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Review Article

Bioengineering Providing Tailor-Made Products

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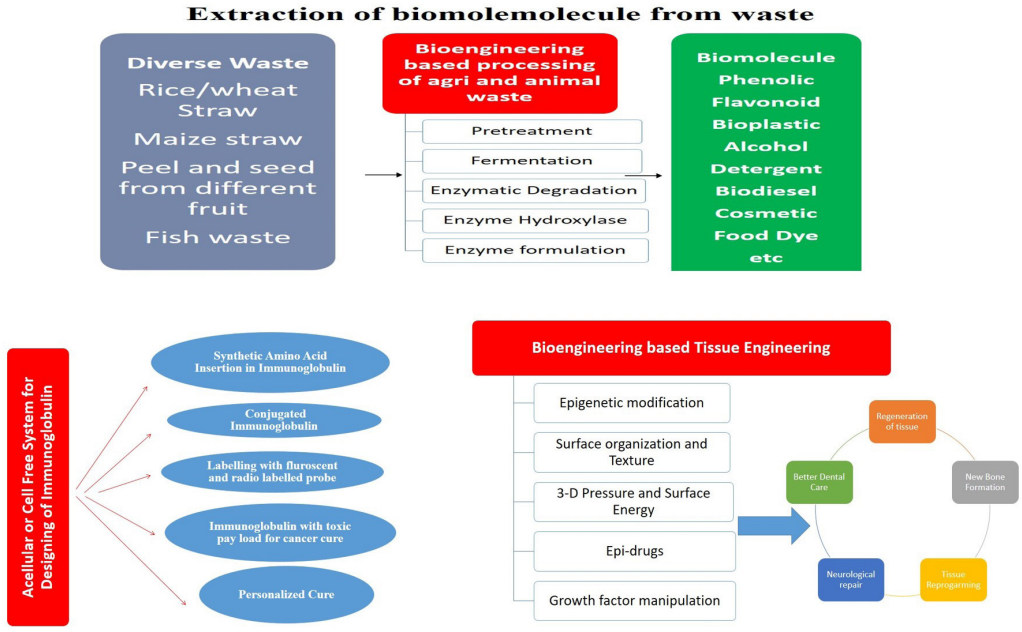
Abstract

Bioengineering is a new discipline of science that apply engineering principles and techniques on the biological process for development of valuable bioactive molecule, designing metabolites and changing cells or cellular metabolites to combat the current as well as future challenges and problem of mankind. This is one of the most challenging fields testing the researcher's intelligence, dedication, skill and creativity and courage needed to contradict convention and cut new pathways through unexplored scientific terrain. Bioengineering has the potential to provide economical processes and methods for marketable and industrial production of commercially important metabolites. Bioengineering is fulfilling the requirement for the mass synthesis of bioactive compounds and valuable products that have applications in diverse fields. These diverse areas range from cutting down the cost of established processes, providing cost effective source material, utilization of agricultural waste in technologies with an aim of strategic fabrication of industrial procedure concerning to commercial production of these valuable biomolecules. Bioengineering is contributing to different processes with wider base and huge application areas, these diverse applications together with their latest development are mentioned in this review.

Bioactive compounds find applications as drug and food additives, agricultural products, beautifying formulation, medicinal, and biofuels. Apart from this, synthetic or designed molecules have been prepared with a health promoting influence. These valuable bioactive compounds include antioxidants like flavonoids, carotenoids and anthocyanins along with natural metabolites like terpene, alkaloids, stilbenes, glycosides, sterols and bio-peptides including sugar moiety bearing glycosides. Bioengineering aids in exploration, biosynthesis, extraction and enhancing biological activities of these molecules which make it one of the most exciting and challenging disciplines in bio-engineered biomolecules. Diverse sources like plants, sea-weeds, bacteria and fungi are utilized for the extraction and synthesis of these mentioned biological products. The extraction and isolation of biomolecules poses difficulties from its natural source for instance these are generally deposited in very low amounts in plants, the qualities and composition differ from, plant to plant, location to location and species to species. Moreover, the synthesis and accumulation of these biomolecules are dependent on age and developmental stage of plant. The chemical synthesis has drawbacks like high-cost, health harm requiring multiple purification steps. This development in genetic engineering has achieved a new milestone, yet employing improved microbes for extracting biomolecules of industrial importance is a preferred alternative. At present, numerous unique approaches for bioengineering of plants and microorganisms have been utilized for the synthesis of natural and synthetic molecules from renewable biomasses. Recent disciplines like developmental engineering, pathway engineering including systems biology and synthetic biology are speeding up the synthesis and the manufacturing of valuable biomolecules. This topic focuses on providing an overview of bioengineering for the bio-manufacturing of bioactive compounds.

Graphical Abstract



Extraction of Active Biomolecules from Agricultural Waste

By-product and rest over waste from agri-industry have high amounts of biomolecules in them like sugars, fibrous compound, biolipids, carbohydrates, bio-protein, carotenoids, phenolics and other compounds, which have array of utilities due to their important bio-properties and might be used as source material for active biomolecules. Conceptually and theoretically, these mentioned biomolecules have potential to offer control and cure for numerous diseases namely abnormal blood pressure, irregular blood sugar level, heart ailments and disorders related to nervous system [1]. Moreover, these have utility as food additive, enhancing their nutritive value, flavor and technical virtues (e.g., modifying water or oil holding power, fortification and gelatinization). Bioengineering techniques play an indispensable role in improving the synthesis, isolation, and utilization of components from agro-industrial waste in a highly effective manner. Because of high specificity, bioengineering methods offer inherent benefits like the synthesis of bio-formulations having high quality and bioactivity with reduced toxication [2]. These bioengineering methods incudes: (1) enzymes assisted isolation that release valuable biomolecules from the material at optimum attributes, making the isolation more precise, (2) biodegradation using engineered microbes or improved strains that bio-transform waste into valuable biomolecules, like alcohol, proteins, bio-proteins, and plastids [3].

Multiple wastes from commercial food crops may be used as source to synthesize bioactive compounds, for example cereal husk, which contain high quantity of phenolics, flavanols, glucosides, and plastids waste from horticulture and eatable from plant source, contain high quantity of phenolics and carbohydrates [4]. Similarly animal wastes contain omega fatty acids (fishery wastes) and diary wastes are rich in bio-proteins [5]. The principle molecules occurring in agro-industrial left-over with potential utility in dietary and drug industry are (1) bioactive peptides, (2) phenolics (3) carbohydrates, (4) Bioactive-enzymes. Bioactive peptides influence the body function depending upon the type and sequences of their amino acids. Peptides displaying antioxidant activity in the human body are in high demand for their harmless and diverse applications [6]. The addition of these peptides in food can slow-down the presence and pace of oxidative reactions, which is of interest as these offer substitute for un-natural antioxidants their associated toxic effects on mankind. The oxygen scavenging properties of plant formulation are reported from diverse plants for example Glycine sp., *Helianthus*, Maize [7], and groundnut powder [8], including from animal unused parts from fishery [9] and chicken husbandry [10].

The antihypertensive action of bio-peptide having ability to suppress the renin activity both inside and outside the cellular environment raising the blood nitric oxide levels have been documented [11]. Due to this, peptides have utility in antihypertensive control and cure, minimizing heart related problems.

Phenolics are well known for their neutralizing oxidative entity, antihypertensive, and combating bacterial growth and chemoprevention [12]. Moreover, phenolics protects from rancidity and development of microbes in eatables, used in drug formulation and grooming products such as hygiene products related to mouth and eyes.

Carbohydrates provide energy for multiple activities of the organism [13]. These occurred abundantly in veggie waste, as different form of cellulose, lignin and glucans, [14]. Starches are an attractive moiety that has application in variable procedures of the industrial interest in different products including dietary products

[15]. β-Glucan has multiple health benefits. The vegetative parts and waste of cereals crop from Hordeum, sorghum after the harvest of economically valuable parts may be used to extract β-glucan [16,17].

Moreover, lipids, namely lycopene-type carotenoids are famous and attractive compounds of nutraceutical importance. Lycopene resides in tomato skin, an unused component from food and agri-industry [18]. Lycopene has an important application as it guards cellular metabolites by neutralizing free radicals and prevents degradation of nucleic acids [19]. All types of waste from tomato can act as potential sources of lycopene, for example the outer peel, including tomato seed [20]. Apart from these tomato waste can act as precursors of vitamin A, hormones, isoprene and dye; hence they are source of different commercial products [21]. The jackfruit's non-commercial parts can act as starting material for multiple valuable biomolecules namely ethanol, bioplastic, absorbents, bakery product and resin for beauty products [22].

Biological Process for Extraction of Biomolecules from Waste

In the above it has been mentioned that the majority of the valuable biomolecules resides in peel or husk. There is a high need to develop and standardize the extraction procedure to harvest these commercially attractive and demanding valuable biomolecules. The traditional extraction procedures like solvent based extraction, thermal and non-thermal energy based processes are costly and time consuming. Apart from these, the tough nature of cell wall and tight bond between different components namely cellulose, hemicellulose, lignin, starch, pectin, lycopene, carotene make the isolation a tedious and tough task.

Bioengineering has developed various microbes or enzyme assisted processes that have the ability to un-bound molecules, rupture cell walls, alter permeability, and hence making intracellular commodities exposed to extraction. The bioengineered microbes can use agri-wastes as sources under specified conditions to grow and produce the desired biomolecules. The active proteins extracted from microbes, plants, and mammalian cells has the ability to speed up bioconversion with high specificity, isomer selective and with desired attributes which are desired for isolation and precise purification of variety of compounds like phenolics, carotenes, isoprenoids, and able to perform their biotransformation to highly demanding products such as biodiesels, detergents and herbal medicine [23]. Few example, worth mentioning here include enzymes that are able to degrade pectinase, amylase, cellulose has been successfully used to extract caffeine and tannins from *Paullinia cupana* seeds [24]. Similar enzymes have been successfully attempted on grape skins for the extraction of anthocyanins and flavanols [25]. The pectin and glycoside degrading enzymes have been used on grape pomace for extraction of multiple products namely alcohols, esters, terpenes, etc. [26]. Proteases and cellulase enzymes have been used to extract Rosmarinic acid from *Salvia officinalis* leaves [27]. An essential oil has also been extracted via enzymatic extraction using Protein, cellulose, and pectin degrading enzyme from *Cyperus esculentus* [28]. Similarly cellulose degrading enzyme has been successfully employed to extract menthol from *Mentha arvensis* leaves [29]. The mango peel has been successfully used as source to extract phenolics by treating with different enzymes capable of degrading amylose, protein, lipid, cellulose, and pectin [30]. Pectinases have been used on the *Citrus maxima* peel for the extraction of flavonoids [31].

Production of Cost Effective Enzymes Using Bioengineering

The fermentative process of by-products provides cost effective microbial enzymes which are widely used to recover compounds of interest [32]. Different researchers documented the successful use of microbial waste to develop hydrolytic enzymes that are able to degrade protein, lipid, and carbohydrates [33,34]. These practices of enzyme extraction using agri-industrial left-over lower down the synthesis of price enzymes, making them more suitable to substitute synthetic and commercial substrates. The overall price pertaining to the recovery of desired biomolecules is also reduced, resulting in economical enzymes extract. However, if the downstream application demands pure enzyme extracts, it may raise the cost of overall procedure [35,36]. Bovine and goat milk are used as source material to synthesize bioactive peptides subjected to the protein degrading properties of different *Aspergillus* proteases [37]. Proteolytic enzymes of fungal origin resulting from the fermentation of wheat bran are successfully used in the hydrolytic cleavage of proteins present in milk. The proteins from milk displayed bacteriostatic and oxygen scavenging activities against a wide range of microbes, therefore an attractive enterprise having utility in biotechnology and industrial processes. The feather meal broth was subjected to solid matrix formation with the *Chryseobacterium* sp. Strain, the resultant protease preparation was used for the synthesis of soy protein hydrolysates [38]. The soybean meal (SBM) and corn husk (CH) were used as source material for the synthesis of valuable biomolecules like proteins and phenolics by the action of cellulolytic complex from fungi [39]. The cellulolytic enzymes were prepared from rice husk and bran via Solid State Fermentation employing *Rhizopus oryzae* CCT 75.

Bioengineering in Antibody Designing

Antibodies are well known for their contribution in disease cure, detection, and in clinical research. Presently, their role as biological probes is central to detect numerous interactions involving biological and biochemical reactions. The increasing clinical relevance of curative and detection antibodies has fueled the development of methods and techniques to provide biomolecules with greater precision for biological targets [40]. The methods pertaining to antibodies biosynthesis including antibody parts using prokaryotic and mammalian cells are well-established [41]. This technology of antibody synthesis demands greater time along with higher prices. To overcome these limitations bioengineering endeavors design and develop precise and highly productive non-cellular antibody generation methods. These systems have been proven as authentic, adjustable and highly influential antibody producing systems, capable of delivering peptides of diverse sources, types, and qualities [42-44]. These systems have been utilized for the manipulation of fragments or whole immunoglobulin [45,46] The prokaryote and eukaryote based systems have been developed for the generation of antibody with desired trait.

Prokaryotic Translation System

At present, cell extracts of bacterial origin are the primary source of recombinant immunoglobulin and complete immunoglobulin [41]. The pioneer work in this area was published by Ryabova and coworkers in 1997 [48]. These researchers evaluated the influence of various chemicals on the production of variable fragments Fv and antigen binding fragments (Fab) [49] which were originally taken from an anti-hemagglutinin antibody [50]. Their findings indicated that the incorporation of protein disulfide isomerase (PI) resulted

in a threefold increase in production of functional Fv antibody arms compared to a control reaction lacking PDI. Additionally, the presence of DNA interacting chaperones K and J enhanced quantity of soluble protein, although it did not affect the yield of active protein. Furthermore, a successful formulation combining different states of oxidized glutathione, PDI, and chaperones was developed, achieving approximately 50% recovery of total scFv antibody fragments with antigen-binding activities. This research laid the groundwork for the synthesis of scFv parts using laboratory environment via translation systems, thereby facilitating the alternation and evaluation of proteins through ribosome display [51]. Following this, anti-lysozyme Fv arms were synthesized by using a similar acellular translation system enriched with glutathione, PI, and chaperones [52]. Different research groups reported the cell-free synthesis of scFv antibody fragments in *E. coli*-based acellular translation set-ups [53,54]. The functional synthesis of Fab parts using a combined *E. coli* transcription/translation system has also been reported by different researchers [55]. The catalytic antibody 6D9 [56,57], specifically reacts with phosphonate analogue and transforms biologically-inactive chloramphenicol precursors to chloramphenicol. The *E. coli* strain BW25113 which is deficient for serine protease activity was used to yield scFv and 6D9 Fab fragments [58]. After this whole IgG immunoglobulin MAK33 with complete activity was generated using similar setup enriched with PDI, DsbC and the ER-specific chaperones Grp94 and BiP [59]. This was followed by the synthesis of the IgG1 trastuzumab in satisfactory yields in range of 1000 microgram per milliliter reaction volume [45]. The IgG1 immunoglobulin, Fv and antigen binding arms were able to interact with human Interleukins, produced in an adaptable transcription/translation system. These systems have the capability to scale up immunoglobulin synthesis from the microliter to liter range. These studies form the basic foundation for production of whole immunoglobulin and sub-parts using bioreactors having capacity of five liter and higher quantity (~800 µg/mL scFv, ~250 µg/mL Fab, ~400 µg/mL IgG). Following this ribosome display, techniques were adopted for the immunoglobulin synthesis and parts [60]. The immunoglobulin output from this synthesis was further increased in grams /liters by use of an improved *E. coli* strain having higher concentration of chaperone [61]. The formation of disulfide bridges and proper assembly of multi-domain immunoglobulin poses challenges in cellular and acellular synthesis set-ups. The presence of mRNA secondary structures and codon degeneracy cause RNA to translate at differential speed, due to this standardization of heavy to light chain ratios is must. The inherent flexibility acellular set-ups, net yield and synthesis of immunoglobulin chains RNA transcripts can be regulated to achieve the accuracy in assemblage of immunoglobulin light and heavy chains in proper orientation and configuration [62,63]. Furthermore, eukaryotic cellular translation set-ups have been employed to produce functional proteins with disulfide bonds, including scFv and Fab immunoglobulin parts, aiming to create a more conducive conditions for the folding of multi-domain immunoglobulin. Although current eukaryotic cellular set-ups exhibit lower productivity compared to *E. coli,* they outperform in the synthesis of soluble full-length proteins [64,65], as well as multi-domain immunoglobulin and those requiring post-translational modifications [66]. Additionally, proteins produced in *E. coli* carry the risk of endotoxin contamination, necessitating extensive purification processes before these valuable target proteins can be utilized in cellular assays or in diagnostic and therapeutic applications [67].

Eukaryotic Translation System

The eukaryotic acellular set-up used for antibody synthesis was usually developed from rabbit and rat lymph node. The serum from rabbit reticulocyte later became the primary translation system for the *in vitro* generation of immunoglobulin parts via ribosome display [68]. Nowadays, ribosome [68] and mRNA display [69,70] are the preferred *in vitro* display techniques, both have potential to translate genetic protein information into its respective phenotype. Following this sequence, scFv antibody arm was synthesized in acellular set-ups from wheat germ [71]. The researcher also standardized different translation parameters for the generation of peptide having disulfide-bridges displaying complete activity utilizing same system. Finally, a DTT-deficient wheat germ extract and the added PDI, found to be optimum condition, capable of satisfactory production of active scFv batch reaction [71].

Soon after, a new insect cell extracts eukaryotic translation system using cultured Sf21 cells was reported [72]. This eukaryotic translation system contains endogenous microsomal vesicles. This property of insect cell extract facilitates the translocation of generated immunoglobulin fragments inside the microsome organelles, providing target proteins contain an appropriate signal sequence [73]. As a result of this translocation event, the protein synthesis and protein folding occurred in different compartments, thus creating the synthesis event similar to *in vivo*. Two subsequent investigations [46,47] have demonstrated the production of functional scFv immunoglobulin parts through the use of the vesicle-containing insect acellular translation method. The importance of two elements is highlighted in the latter optimization of these systems: It was discovered that the addition of glutathione, the removal of the reducing agent DTT, and the protein sequence needed for translocation of desired proteins were important components. Unlike previously reported cell-free translation systems, the synthesis of functional target proteins did not require the inclusion of exogenous enzymes or chaperones, nor did it require prior treatment of the lysate with iodoacetamide. It was demonstrated that functional scFv molecules may be produced by starting with one antibody fragment model that binds to fluorescein [74,75]. It was demonstrated that PCR-amplified DNA templates could be used to create functional scFv molecules [47]. It has been discovered that adding the honeybee melittin signal sequence to antibody molecules causes the mobilization and accumulation of immunoglobulin parts in microsome. Signal peptide-induced translocation of the majority of the scFv antibody fragments examined in these investigations was found to positively impact protein functionality [46,47]. Similarly, employing a similar insect-based translation system, this positive effect was also demonstrated for the production of antigen binding arm [74]. Based on these studies, one may reasonably contend that proteins performing the synthesis and rearrangement of sulfide links *in vivo* [76], present inside the vesicle and are fully active.

Limitations of Acellular Systems

Each of these systems have their own merits and demerits, like bacterial expression systems are cost effective and deliver high quantity of simple recombinant immunoglobulin. Bacteria suffer from drawbacks like unable to incorporate changes that happen after translations of protein product [77]. Moreover, this system may synthesize non-active protein with the right molecular structure, resulting in synthesis of inclusion bodies. Endotoxins, generated

by bacteria in due course of expression, are cumbersome and hard to remove, this makes immunoglobulin purification challenging. These difficulties can be solved using Mammalian expression systems which are capable of synthesizing glycosylated as well as chimeric antibodies. These set-ups have higher costs and procedures are labor intensive. Mammalian expression systems are used for the most antibodies to manufacture glycosylated and complicated protein structures. The mammalian system has the unique capability to produce humanized native protein and perform modification at post translation level. These systems are expensive and require complex and time-consuming procedures, resulting in low production. The plant-based expression systems overcome these drawbacks and have lesser synthesis price as well as higher production. However, the glycosylation pattern of these plants is different from the human system, this adversely influences the immune reaction of recipients. This limits the plant-based system's capability to act as a production system for all proteins. The recombinant proteins can be accumulated in different parts of plant like Root, leaves, stem, fruit, and seeds. The extraction and isolation of recombinant protein is tedious due to the presence of phenolic and protein degrading enzyme, which might be non- tolerable by patients [77].

Conjugated Peptides

The synthesis of conjugated peptides, which is bounded with functional moiety like dye and metal ligands, or peptides attached to drugs and other proteins, has enormous application in biological, biotechnological, and biomedical usages [78,79]. For example, there is a high demand for potent antibody-drug conjugates, capable of delivering cytotoxic dose to malignant cells, has potential to revolutionize chemotherapy. Moreover, antibodies with cytokines or immune-reactive peptides can act as vaccines [80]. Kanter synthesized two scFv fusion proteins via cell-free extract which safeguards against a B-cell carcinoma. A tumor-derived Fv bound to macrophage stimulating factor (GM-CSF) and another Fv connected to a peptide produced from interleukin-1β have both been demonstrated to function as vaccines. To increase immunogenicity, both vaccines were chemically bound to keyhole limpet hemocyanin protein. Similar in efficacy to the traditional immunoglobulin generated in mammalian cells, these vaccines induced tumor-specific immunoglobulin activation in mice. After that, a different scFv construct was created that included fused tetanus toxin fragment C (TTFrC) [81]. The fusion protein that was produced using *E. coli* setup after synthesis provided immunity to mice against tumor and elicit anti-tumor humoral responses. These instances amply demonstrate the remarkable ability of these acellular set-ups to speed up synthesis of personalized vaccines. In the coming time, personalized vaccines shall be developed via PCR which are utilizing the transcripts from patient´s own DNA coding for immunoglobulin diversity arms [81]. The synthesis and scientific information of conjugated peptides are growing at fast pace and nearly twelve different monoclonal antibodies coupled with active load for tackling carcinogenic cells are in clinical trials [82]. The escalating requirement for cost-effective and sustainable biomolecules with sustainable carbon usage pathways and green energy regeneration can be obtained from these systems [83]. These systems are so flexible that they are equally suited for commercial to lab scale synthesis of target molecules. This is beneficial for tailored synthesis of medicine; it will be best suited for customized need-based synthesis of medicine for multiple niche drugs with low requirement for drug manufacturing industries to warrant building expensive cell-based infrastructure to produce them [84]. Acellular systems can be utilized at such a small scale that tailored on-demand production of drug for single patient would be feasible [85].

Labeling of Immunoglobulins with Synthetic Amino Acids

The acellular translation systems offer user-friendly tools to engineer the target protein with enhanced and targeted functions. The acellular translation systems enabled the labelling of target immunoglobulin with single or multiple synthetic amino acids co-translationally [86,87]. Whereas *in vivo* methods show limitation like toxicity or some amino acids unable to enter the cell, translation systems offer a reliable substitute, as they remain un-affected from cytotoxicity caused by amino acid substitutes [88]. The natural side chains generally belonging to cysteine and lysine of given proteins are used in synthetic posttranslational modification techniques to chemically conjugate with a specific payload. It adversely affects protein dissolubility and performance as the label is statistically incorporated into the protein at multiple sites, producing a non-homogeneous labeled product [89]. Due to these drawbacks, site-specific labeling techniques have evolved, for ease to incorporate an amino acid analogue at a single, defined location into a polypeptide chain [88,90]. The application of azide-alkyne click chemistry has proven effective in generating scFv-fusion proteins. In a notable study conducted in 2009, researchers illustrated the synthesis of immunoglobulins that were site-specifically labeled with *Gaussia princeps* luciferase using acellular set-ups [81]. Besides the production of conjugated protein, tagging at desired position can facilitate the attachment of immunoglobulin to ligands, probe or toxic payloads [91]. In this regard, bacterial set-ups have been shown to synthesize antibody-drug conjugates by incorporating p-azidomethyl-L-phenylalanine an analogue of phenylalanine into an IgG molecule, which is subsequently conjugated to DBCO-PEG-monomethyl auristatin, a cancer-targeting payload [92]. Alternatively, this *E. coli*-based system has also been employed for the labeling of scFv molecules with ^{11}C, a crucial element for positron emission tomography (PET) [88]. In another strategy, Fv immunoglobulin were co-translationally tagged by introducing lysine analogue using insect lysate during translation reaction [47]. This residue-specific labeling technique allows for the insertion of the fluorescent amino acid at multiple sites within the protein. Furthermore, specific amino acid can be labelled stop codon TAG sequence at the 3'-end transcript encoding the Fv arms. The addition of fluorescent amino acids has advantages like protein analysis can directly be performed using fluorescence spectrometry procedure, avoiding radiography analysis, therefore freedom from safety measure related to radiography. Recently studies were performed to analyze the effect of azide conjugation on anti-tumor necrosis factor α (TNFα) Fab (FTN2). The azides consist of synthetic amino acid namely; N6-((2-azidoethoxy) carbonyl)-l-lysine (Azk), Azidohomoalanine (AHA), para-azidomethyl-l-phenylalanine (pAMF) and para-azidophenylalanine (pAzF), these are analogue of lysine, phenylalanine, and alanine. The study claimed the precise insertion of amino acid at pre-determined site. The influence of inserted amino acid on different activities of FTN2 like its expression, overall shape, and activity was analyzed. The result claimed that the impact of Azk incorporation at different sites of the anti-tumor necrosis factor α (TNFα) Fab (FTN2) did not affect its expression, structure, and functionality. This research paves the way

for the precise labelling and synthesis of commercial conjugates such as fluorescent labels, radio-labels, and drugs enabling tracking of the synthetic protein or drug to be used in disease cure [93].

Tissue Programming and Regeneration

The other exciting area of bioengineering includes tissue and organ regeneration, which includes the method to regenerate tissues and organs by applying different biomaterials *in vivo* or *in vitro* models [94]. The Epigenetics procedures alter gene expression without alternation in the genomic sequences. Epigenetic phenomenon causes chromatin reprogramming in turn causes activation or inactivation of genes. Post-translation changes in histones involve acetylation, phosphorylation, methylation and ubiquitination of amino acids such as lysine, arginine, and serine respectively. Acetylation of histones activate genes transcription and is mediated by the specific transferases enzyme which add acetyl molecule to histones using specific deacetylases (HDACs) that cause removal of same. Similarly, during DNA methylation, methyl molecules are added to specified cytosine nucleotides of DNA strand, changing the activation of the DNA [94]. It's noteworthy to mention that epigenetic phenomenon is reversible and could act as potential treatment models for improving personalized medicine. The interfacial energy, geomorphology and arrangement of atoms on the surface are important factors that significantly impact the epigenetic pattern in cells and have an impact on tissue maturation and regeneration. Atomic arrangement and topology of the surface both affect surface energy, which is a crucial aspect of a cell's reaction to a biomaterial. Surface hydrophobicity controls cell attachment; less hydrophobic cell surface is better suited for integration with body liquids and protein attachment. Protein attachment to a surface modifies its energy and affects how cells react. According to [95], hydrophilicity was found to be linearly dependent on surface energy and to accelerate the osteoblast cell dispersion and elongation on glass slide polished with fibronectin, or a high energy surface. Glass has greater specific energy and more preference for water, making it water attractant and causing the escalation of water to decrease the interfacial energy. Similarly, the degree of surface unevenness raises surface energies given the surface is hydrophilic. Uneven titanium implants resulted in better bone calcification and mechanical stability because of greater surface energy due to better wetness that cause enlarge surface contact area as compared with smooth surface as a result of increased wetness, provide large surface contact area [96]. Surface energy influence multiple cellular processes like growth, differentiation, mineral deposition, including osteogenic maturation [97].

Apart from surface energy, cell function and differentiation are also affected by topography, atomic arrangement, pressure regime, and interfacial energy of material. The surface topography of a substrate at micro and nano-level meditates epigenetic modification in cells. When cells are cultured using micro-level substrates results in better redirecting of cells into induced pluripotent stem-cells (iPSCs) for utility in genetic correction treatment. Any surface with width, spacing and grooves size of ten micrometer increases redirection of cells into iPSCs. The aligned nanofibers also give a similar outcome [98]. It is interesting to mention here that cellular manipulation at nanometer scale reported more damage by changes in surface topography when compared to microscales or macroscales [99]. The scaffolds designed surface manipulated at nanoscale level may be useful in engineering of gums tissue [99]. Hydrostatic pressure is an influential signal for differentiating mesenchyme stem cells (MSC) into ultra-active osteoblasts and hence a method of choice for cell engineering. It has been proved that initiation of osteogenesis requires certain levels of minute force and frequency, i.e. a pressure 'switch', that is related to the physiological forces sensed by cells in their natural surroundings. A more detailed study is required to explore the underlying mechanism [100].

The change in the degree of histone acetylation is a governing factor that determines cell's reaction to matrix surface and architecture. Stem cell (Sc) cultured on micro-patterned, flexible substrates shown to have a low HDAC activity and heavy histone acetylation resulting in high level of gene expression in them. The physical pressing and pulling of the matrix modify the nucleus morphology and way of the histone acetylation depending upon the direction of applied force [101]. The elasticity of the matrix also affects the cells directly layered on matrix [102]. The Scs cultured on micro-grooved poly dimethyl siloxane matrix had a stretched nucleus morphology and low HD affects with high level of histone acetylation and gene transcription [101]. The Mammary epithelial cells culture using plastic matrix synthesize stress fibers and grow as a single-layer on the matrix, whereas same cells cultured using three dimensional laminin rich extracellular substratum with two dimensional design have a rounded shape. Therefore, histone acetylation has correlation with cellular shape [103]. The mesenchymal stromal/stem cells (AMSCs) from adipose-tissue cultured using 3D porous titanium discs and 2-D tissue culture polystyrene dish *in vitro* showed a difference in histone methylation, and expression of bone extracellular proteins genes [104].The levels of acetylated histone H3 cancer stem cells is higher when cultured in monolayer, and upon culturing cells in low-adhesion conditions (holospheres), H3 histone acetylation is lowered [105]. The level of histone methylation is also dependent on the surface topology. The titanium dioxide (TiO_2) with 70 nm furrow affected the histone methylation to promote an osteogenic differentiation of human adipose stem cells hASC [106]. The culturing of cells on steep furrow results in stronger histone acetylation of fibroblasts in comparison to smooth exterior. This increase correlates with lesser histone deacetylase activity and leads to formation of more contrasted cells owing to mesenchymal-to-epithelial transition and activation of epithelial-associated genes [98]. Furthermore, histone methylation levels were influenced by the surface characteristics of the matrix. Specifically, titanium dioxide (TiO_2) having 70 nm furrowed surface, increased histone methylation, causing human adipose stem cells (hASC) to differentiate in osteogenic cells [106]. The type of biomaterials also modifies cellular epigenetic patterns, titanium and TiO_2 are highly studied materials, whereas silica, glass, and graphene are least studied. The DNA destruction response and the phosphate incorporation of Histone H2A.X (γH2AX), are tightly linked with epigenetic modification. During DNA damage, γH2AX is the first to activate, hence act as an important marker. The effectiveness of γH2AX during damage can be regulated by acetylation of histone which is an epigenetic phenomenon. Increased levels of acetylation at 56 amino acid lysine (H3K56ac) enhanced the DNA repair in SCs [107]. Therefore, γH2AX/H3K56ac interaction is considered crucial in controlling cell's sensibility to DNA repair. Beside this, exposing cells to TiO_2 particles influence histone acetylation resulting in more damage to genomic DNA.

Histone acetylation is induced by the silica substrate, which also promotes nuclear elongation and cell alignment [108]. Silicon wafers

featuring microfurrow cause acetylation of histone, hence increasing the cells' sensitivity to HDAC inhibitor treatment. Gene expression in osteoblasts treated with silica, calcium oxide, phosphate, and hydroxylapatite (HAp) nanoparticles revealed a distinct expression. There was a documented increase in DNA methylation of the ALP promoter region and decrease in alkaline phosphatase activity [109]. By inducing the microRNA miR-30c, nanobioglass ceramic particles increased osteoblast development by reducing HDAC enzyme levels [110]. Cell development and differentiation were accelerated using graphene, a substrate consisting of 2-D carbon atom structures [111]. In cellular reprogramming experiments, it was found that the presence of graphene increased histone methylation at the gene promoter region responsible for the mesenchymal to epithelial cell transition in mouse fibroblasts grown on glass [111]. Additionally, graphene improved the staining of alkaline phosphatase in iPSCs, suggesting a possible instrument for the regeneration of bone tissue.

Gene Therapy

RM focusses on utilization of biomolecules to bring desired changes in cells with an aim of enhanced tissue revival by exploiting specific biochemical processes. Multiple organs can be targeted for whole body phenotype using epigenetic processes. The target organ will be responsible for epigenetic remembrance for complete body, restoration of their un-regulated states to normal may lead to correct tissue regeneration state. This is approachable by eradicating damaged or dead cells by using specific drugs [112] or by engineered chimeric antigen receptor (CAR) T-cells specialized in removing dead cells [113]. Bioengineering of these cells involves molecular-targeting, remodeling of chromatin, and reprogramming. The epigenetic states of damaged cells can be altered via epigenetic drugs [114] that alter DNA methylation or histone editing states in the cells. Moreover, drugs that manipulate target proteins, with active role as memory controller might be utilized. Moreover, CRISPR-based editing technology can also be used to alter the chromatin states of target cells [115]. The scaffolds carrying micro molecules such as latest epigenetic drugs (epi-drugs), may cause cell to differentiate and regenerate by improving and reprograming of epigenetic process [116]. The FDA has permitted Silica to be applied in a delivery tool for 5-aza, an inhibitor of DNA methylation. Scaffolds have wonderful properties of drug immobilization of micro-molecules like nucleases, or viruses, therefore have great potential to act as model delivery system in RM, hence can deliver molecules to the target site having tissue damage [117]. The structural features of the scaffold can additionally contribute to the induction of epigenetic changes pertaining to cellular behavior, gene expression, including tissue regeneration. The extracellular delivery of micro-molecules, like HDAC is carried out, and fabricated in 3D-scaffolds, along with microparticles and genetically altered cells [117]. Local delivery methods also minimize the possible harmful consequences of the HDACi [118]. Histones fabricated on microspheres aid in adhesion, proliferation, and network formation. [119] This serves as a new strategy to use epigenetic substances to get desired surface changes in designing of scaffold and tissue revival [120]. The different FDA approved epi- drugs are undergoing clinical trials for the treatment of cancers [121]. HDACi has the ability to inhibit HDACS hence prompt gene-transcription. HDACi, namely TSA, Valproate, and MS-275, have been studied for their tendency to influence osteogenesis and found promising molecules for specific as well as systemic bone-repair [122]. The animal-based studies related to rheumatoid arthritis, revealed a beneficial effect of HDACi on both bone and inflammation, this may be useful in the treatment of bone and tissue damage simultaneously, therefore, open up a novel approach in bone regeneration [118]. The bone marrow cells treated with either TSA or 5-aza-dC programmed to develop into osteogenic and chondrogenic cells respectively [123]. This imply that epigenetic compounds can be used to induce cellular differentiation into separate cells lineage from mother stem cells. The application keratin and 5-Azacytidine on cell-surfaces made the hMSCs differentiate into a cardiomyocyte lineage [124].

Valproic acid (VPA) presents a secure molecule with clinical application and induces *in-vitro* nerve regeneration using a silicon tube joining two neuron dendrites. The presence of VPA in the silicon tube forms micro-climate that aid in correct shape and orientation of nerve bundles [125]. The HDACi treatment of Collagen sponges and porous calcium phosphate scaffolds initiated woven bone formation and new bone adjacent to scaffold [103]. The addition of HDACi to collagen preserves the euchromatin structure, hence guards against undesirable effect of the muscle tissue [126]. The HDACi can be useful in gene transfer using lentiviral vector as it was found to restore genes expression and act as novel tool to improve *ex vivo* gene manipulation [127].

The porous type I collagen (COL) scaffold activated with heparin (HEP), and different growth factors related to fibroblast (FGF2), and vascular endothelial cells (VEGF) (COL-HEP/GF) showed better pre and postnatal skin regeneration in a fetal sheep full thickness wound model. The spina bifida is a developmental disorder and causes missing tissues (skin) and expose the spinal cord to the amniotic fluid, which can negatively impact neurological development. Medical sealing of the skin reduces the nervous damage but results in permanent tightness in tissue. COL-HEP/GF affects different bioprocess concerning wound cure. The multiple gene clusters linked with spina bifida that regulate cell signaling and extracellular matrix arrangement, was identified using protein-protein network interaction, hence help in this abnormality [128]

Future Considerations

With the emergence of latest approaches like next generation sequencing technology, metabolomics, metagenomic and multiomic technology, the gigantic information pertaining to microbes and their functioning is generated every day. It is plausible to argue that future endeavor of bioengineering will further reduce the cost of biomolecules and bioprocesses as well as better management of wastes including E-waste. Better and cost-effective setups will be developed for designing synthetic. There are diverse sources of waste from agricultural and animal origin, these fields demand more serious and elaborative studies for development of processes for waste processing from these sources. There should also be more emphasis to lower down the cost of process pertaining to extraction and purification of biomolecules from waste, every extracted component must be evaluated for its possible application in different fields to its maximum potential.

Immunoglobulin holds the potential to treat old and current medical issues of the global community. The conjugated antibody will provide more accurate disease detection and cure. The expression system based on insect and plants are among the favorable options for their synthesis. The systems need to improve in a drastic way as they are still at initial level of development, the revolutionary influence of

these immunoglobulin is expected in healthcare as they are advanced, effective, and more economical. To meet the need of increasing demand of conjugated antibodies and shorten the time required for their development various workflow have been developed. The new acellular based interconnected workflow model for immunoglobulin biosynthesis and assessment to ease the screening of newly developed immunoglobulin for accelerating immunoglobulin discovery have been reported recently [129]. This workflow holds unique advantages like; cell-free amplification and assembly of coding transcript (DNA), systems capable of disulfide containing Immunoglobulin from linear DNA templates, Luminescent based Assay to identify rapid protein-protein interaction, characterization of crude protein purification and acoustic liquid handling for a highly parallel and miniaturized workflow. The developed workflow is so efficient that antigen binding of several immunoglobulins can be evaluated within 24 h by a single technician. The developer claims to test the workflow previously and newly developed immunoglobulin [129]. A better design with compatible payload using this type of workflow can accelerate healthcare diagnostics and drugs in a revolutionary way in near future.

Tissue engineering and regeneration helps in recovery of the lost and accidentally damaged organs of the human body. Although tissue engineering has been discussed in light of organ and tissue engineering, this field is equally useful to multiple biological processes. Epigenetic memories are universally occurrence to replenish maintenance and working of physiological and pathological condition. Especially, T-cells having importance in adaptive immunity, behave like stem cells in different ways [130] like origin, lineage determination, and cell exhaustion. As such, tissue and organ manipulation may be utilized to alter immunity in old patients and rejuvenate their overall immunity. More exploration and understanding of the epigenetic phenomenon will be useful for developing better delivery systems for epi-drug. The revolutionary development pertaining to personalized treatment is still awaited.

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