonin.

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Introduction

Melatonin, the "hormone of darkness," is a ubiquitous hormone primarily synthesised by the pineal gland. It plays a pivotal role in regulating circadian rhythms, sleep, mood, reproduction, and ageing [1-3]. The secretion of melatonin is suppressed by light and enhanced by dark, thereby allowing the body to distinguish between day and night [4, 5].

Melatonin (N-acetyl-5-methoxytryptamine), an endogenous indole-amine, was first isolated by Lerner et al. in 1958 from bovine pineal extracts and was named based on its ability to aggregate melanin granules and thereby lighten the colour of frog skin [6]. It is synthesized from the amino acid tryptophan, which is first hydroxylated to 5-hydroxytryptophan and then decarboxylated to 5-hydroxytryptamine (serotonin) by the enzymes tryptophan hydroxylase and tryptophan decarboxylase respectively (Fig 1-A). The enzyme arylalkylamine N-acetyltransferase (AANAT) produces N-acetyl serotonin, the immediate precursor of melatonin, and it is the rate-limiting step in melatonin biosynthesis (Fig 1-A). Finally, hydroxyindole-O-methyltransferase (HIOMT), through

methylation, converts N-acetyl serotonin to melatonin [7-10]. Then, it is released into the bloodstream, reaching different body fluids, cellular compartments, and tissues [11]. Melatonin is synthesized in the pineal gland and the extra-pineal sites, including the retina, harderian gland, gastrointestinal tract, human and murine bone marrow, skin, and lymphocytes, placenta during pregnancy [9, 12, 13]. It is primarily metabolised in the liver to 6- hydroxy melatonin by cytochrome P450 mono-oxygenases, and then it is coupled with sulfate to form 6-sulfatoxy-melatonin, which is the main metabolite excreted in urine (Fig 1-B) [14-16].

A) Melatonin biosynthesis

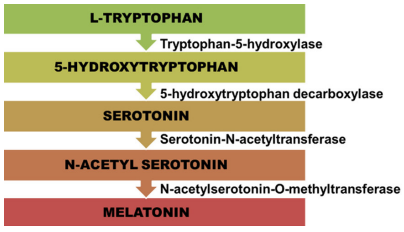

Figure 1: Melatonin biosynthesis and Metabolism

B) Melatonin metabolic pathway

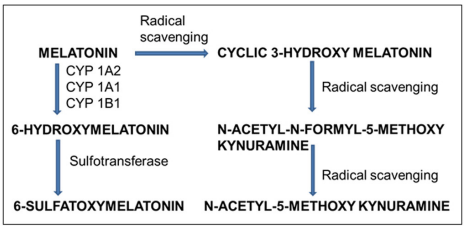

FIGURE 2: The pleiotropic effects of melatonin

Melatonin plays a vital role in regulating circadian rhythms (CRs), receiving signals from the suprachiasmatic nucleus (SCN) and communicating with the rest of the body. CRs are physiological and behavioral cycles that repeat every 24 hours. They are organized by the endogenous biological pacemaker, the SCN, located in the anterior hypothalamus. CRs are modulated by endogenous, environmental and behavioural factors [17]. Circadian rhythm sleep-wake disorders (CRSWD) can be misdiagnosed as insomnia and daytime hypersomnolence. In this disorder, impaired functioning is primarily caused by a misalignment between the internal circadian clock and the 24-hour social and physical environment. CRSWDs are categorised as delayed sleep phase disorder, advanced sleep phase disorder, irregular sleep-wake rhythm, free-running disorder, jet lag, and shift work disorder [18].

Melatonin acts as a pleiotropic modulator having antioxidant and neuroprotective effects, affecting reproductive, immune, cardiovascular, and mood systems and the entire physiology of the body (Figure 2) [19-20]. Melatonin scavenges excessive free radicals generated in the body by anti-excitatory and anti-inflammatory properties. So, disruption of sleep during ageing and even during fetal development (during pregnancy) are potential risk factors for melatonin production, thereupon causing the onset of many disorders like sleep disorders, cognitive impairments, mood disorders, dementia, breast cancer, neurodegenerative disorders, etc. [9, 21-24].

Melatonin is involved in each phase of ovulation, fertilisation, and embryo implantation, and it is also a pregnancy regulator [25]. Higher levels of serum melatonin are reported after 24 weeks and again after 32 weeks of implantation [26,27]. Maternal melatonin enters the fetal circulation transplacentally, influencing the internal rhythms and neurodevelopment. Melatonin receptors are widely distributed in the fetus from early fetal development [28]. Research suggests that melatonin deficiency in pregnant women can lead to problems with fetal development and may increase the risk of certain complications, such as preterm birth [29].

In recent years, several studies have been carried out to understand the role of melatonin in human reproduction and assistive reproductive technologies (ART) and related disorders like polycystic ovary syndrome (PCOS) [30-33]. According to the World Health Organization (WHO), PCOS is an endocrine disorder affecting approximately 8-13% of women of reproductive age, and up to 70% of affected women remain undiagnosed worldwide [33]. In PCOS patients, lower melatonin concentrations are reported in the ovarian micro-environment, which leads to abnormal circadian rhythm and impaired embryogenesis [34]. Treatment of melatonin for six months in PCOS women has restored the menstrual cycle by bringing down the androgen levels and boosting the FSH levels [35]. Melatonin supplementation in PCOS oocytes cultured in vitro increased the expression of oocyte maturation-related genes and antioxidant-related genes while reducing total intracellular reactive oxygen species (ROS) levels and apoptosis [36]. Additionally, in in-vitro fertilization (IVF) procedures, melatonin supplementation reduced the number of poor-quality embryos [37].

During ageing, melatonin secretion progressively declines, disrupting its level and setting in various age-related circadian rhythm sleep disorders [38,39]. In aged people, the nighttime levels are almost indistinguishable from those obtained during daytime.

In light of the widespread role of melatonin in body functions, the challenges and advancements in detecting melatonin levels are discussed in this review that will help diagnose or treat melatonin deficiency and related disorders. The detection of melatonin levels is essential in the current scenario because the growing prevalence of CRSWD, sleep disorders, mood disorders, cancers (especially breast cancer), and reproductive and other neurodegenerative disorders are increasing day by day [9,40-42]. Detection of melatonin levels holds promise as a biomarker for these medical disorders, and thereby, it can aid in disease diagnosis, prognosis, and treatment monitoring.

It is also pointed out that several food items are also rich sources of melatonin, including tomato (4.1-114.5 ng/g), strawberry (1-11ng/g), rice/barley (300-1000 pg/g), walnut (3-4 ng/g), and olive oil (53-119 pg/ml) etc. [9,43-45]. Labelling the melatonin content in natural and enriched natural food will be good. Melatonin and its precursors in various food items, edible plants, and plant-based products may be a natural source for countering or reducing melatonin deficiency, insomnia, circadian sleep disorders and REM sleep behaviour disorder [46].

Prevalent techniques for melatonin measurement

Melatonin synthesis in night/dark hours is a universal phenomenon across species [4,5]. In humans, the secretion starts soon after sunset and reaches its peak concentration in the middle of the night, around 2:00 - 4:00 am, and decreases gradually during the second half of the night [47,48]. The time of day that melatonin first increases above its baseline level is called Dim Light Melatonin Onset (DLMO) [49]. The measurement of DLMO can give the time of day that melatonin production starts. So, delayed and advanced sleep phase disorders can be characterized by delayed or advanced DLMO. Nowadays, researchers are increasingly interested in DLMO measurement to compare daytime levels in physiological and clinical states [50-52].

Many distinct assay methods have been developed over the years, including bioassay [53-55], physico/chemical (chromatographic and mass spectrometric techniques) [16,56,57], immunological methods such as radioimmunoassay (RIA), and enzyme-linked immunosorbent assays (ELISA) and a novel method called fast-scan cyclic

voltammetry (FSCV) to determine melatonin [8,16,58,59] (Table 1). Mass spectrometric methods are more precise and potentially the most sensitive among these. Still, the main drawbacks of this method are that it requires expensive equipment and highly trained personnel and is not suitable for large sample numbers. Additionally, effective immunological methods have been developed for melatonin detection offering sensitivity and specificity and are cost-effective immunological methods have been developed for melatonin detection offering sensitivity and specificity. Cost-effective immunological methods have been developed for melatonin detection, offering sensitivity and specificity, and immunological methods have been developed for melatonin detection, offering sensitivity and specificity which are cost-effective and suitable for large sample numbers. For these reasons, immunoassays of melatonin, especially the ELISA method, have become the choice of researchers to measure melatonin [56]. However, the selection of the detection method depends on factors like the condition collection study's purpose, considerations for sample collection frequency, lighting conditions, and body posture to evaluate circadian rhythms effectively [16].

Table 1: Different assays developed for melatonin detection and their properties.

Method for melatonin detection	Properties	
	Advantages	Disadvantages
Bioassay [53,54,55]	Bioassays are based on melatonin-induced pigment aggregation in amphibian dermal melanophore.	Low sensitivity of about 100 ng The complex procedure as an analytical tool
HPLC [16,57]	High sensitivity & high specificity Allows detection of low conc. of melatonin in small samples.	It requires complex sample preparation specialized equipment trained personnel highly expensive
GC-MS [56]	High sensitivity & specificity.	Sample derivatization is often needed for melatonin volatilization adding complexity to the process, It requires specialized equipment, trained personnel, It is not cost effective for running multiple samples
RIA [58]	High throughput application, relatively specific and produced sufficient sensitivity.	Use of radioactive material is a notable disadvantage
ELISA [58,59]	Cost effective, easy to use, and widely available.	Limited specificity & sensitivity, potential cross-reactivity
F-SCV [16]	Innovative method to determine melatonin in immune system, high sensitivity and allows the observation of rapid changes in melatonin level in tissue	Limited spatial resolution Require specialized equipment & expertise Potential interference

In the RIA method, a calibration curve is generated by the competitive binding of radiolabeled and unlabeled melatonin to a known amount of antibody against melatonin. The calibration curve constructed allows the determination of unknown melatonin concentrations in biological samples [56,60]. The drawbacks of the RIA method include potential health hazards, expensive radiolabeled ligands, and the need for a costly radio-isotope license to purchase and perform the assays.

ELISA is a technique that utilises antibodies to detect and quantify specific compounds. In the case of melatonin ELISA, antibodies are designed to bind specifically to melatonin [61]. Two types of melatonin ELISA kits were developed: competitive indirect ELISA and direct ELISA. Competitive ELISA involves simultaneous competing antibodies or proteins in different ways.

Even though melatonin ELISA kits are commercially available for measuring melatonin levels in biological fluids such as blood and saliva, the quality and accuracy of these kits vary significantly. Some kits are not sensitive enough to detect low concentrations of melatonin, and some kits produce unrealistically high daytime melatonin levels, which leads to inaccurate interpretations [59]. Researchers should be cautious while developing or selecting a melatonin kit. It is essential to consider the sensitivity and assay range to get accurate results for the specific research needs [8,62].

Challenges in the development of melatonin kit

Antigenicity and Antibody production

Melatonin, with a small molecular weight of 232.278 Da g/mol, displays low immunogenicity. The preparation of antibodies is the most crucial step in developing a detection kit. However, due to the low antigenicity of melatonin, it must be coupled to a molecule of suitable immunogenic molecules such as bovine serum albumin (BSA), ovalbumin (OVA), and thyroglobulin [8]. Thus, either coupling of melatonin or its analogue with suitable carrier proteins is used to develop specific melatonin antibodies [63-68]. Proteases digest the protein into peptides after the internalisation of the antigen by presenting antigen-presenting cells. The peptide is bound to class 2 MHC molecules on the cell membrane and presented to B lymphocytes, which produce an antibody against each epitope [8].

The conjugation points on melatonin or its analogue where the protein binds have a major impact on antibody specificity. Kit manufacturers generally do not disclose sufficient details about antibody production, such as used epitopes on melatonin analogs and their characterization details, that can help new investigators replicate the study [8,58].

Sample collection and preparation

The specificity of the assay mainly depends on the antibody used for the ELISA, but the sample presented to the antibody is also critical [8]. Blood and saliva are the samples collected for melatonin assays. The direct functioning of the circadian system and the pineal gland is analysed by measuring melatonin in blood plasma. For this, the blood samples need to be collected in lithium/heparin tubes and plasma separated immediately and stored and frozen (-20°C) [69]. Sampling multiple times is essential to derive circadian patterns; blood is the best sample for this purpose although it is an invasive procedure[70].

Melatonin levels in saliva are approximately 30% of the plasma melatonin levels, and this can be easily sampled, and approximately 3ml is needed for the assay [69,70]. The advantage of saliva sample collection is that it is non-invasive and requires minimal sample handling. However, saliva melatonin measurement cannot be used to infer the production of melatonin by the pineal gland, and they are only valid indicators of 24 hr rhythmicity of the hormone [71].

Another noninvasive measurement of melatonin is done by detecting 6-sulfatoxy melatonin in urine. This is the major liver metabolite of melatonin and is a valid index of pineal melatonin production. It does not require sample pre-treatment and is stable at room temperature. The only disadvantage of this is that the samples are integrated over several hours, and so phase assessment is less accurate [69].

Advancements in detection techniques

Today, there is a need to develop a low-cost, simple, and fast detection method for melatonin. Lateral flow immunoassay (LFIA/ LFA) strip is a simple technique that doesn't require an extraction procedure, requires less time, and has less cost. The working principle of LFA is that "the sample which contains the analyte of interest moves by capillary action through various zones of polymeric strips, on which molecules that can interact with the analyte are immobilised. The critical components of the LFA include antibodies and membranes, which react with the analyte bound to the conjugated antibody and thereby detect specific substances or antigens in the biological fluids [72].

In 2019, Chen et al. developed a low-cost, simple, fast, and quantitative lateral flow immuno-chromatographic assay (ICA) for melatonin detection in healthy foods [73]. The ICA yielded a visual limit of detection (VLOD) of 10,000 ng/ml and LOD of 50 ng/ml. It could be an excellent tool for melatonin detection in food. The main challenges of lateral flow immunoassays for detecting melatonin include low sensitivity due to transient reactions triggered by capillary action on nitrocellulose membranes [74].

Recently, a sensitive and straightforward colorimetric assay was developed using an aptamer-gold nanoparticle (AuNP) probe, which gives visually distinct color changes on salivary melatonin detection. The ultralow level of salivary melatonin was detected with LOD of about 0.0011nM using truncated 36-mer MLT-A-2 aptamer-AuNP. Aptamers are single-stranded DNA or RNA oligonucleotides. Compared to antibodies, aptamers exhibit greater stability, do not elicit immunogenicity, and modification has capability that could facilitate enhanced sensitivity and specificity [75].

Conclusion

Melatonin level has a pivotal role in the circadian rhythm disorders. Several methods, such as HPLC, gas chromatography-mass spectrometry, RIA, and ELISA can detect the level of melatonin in blood plasma, saliva, and urine. Because of reasons such as cost, specialized equipment, and use of radioactive materials, the assay methods, except ELISA, are not followed by researchers, and they are focused on developing a specific ELISA method for detecting melatonin. So far, researchers have developed ELISA kits for research purposes only, and many of these kits do not satisfy the precision and sensitivity we need for melatonin detection. Additionally, the multiple sample collection at different phases of the day is crucial for the analysis, so relevant research is required for the development of a single Indigenous or home-based kit that would help detect melatonin at different times.

Furthermore, future research efforts should focus on specific antibody-antigen preparations, which determine the specificity of the ELISA, or should move on to aptamer-based methods, which don't require antibody preparation, and finally make the kit that could be used for clinical purposes.

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Abbreviations used: HPLC - High Performance Liquid Chromatography; GC-MS - Gas Chromatography - Mass Spectrometry; RIA – Radioimmunoassay; ELISA – Enzyme Linked Immunosorbent Assay; F-SCV – Fast-Scan Cyclic Voltammetry.

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Navigating Frontier Science: Advancement and Prospects of Nanomaterial Applications in Cell-Tissue Engineering

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Abstract

Tissue engineering (TE) stands as a beacon of interdisciplinary collaboration, integrating principles from engineering, materials science, and medicine to engineering biomaterials tailored for tissue repair, replacement, or regeneration. Despite significant advancements, the search for viable alternatives to traditional implants persists, emphasising the crucial need for ongoing research. However, recent breakthroughs in nanomaterials have heralded a transformative era in TE. These state-of-the-art materials possess exceptional mechanical, electrical, and drug-delivery capabilities, poised to revolutionise the field. Nanomaterials with dimensions below 100 nm intricately mimic natural tissue properties, seamlessly navigating cellular barriers and facilitating precise bioactive agent delivery. Moreover, beyond tissue engineering, nanomaterials have found extensive applications in clinical domains such as cancer therapy, gene therapy, and drug delivery, further underscoring their versatility and impact.

This comprehensive review delves into the pivotal role of nanoparticles (NPs) in tissue engineering, shedding light on their transformative influence in advancing regenerative medicine across diverse domains. Specifically, their applications in bone, skin, and nerve regeneration are explored, highlighting their potential to address critical healthcare challenges. Through a synthesis of theoretical insights and empirical evidence, the mechanisms underlying NP-mediated tissue regeneration are elucidated and discussed across the current challenges and future directions in this novice field of medical science. Ultimately, this review provides a nuanced understanding of how nanomaterials reshape the landscape of tissue engineering and regenerative medicine, offering promising avenues for clinical translation and patient care.

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Introduction

Tissue engineering (TE) and biomaterial sciences have gained strong attention in the last decade due to their widespread applications in various tissue regenerative therapies (skin, bone, nerve, etc.). This has emerged as a promising field considering the failures/drawbacks of cell therapies, which focused on injecting live/active cells into the damaged area. Limitations such as loss of cellular activity and cell death on/before reaching the area under repair, failure to reach the site of injury, and prolonged regeneration paved the path for developing 3D tissue engineering scaffolds (Fleischer & Dvir, 2013) to mimic their in vivo counterparts. Thus, tissue engineering and Regenerative Medicine (TERM) is a biomedical tri-discipline of three different sciences – biology, chemistry, and engineering.

The 3D scaffolds mimic the structure and functions of the extracellular matrix (ECM) and provide a platform to repair, restore and regenerate the human body's damaged or injured tissues and organs. Furthermore, the advancement in TE leads to the delivery of suitable cells and growth factors to the site of injury by incorporating it with bioma-

terials. The three components, i.e., scaffolds, biologically active molecules, and cells, known as the TE triad, are the functional unit of TE and enhance regeneration and rate of healing (Figure 1). In addition to biodegradability, non-toxicity and biocompatibility, an ideal scaffold must possess qualities like adequate porosity, mechanical strength, cytocompatibility, etc.

Integrating nanotechnology with TE brings forth a new set of biocompatible scaffolds like nanoparticles, nanocrystals, nanorods, nanowires, nanotubes, carbon nanotubes, and others (Kim et al., 2013). Nanomaterials are natural or synthetic materials that contain particles in the size range of 1nm - 100nm in at least one dimension (Colson & Grinstaff, 2012). Excellent chemical and physical stability, high surface area, biodegradability, biocompatibility and low immunogenicity make the nanomaterials a preferred material choice in TERM (Danie Kingsley et al., 2013). The incorporation of nanoparticles in biomaterial scaffolds enhances its mechanical strength, degradability, transport and controlled release of bioactive molecules to specific targets (Sahoo et al., 2010; Wang et al., 2009). Since nanoparticles can cross the cell membrane, they can directly deliver proteins to the cells. Thus, bioactive molecule-encapsulated nanomaterials have great importance in tissue engineering (Pawelec et al., 2021), cancer therapy (Gmeiner & Ghosh, 2015), drug delivery (Yang et al., 2017) and gene therapy (Keles et al., 2016).

This chapter focuses on the types, synthesis and functions of different nanoparticles such as metallic NPs, ceramic NPs and polymeric NPs and the applications of nanomaterials in tissue engineering of bone, skin, nerve, etc.

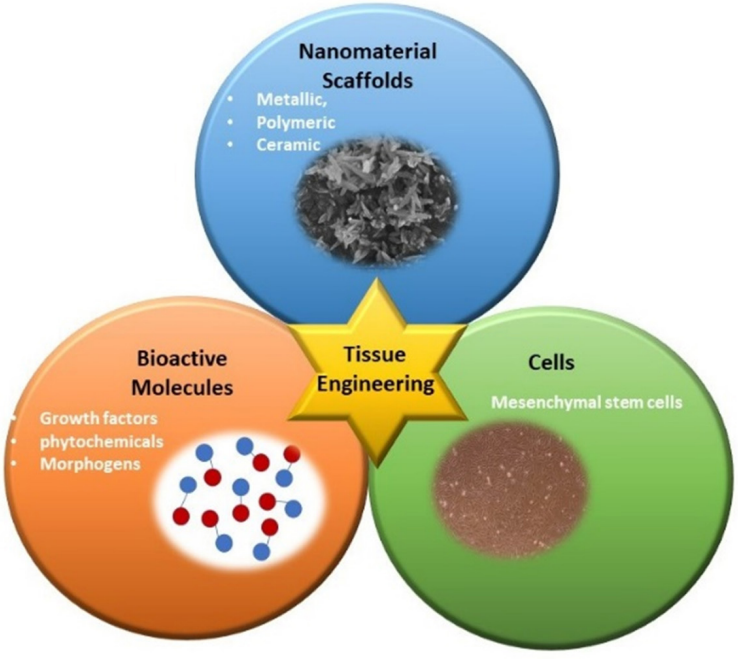

Fig 1: Schematic representation of the three main components of tissue engineering (Triad of Tissue engineering)

Nanomaterials used in TERM

TERM uses the advantage of 3D scaffolds, which act as a platform (ECM) for the cells of damaged organs to grow, proliferate and differentiate into desired cell types. Researchers use different techniques and strategies available to improve and develop an ideal scaffold for TERM. Nanotechnology has been used widely in modern medicine and recently for developing novel biomaterials in tissue engineering. Metals, ceramics, and natural and synthetic polymers are generally used to generate nanomaterials. Scanning electron micrographs depict these three types of nanomaterials (Figure 2).

1. Metallic Nanomaterials

Metallic and metallic oxide nanoparticles such as gold, silver, copper, palladium, titanium oxide, aluminium oxide, etc., are of great importance for applications such as antimicrobial, anticancer, antioxidant, wound healing, antiparasitic, thrombolytic and anticoagulant compared to other nanoparticles (Vimbela et al., 2017). Metal nanomaterials can be classified into three groups: metallic NPs, metal oxide NPs and magnetic NPs. Among these metallic NPs, gold NPs and AgNPs possess unique characteristics like antibacterial activity, electrical conductivity, and mechanical properties, which, together with these properties, make them acceptable for tissue engineering applications. By modifying suitable functional groups of metallic nanoparticles, different bioactive molecules such as growth factors, ligands, antibodies, and hormones can be conjugated and coupled for drug delivery purposes.

1.1 Gold nanoparticles

Gold nanoparticles are the colloids of nanosized gold particles first synthesised by the scientist Turkevich as monodisperse spherical nanoparticles (Dong et al., 2020). Later, the methods were modified by G. Frens as he discovered that the mixing of various proportions of citrate and HAAuCl4 resulted in the development of controllable-sized nanoparticles. Other methods, such as seed growth, ultrasonic spray pyrolysis (USP), and green synthesis developed later, are also widely used. Green synthesis of nanoparticles is the ideal method for creating nanoparticles in biological applications since it is environmentally acceptable and economical. Bacteria, fungi, algae, and plants can synthesise GNPs with the help of potent reducing/coupling agents present in them.

AuNPs coupled with bioactive molecules are widely used for drug delivery applications. Quercetin-conjugated gold nanoparticles induced the apoptosis of breast cancer cell lines by inhibiting EGFR/PI3K/Akt–mediated pathway (Balakrishnan et al., 2016). Growth factor receptor inhibitors have been used widely to stop the growth of tumour cells by coupling them with NPs. In cancer therapy, in addition to the conjugation of epithelial growth factor inhibitors to the gold nanoparticles, photothermal therapy also contributed to the inhibition of cancer growth (El-Sayed et al., 2006).

Gold nanoparticles are extensively used in bone tissue engineering as it has been found that they induce the differentiation of stem cells into the osteogenic lineage, thereby increasing bone regeneration. Mesenchymal stem cells are multipotent cells that can differentiate into other types of cells, such as bone, cartilage, muscle, adipocytes, etc. Studies by Zhang et al. showed that the action of gold nanoparticles induced osteogenic differentiation and mineralisation of primary osteoblast cells through the ERK-MAPK pathway(Zhang et al., 2014). In another study, Choi et al. reported that AuNPs induced osteogenic lineage on human adipose-derived mesenchymal stem cells by activating the

wnt / β – catenin pathway (Choi et al., 2015).

Gold nanoparticles are also successfully used in skin tissue regeneration applications such as wound healing. The antibacterial effect of AuNPs in wound dressing helps kill bacteria in infected wounds. Similarly, studies of Yang et al. depicted the impact of modified AuNPs loaded PCL/gelatin electrospun scaffolds on multidrug-resistant (MDR) bacteria in infected wounds. The inability to use conventional antibiotics in such cases offers AuNPs incorporated scaffolds a significant advantage (Yang et al., 2017).

1.2 Silver Nanoparticles

AgNPs are renowned for their antimicrobial activity against bacteria, fungi and viruses and their application in households (soap, paste, food, etc.), pharmaceutical and cosmetic industry, food packaging and textile industry, etc. (Ahamed et al., 2010). Along with antimicrobial activity, AgNPs are also used as an anti-inflammatory and antitumor agent.

Silver nanoparticles are currently synthesised using three methods: physical, chemical, and biological. Chemical reduction is an extensively used method where nanoparticles are synthesised with the help of organic or inorganic reducing agents such as sodium citrate, ascorbate, Tollens reagent, and N, N- dimethylformamide (DMF). On reduction, silver ions in aqueous or non-aqueous solutions are converted into metallic silver; NPs are then formed via nucleation and subsequent growth. Advantages such as low cost, ease of production and high yield make the chemical method more popular (Quintero-Quiroz et al., 2019; Suriati et al., 2014). Standard physical methods for synthesising AgNPs are evaporation and condensation, irradiation, laser ablation, gamma irradiation or ultrasonic irradiation and lithography. In the physical method, instead of chemicals, radiation is used as a reducing agent; hence, it is not toxic compared to the chemical method. However, the method is unpopular due to the slow reaction rate and low yield.

Drawbacks of chemical and physical methods resulted in the development of biological processes, which evolved as a boon for producing AgNPs. In this green synthesis method, biological agents (bacteria (Samadi et al., 2009), algae (El-Rafie et al., 2012), yeast (Salem, 2022) fungi (Bhainsa & D'Souza, 2006)) replaced reducing agents as well as stabilisers. Bacteria like Escherichia coli (Divya & Mini, 2014), Pseudomonas aeruginosa (Kumar & Mamidyala, 2011), Bacillus amyloliquefaciens (Fouad et al., 2017), Brevibacterium casei (Kalishwaralal et al., 2010) and fungi such as Trichoderma viridae (Mohseni et al., 2019a), Fusarium oxyporum (Campora & Ghersi, 2021) were generally used for this purpose. Due to the antibacterial and anti-inflammatory effects, Silver NPs became an inevitable component of wound dressings along with natural or synthetic polymers. Antimicrobial dressings developed from PCL/PVA nanofiber conjugated AgNPs showed quick angiogenesis, epithelialisation and remodelling in male Wistar rats compared to silver sulfadiazine (SSD) conjugated wound dressing (Mohseni et al., 2019b). AgNPs have gained much attention in artificial implant development since they can replace conventional antibiotic treatment.

2. Polymeric Nanomaterials

Polymers are solid, non-metallic macromolecules composed of repeating units of small monomeric units that form extended chain-like architecture with varying character-

istics based on their composition. Better biocompatibility, good biodegradability, less toxicity, and the ability to protect the biologically active molecules against enzymatic degradability make the PNPs the best candidates for TERM applications. PNPs of different sizes and shapes are available, such as nanofibers, nanospheres, nanogels, micelles, nanocapsules, polymersomes (Nicolas et al., 2013).

To develop suitable PNPs for TERM application, researchers were synthesising and developing biocompatible and non-toxic polymers such as polyamide, polylactic acid (PLA), polyglycolic acid (PGA), PLA–glycolic acid copolymer (PLGA), polycaprolactone polyester, polyanhydride, polysaccharides, proteins etc. Polymeric nanoparticles attracted more attention in TE due to the importance of targeted drug delivery. It has some advantages like controlled release, targeted delivery, drug protection from the external environment, etc. Scientists are working hard to develop more suitable drug-delivery vehicles using these synthetic polymers. Polymeric micelles are promising vehicles for bioactive molecule transport due to their advantages, such as higher cargo capacity, excellent stability, controlled drug release, and non-toxic nature (Ahmad et al., 2014). Tallian et al. studies showed pH-dependent drug release from silk fibroin nanocapsules loaded with human serum albumin (HSA). Here, pH is associated with the release of HSA from nanocapsules (Tallian et al., 2018).

3. Ceramic Nanomaterials

Ceramic materials are suitable for producing hard tissue implants such as cartilage and bone. They are preferred over metallic and polymeric scaffolds due to their higher stability and superior chemical and thermal properties. Bioactive glass nanoceramics (nBG), bioresorbable nanoceramics, and bioinert nanoceramics are the different classes of nano bioceramics.

Biogels (nBG) are synthesised mainly by sol-gel technique, but other methods like microemulsion, laser spinning, and gas-phase synthesis are also helpful (Taygun & Boccaccini, 2018). nBG are desirable candidates for bone tissue engineering because they can induce differentiation of osteoblast cells. Moorthi et al. reported that nBG synthesised via sol-gel method induced differentiation of rat osteoprogenitor cells by stimulating the ERK pathway and cell cyclin-c gene expression (cyclin c). Furthermore, nBG also activated Runx2 genes of osteogenic progenitor cells and thus induced differentiation (Moorthi et al., 2012). Again, in another study, nBG induced the differentiation of osteoblast cells by stimulating micro RNA -30c of human osteoblast cells (MG63) and downregulating the genes such as TGIF2 HDAC4 genes involved in the negative regulation of osteoblast differentiation (Moorthi et al., 2013).

Hydroxyapatite, calcium aluminate, calcium carbonate, tricalcium phosphate, bicalcium phosphate, octa calcium phosphate etc., are some of the bioresorbable nanoceramic materials. Hydroxyapatite is the most widely used nanoceramic material for bone tissue engineering since it is the major component of natural bone (50-70%), also known as a bone mineral. Strontium, silicon, cadmium, silver, zinc, copper, magnesium, etc., have been incorporated with hydroxyapatite to modify the properties of nHA (Cox et al., 2014; Hidouri et al., 2018). In a recent study, Wang et al. developed a novel nanosphere consisting of strontium-doped hydroxyapatite/silk fibroin (SrHA/SF) biocomposite via ultrasonic precipitation method. The sr-containing nanosphere showed higher osteogenic differentiation potential than the Hydroxyapatite/silk fibroin nanosphere (HA/SF). SrHA/SF thus proved to be a potential bone defect-filling biomaterial in bone tissue

engineering (Wang & Wang, 2006).

Titanium oxide (TiO_2) and zinc oxide (ZnO_2) are bioinert nanoceramic materials with diverse medical applications. They can be synthesised using methods such as the sol-gel process, hydrothermal, solvothermal, or emulsion precipitation.

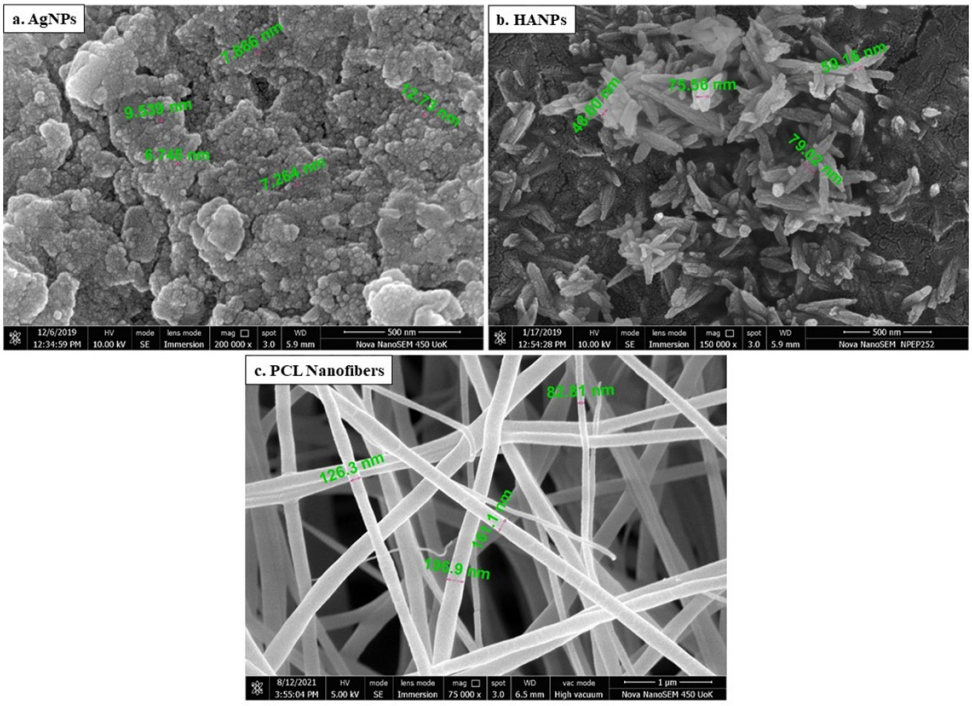

Fig 2; Scanning electron micrographs of a. metallic nanoparticles (AgNPs), b. ceramic nano particles (nano hydroxyapatite/ HANPs) and c. polymeric nanofibers (PCL nanofibers).

Applications of nanoparticles in tissue engineering

Robert Langer and Joseph Vacanti defined tissue engineering as an interdisciplinary field that applies the principles of engineering and life sciences towards developing biological substitutes that restore, maintain, or improve tissue function (Fisher et al., 1993). A functional tissue engineering triad consists of a scaffold, bioactive molecules (growth factors, antibiotics, ligands) and cells. Nowadays, many nanomaterials have been used in tissue engineering for tissue/organ regenerative purposes, especially in skin, bone, cartilage, neural, cardiac, liver, tendon, etc. this is because the materials in their nano form possess better biocompatibility and stable physical and chemical properties than the actual material from which it is produced.

a. Bone Tissue Engineering

Demand for functional bone scaffolds is increasing day by day due to bone-related diseases and defects such as osteoporosis, osteoarthritis, bone cancer and bone fractures. Conventional treatments use autografts and allografts for regeneration, but their drawbacks such as limited availability, difficulty in shaping, donor site morbidity have made these treatment strategies unsuccessful. A functional bone scaffold should contain four elements: scaffold, osteogenic progenitor cell and osteoinductive growth factors with

excellent mechanical strength. The development of 3D bone grafts, with compositions similar to that of natural bone through tissue engineering, was a milestone in orthopaedic surgery. Recently, nanoparticle-based bone scaffolds gained much attention because of the biological nanostructure of bone. About 70% of the natural bone matrix consists of nanocrystalline hydroxyapatite (calcium phosphates) with a size range of below 100nm.

Nano-encapsulated bioactive molecules as bone tissue engineering scaffolds are of great importance. Studies on silica nanomaterial scaffolds revealed their ability to encourage bone cell growth and the targeted delivery of bioactive molecules encapsulated in the mesopores. By encapsulating in mesopores, small biomolecules like growth factors, dexamethasone, vitamins, vaccines and mineral ions can be delivered to the target sites (Eivazzadeh-Keihan et al., 2020). In another case, amino acid ester polyphosphazenes entrapped nHA composite microsphere scaffold has been developed for bone tissue engineering application as a load-bearing scaffold. The composite microsphere sintered into a 3D scaffold showed osteoblast cell adhesion, proliferation, alkaline phosphatase expression, and bone regeneration (Nukavarapu et al., 2008).

Due to their higher mechanical and electrical characteristics and biocompatibility, carbon nanotubes (CNTs) are considered ideal for bone graft development. A study has observed that CNTs support the proliferation and adhesion of osteoblasts but inhibit or decrease the adhesion of other cell types (Price et al., 2003).

As a new class of bone graft material, nanocomposite scaffolds play a central role in bone graft evolution. In a study, 3D-printed porous scaffolds were synthesised using poly lactic-co-glycolic acid (PLGA) and TiO_2, where the addition of TiO_2 enhanced the wettability of the scaffold surface. They showed improved osteoblast proliferation and ALP activity compared to pure PLGA (Rasoulianboroujeni et al., 2019). Again, in another study, the porosity of poly (L-lactic acid)/β-tricalcium phosphate (PLLA/β-TCP) nanocomposite scaffolds was improved by combining thermal-induced phase separation and salt leaching techniques. The β-TCP nanoparticles enhanced the mechanical properties and bioactivity of the PLLA matrix; furthermore, the scaffold improved MG-63 osteoblast proliferation, penetration, and ECM deposition (Lou et al., 2014)

b. Skin Tissue Engineering

Skin, the human body's largest organ, functions as the first line of defence against pathogen entry. In addition to protecting against microorganisms, it is also involved in temperature regulation, water and electrolytes balance and responds to external stimuli. So, to protect the body, the skin has the capacity to repair and regenerate continuously with the help of stem cells present in it. Skin wounds/damages are classified into two; acute and chronic wounds. Acute wounds are minor wounds such as small cuts and burns, which will undergo rapid healing and will not retain a scar on the skin after healing. On the other hand, chronic wounds such as accidental wounds, diabetic wounds, severe burns and cuts, leads to severe inflammation and scarring and will take several months for complete healing. So for the regeneration and healing, the large number of scaffolds have been developed from natural biomaterials like collagen, chitin, hydroxyapatite, chitosan, alginate, gelatin, polypeptides and synthetic biomaterials like, poly (ε caprolactone) (PCL), poly(lactide-co-glycolic) acid (PLGA, polylactic acid (PLA), and poly (ethylene glycol) (PEG).

Nanocomposites prepared from both synthetic and natural polymers act as dressing

materials for wound healing and repair. These wound dressings work as an ECM and thereby provide a platform for neighbouring cells' penetration, growth, and proliferation. Furthermore, nanocomposites with antibacterial agents such as metallic nanoparticles may reduce further infection and inflammation of the wounded site.

Silver nanoparticles coated bacterial cellulose nanofiber scaffolds (AgNP-BC) comprises a new wound dressing material developed by Wu et.al, which showed significant antibacterial activity against Staphylococcus aureus. On applying AgNP-BC on a second-degree rat wound model, a significant reduction of wound flora and low inflammation was observed in the healing sequence. Further, in another report, Tasar silk fibroin nanofibrous mats were prepared and coated with silver nanoparticles to enhance their therapeutic efficacy (Wu et al., 2014).

Nano Phytochemicals (plant secondary metabolites) incorporated in wound dressings have come into vogue recently since most of these phytochemicals show anti-inflammatory, antimicrobial, anticancer, antioxidant, and wound-healing properties. In one study, curcumin nanoparticles were combined with a tripolymeric composite (chitosan, poly-γ-glutamic acid, and pluronic) that can be used as a delivery device for wound healing. In vivo experiments on Sprague–Dawley rats showed neo-collagen regeneration and tissue reconstruction (Srivastava et al., 2019).

Since nano encapsulated material helps in the targeted delivery, they set out a favourable approach in skin TE. The encapsulated materials act as a drug delivery system, allowing a more targeted drug delivery; further, coating the nanoparticles with polymers increases the amount of drug-loaded. Zahiri et al., 2020 synthesised a curcumin loaded chitosan nanoparticle (NCs/Cur) combined with an electrospun polycaprolactone (PCL) and gelatin (Gela). Human endometrial stem cells (EnSCs) seeded scaffolds showed good biocompatibility with the host immune system and wound healing ability in a full-thickness excisional animal model (Yang et al., 2017).

c. Neural Tissue Engineering

Regeneration of damaged nervous system is a massive challenge due to the complex physiology and limited regenerative capacity of nerve cells. Generally, polymers such as synthetic and natural polymers are used for regeneration purposes. Due to the advantages of biocompatibility, biodegradation, and mechanical properties, polymers are considered superior materials compared with ceramics and metals.

Nanotechnology helps develop nanofiber and nanotube scaffolds with exceptional biocompatibility and conductivity properties to improve neuron activity. These materials are used to encapsulate neural stem cells and Schwann cells to enhance nerve repair and regeneration. In a study, Yang et al. checked the efficacy of aligned poly (L-lactic acid) (PLLA) nano/microfibrous scaffolds using neural stem cells (NSCs) as a model cell line. Cell elongation and orientation were similar on both scaffolds; however, differentiation of NSCs was higher on nano PLLA fibres than on microfibres (Yang et al., 2017). Because of the controllable features, nanocomposite materials are the chief materials of choice amidst other materials.

Shokrgozar et al. developed chitosan/Poly (vinyl alcohol) reinforced single-walled carbon nanotube (SWNTs) nanocomposites for neural regeneration. By incorporating SWNTs into the polymeric scaffold, the scaffold exhibits better morphology, porosity, and mechanical properties. In addition, it enhances the biocompatibility and proliferation of human brain-derived cells and U373 cell lines (Shokrgozar et al., 2011).

Recently, a novel research strategy with conductive nanomaterials has been developed in neural tissue engineering. A new hydrogel scaffold was constructed from poly (2-hydroxyethyl methacrylate) (pHEMA), loaded with multi-walled carbon nanotubes (mwCNT) to introduce conductivity. The SHSY5Y neuroblastoma cells-seeded scaffold showed good viability and proliferation compared to the pure poly (2-hydroxyethyl methacrylate) (Arslantunali et al., 2014).

Future perspectives

In this review, different types of nanomaterials and their applications in various disciplines of tissue engineering have been highlighted. The drawbacks of traditional therapies for treating damaged or diseased tissue boost the evolution of tissue engineering and regenerative medicine. However, the hunt for better and ideal scaffolds with the potency to deliver biological molecules to the damaged sites is in pursuit. Unlike their bulk materials, nanomaterials exhibit higher biocompatibility/cytocompatibility, drug encapsulation, etc., all of which make them excellent materials for tissue engineering applications. Nanomaterials, such as nanotubes, nanocrystals, nanorods, nanofibers, and nanocomposites, were all developed for TE applications to achieve structural and functional re-establishment of injured or defected tissues that have lost their normal function. Recently, researchers are using nanotechnology to construct or regenerate whole or part of organs such as the heart, kidney, bone marrow, spleen, liver, and pancreas in vitro, but studies are in the premature stage. If so, the construction of functional organs in vitro will have a powerful impact in regenerative medicine and allied fields. Although using nanotechnology in TE hastens tissue repair, numerous questions/doubts and challenges remain unanswered. Still, researchers need to be fully aware of the cellular level of interactions with nanoparticles, subsequent immune response, potential toxicity, and effect on reproduction and foetal development. Comprehension of the primary mechanism of interaction between nanomaterials and cells at the molecular level will venture into great strides in the future of TERM.

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