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Biocatalysis and Biotransformation for Pharmaceuticals Synthesis

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Abstract--- *The strategies of biological manipulation bio-catalysis for synthesis of pharmaceuticals has raised in previous years. This review covers the following methods to candidate catalysis genes coding to enzymes in high expression to modified the microorganisms genetically. The operation will tend to create a new bio-therapy used as catalysts for synthesis a new pharmaceutical assessable product, on both a laboratory and a commercial scale.*

Keywords--- *Biological Manipulation, Pharmaceuticals Synthesis, Bacteria.*

I. INTRODUCTION

Qualitatively, Bacterial enzymes have several significant effects on health, like radical scavenging activity, anti-allergic effects, anti-tumor, anti-platelet, anti-microbial, anti-oxidant, anti-viral, anti-ischemic, anti-inflammatory, in addition to (1) the estrogenic effects.

Often, the sitting employments have been coherently limited because of the reduced solubility, also immovability in the hydrophilic media. Furthermore, (2) the bio-transformation and the bio-catalysis related to metabolic products were provided for modifying their structure, also increasing the wild structures dissimilarity, that might be altering the physicochemical charterers in addition to improving the bio-equivalence have been differenced of biological properties of maternal compounds.

It can take advantage of an expanded range of strategies for the introduction of versatile into the starting gene(s) are available, and these can be widely branched into two classes; (i) non-re-combinative models and (ii) re-combinative methods, and can determined from creating libraries with as few as 200 variants to many tens of thousands of distinction structures (3, 4).

Moreover, its deciding where to introduce (5,6) mutations or where to run a new recombination is vigorously being scanning by forming experiments and computational methods. These models are exactly being successfully used for combining of novel proteins for bio-catalysis (7) and a new life for cellular compounds (Fig.1).

The review has shown bio-catalytic structural modification in cell by multiple sorts of enzymes or other microbial products, the approach and probable mechanism will discuss at different models (5,7).

Microbial growth is rapidly having ability to rise products and easy to be scale-up. Therefore, metabolic engineering of microorganisms provided a substitutional method of support valuable wild products that occur at low levels in wild type one, in exchange for bio -catalysis synthesis.

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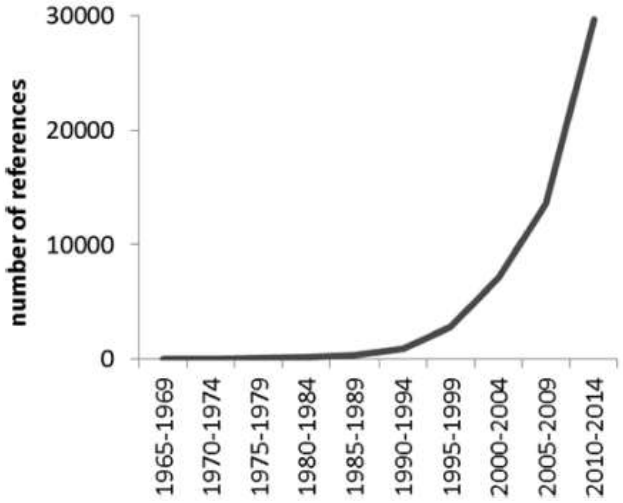

Figure 1: Number of Publications and Patents Discussing "Pharmaceutical Biocatalysis" for each 5 Year Period of the last 50 Years

A. Genetic Modification Models

1. New Genetic Recombination

Genetic recombination utilized with novel mutagenesis and recombination approaches which are recently indicated. Detailed outline regarding the new methods to create additional distinctive mutant libraries has been provided for modifying a lot of bio-catalyzed optical pure amines with the use of various enzymes (amine transaminases, imine reductases, amine dehydrogenases, and reductive amidases (8).

The microbial transformation can be considered as one of the efficient tools along with enzyme with regard to structural modifications regarding bioactive wild and artificial compounds. Previous explicative was meander developed microbial factories for posing benefits like high level productions on the wild product's bio-transformation, simple cultivation (9), fast growth, and simple genetic manipulations. (Fig.2)

For example, *Aspergillus* and *Bacillus* have been the significance of selection in the directed evolution approaches has been specified such as β-lactam acylases (9).

2. Gateway in Gene Cloning

Last a few decades, the survey of proteins function defiantly, includes the utilizing of a cloned differenced genes for proteins expression as well as functional assays. The approach has been of high importance in the case when datum detect related to the function has been limited. Also, it specified new proteins which are revealed through the genomics requiring fast approaches in comparison to conventional single gene applications, the productions have been required for rapid, reliable, and flexible systems of cloning, such processes of the open reading frame (ORF) clones might be used with the elevated-throughput proteomics platforms, like cell-based assays and protein microarrays, for answering certain biological questions. (10) Such datum might be providing the setting with regard to DNA cloning, discussing the main systems of high-throughput cloning (Creator™ DNA Cloning System, Flexi® Vector Systems, and Gateway® Technology), also comparing them in other sides (fig. 2). For example, given that

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twenty different amino acids can occupy each regions in a protein, the number of possible different of even a small, 100-residue catalytic enzymes are 20100 - more than the number of atoms in wild protein (11).

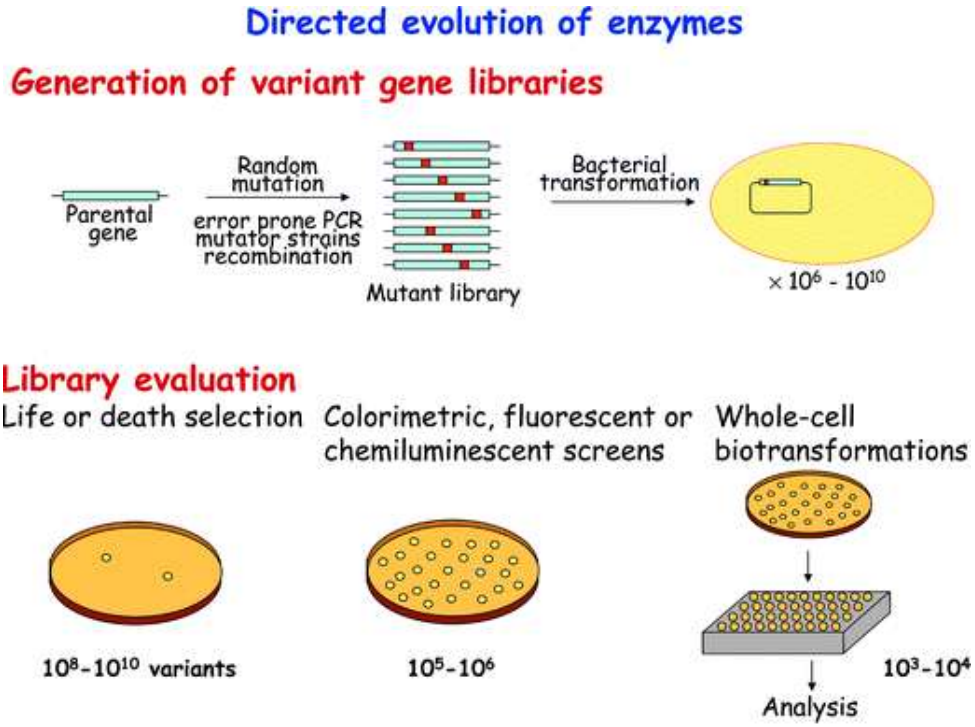

Figure 2: Direct Evolution Catalytic Enzymes

With the use of expression clones, the proteins might be created in the cell-free (*in vitro*) or the cell-based (*in vivo*) models. The latter are relying on providing plasmid in the cell, while translation machinery and cellular transcriptional is going to lead to protein synthesis. Eukaryotic and prokaryotic systems were utilized for the protein expression, some of them are yeast, insect, bacteria, plant and mammalian cells, whereas in the former is considered a collection regarding all the compounds for the translation, also requiring RNA as template. Furthermore, there are 2 steps to show the cell free protein expression (RNA's transcription, after that translation) or combined in one step, with the use of pairing systems.

In comparable scope, such distinctive issues regarding the microbial bio-production of the cholesterol to coprostanol, which is metabolite feebly that can be absorbed via the intestine of humans, enabling it to have effect on cholesterol metabolism as well as the modulation regarding the levels of cholesterol. Such bio-transformation has been examined in available schooling cholesterol-metabolizing bacteria, also in their related genes. Therefore, using it is providing the ability for designing new hypocholesterolemic approaches which might be finished to the departed explicative prescription related to statins. It considering a leap in pharma synthesis which it takes advantage of microflora bacteria modification and result safety drug has replaced other medications and surgery solutions (12).

In example, the following strategy had achieved in vitro was transforming bacteria *E.coli*, *Streptococcus Sp.*(microflora, probiotic) by the existence many copies of an genes´s enzyme referred to as the bile salt hydrolase

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were leaded to remove gallbladder stones which consist of 70% cholesterol and treat hypercholesterolemia when over expression of bile salt hydrolase genes was released. It might profiter specific species regarding indigenous microflora, involving several bifidobacteria and lactobacilli, also it comes with the capability for deconjugating the bile salts for achieving such actions is on the basis of bile salt hydrolase (BSH; cholylglycine hydrolase; EC 3.5.1.24) which is catalyzing the hydrolysis regarding glycine-and/or taurine-conjugated bile salts to amino acid residue and bile acid(13) .

Probably, it can put many solutions to treat many diseases when it has accurately employed genes of catalysis enzymes treatment , it similarity have identified a genetic determinant in probiotic bacteria that contributes to bile salt resistance by examined this possibility by analyzing hydrolysis of conjugated salts in media using 0.5% Ursodeoxycholic acid was using cholesterol of gallstones (13,14) like dissolved therapy and *bsh* genes estimation depend on, and studied the transformational bacteria to push up bacterial *bsh* gene expression to highest level to convert to gallstones cholesterol dissolving drug (table 1).

Table 1: Explain the gallbladder stones reducing concentrations in bacterial media in vitro for several species of wild type and transformer (microflora and probiotics) strains which have bile salt hydrolase genes expressed for biocatalysts enzyme responsible for cholesterol reducing

Bacterial grow thing media types	***S. parasanguinis Reducing ratio%***	***E.coli Reducing ratio%***	***S. mutans Reducing ratio%***	***S. byogenes Reducing ratio%***	***S. salivarius Reducing ratio%***	***S. feacalis Reducing ratio%***
Wild type bacteria without stone, bile salt and free cholesterol	**1%**	**1%**	**1%**	**1%**	**1%**	**1%**
Wild type bacteria with stone, and 0.5% bile salt in media	**16%**	**64%**	**47%**	**45%**	**78%**	**61%**
Transformer type bacteria with stone and 0.5% bile salt in media	**24%**	**82%**	**56%**	**46%**	**82%**	**63%**

I. Hyper-epigenetic Modification

The epigenetic modifications showing the inherited change in the gene's expression with no change in the DNA backbone or the sequence, involving X-chromosome inactivation, histone modification, DNA methylation, genome imprinting, as well as microRNA regulation, in which the DNA methylation, also histone modification have been of high importance in the cancer and neurological diseases (15,16).

Development models and epigenetic drug discovery were of high interest recently. The possibility for using new therapeutic approaches is efficiently reverse transcriptional in addition to epigenetic abnormalities which have been caused in a lot of human's disorders (17). Epigenetic targets have been of high importance in drug ability for drug able targets to emerge in 10 years. Such purposes have been turreted for the oncology researches, also have certain effect on the cardiovascular, neurological, metabolic, and inflammatory disorders (18,19). Effective epigenetic drug studies requiring tools of high-quality, involving epigenetic assays, validated antibodies, genetic recombinant with regard to proteins and enzymes modification (fig.3).

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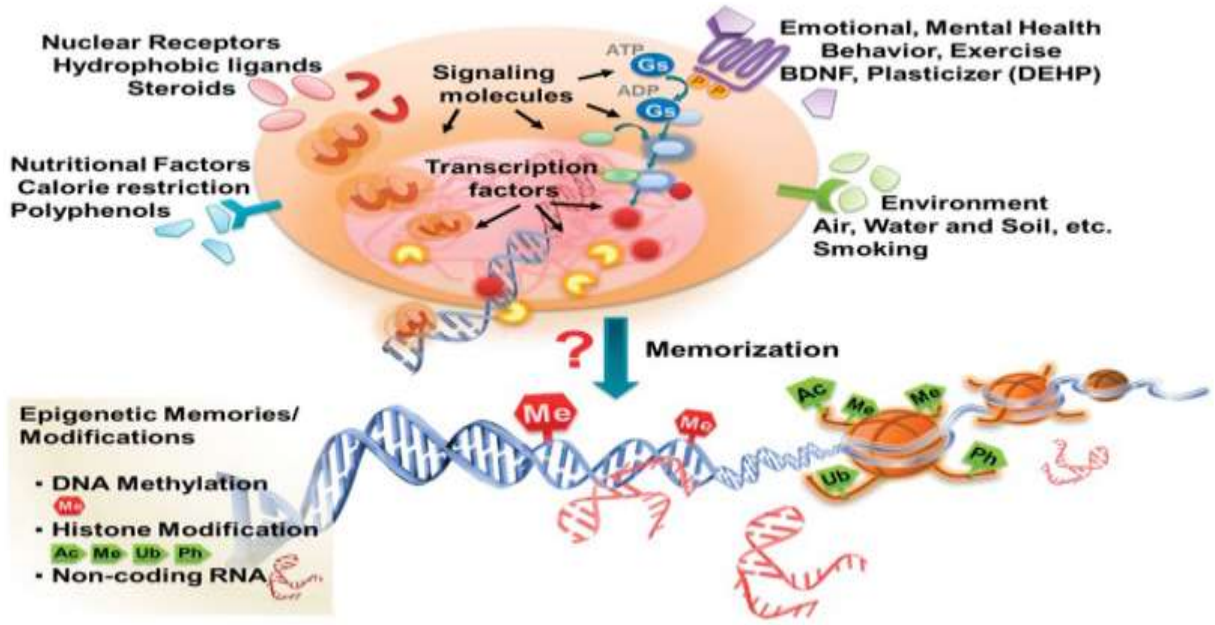

Figure 3: Signal-governed epigenomic changes. Environmental cues can be transduced into cells, relayed into nuclei, and memorized as epigenetic marks. The unknown mechanism by which external signals are translated into epigenetic codes is marked by a red question mark.

Understanding how these catalytic enzymes are regulated in both normal physiology and disease. It has been indicated the biological systems approach with the use of microarray analyses regarding methylation patterns and gene's expression are taken a well fundamental importance and may offer therapeutically odds (20,22).

II. Other Epigenetic Models

Epigenetic marks have been stablishing causal relationship with genes transcription ways molecular procedures, that might be modifying the precise genomic regions. Therefore, considering a novel model such as CRISPR/dCas9-based toolbox with regard (22,23)to direct gene regulation and epigenetic editing. It promoted a system related to the expression of orthogonal dCas9 proteins that are fused to different effect or domains, also involve multi-gRNA system with regard to the simultaneous targeting dCas-9 orthologues (24)for up to 6 loci. The technology of CRISPR-Cas9 has been rapidly changing the perspective for the way that bioengineers and scientists are studying and manipulating genome. Taken from bacterial adaptive immune system, the CRISPR-Cas9 was co-opted and aim for various functions such as regression or simulation of gene expression (referred to as CRISPRi or CRISPRa, respectively like RNA interference (RNAi) (25)or the gene over-expression vectors leading to initialize exploring the potential of CRISPR in developing and evaluating broad-host-range CRISPR/Cas9 gene-editing tools for the purpose of enhancing genetic-engineering abilities of the biopharmaceuticals faces(26).

II. POST-TRANSLATIONAL MODIFICATION (PTM) MODEL

The number of the verified bio-pharmaceuticals, in which the product quality attributes are of high importance, and steadily increasing. In various expression hosts (27,28,29), bio-pharmaceutical's productions are facing a lot of limitations in terms of PTM, whereas various bio-pharmaceuticals demanding various specifications and forms of

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PTMs for functioning properly(30). With the development of the technologies related to genetic engineering, there is a possibility now for addressing general in addition to host- or biopharmaceutical- specific product quality problems.

A. Proteins Engineering

Designing novel catalytic enzymes or proteins have needed protein engineering is liked desirable or new function. It is standed up recombinant DNA promoting for changing the amino acid sequences. Multiple changes in protein engineering trials have been provided, due to the rapid advancements in the biological sciences, particularly, the technologies of recombinant DNA (31).

The other and most significant factor affecting the speed of come true for bio-catalytic procedures has been the protein engineering (32).

Bio-catalysis has been moving through 3 phases. These phases have precipitated for modifying or engineering a protein; the properties were changed for suiting our requirements. Its summarize need some key points here to classify bio-catalytic according to last phases. The first phase has involved naturally occurring biocatalysts for mediating the required transformation. Also, the chemical modifies used for activating type enzyme's normal behavior for the purpose of converting substrate to required product. The second phase has been the techniques of protein engineering that are incited via structural datum utilized (34) for expanding substrate scope regarding bio-catalysis to the non-wild compounds (table 2).

Table 2: Classification of Biocatalysis types

Classification of types of **Biocatalysis**			
1	**Traditional Biocatalysis**	Conversion of natural products into other natural products.	Uses natural reactions and pathways.
2	**Broad-substrate range Biocatalysis**	Conversion of chemical intermediates (non-natural products) into other chemical intermediates.	
3	**Multistep Biocatalysis**	Natural products into fuels, materials and chemical feedstocks (non-natural products).	Uses non-natural reactions and pathways.

The 3rd phase rev biocatalyst optimization steps using the fast generation regarding enzyme´s mutants with the novel techniques of molecular biology selective and combined pressure through screening conditions enabled for the enzymes for having the required properties and at fast rate(35).

The fourth phase dose mean the proteins' have get chemo-catalysis counterparts consisted of extremely large 3D structures which does multiple fulcrums of reacted with the substrate, through protein engineering can make a

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computational design approach to the D-amino acids have been included. Also, all the amino acids have many forms to appear in levorotary (L) or dextrorotary (D) forms. These structures have helped to degradation resistance properties that come from such approach as D-proteins have not been specified via their L-protein interaction partners (like proteases).

Since the proteolytic degradation has been a main obstacle to deploy proteins as the pharmacological agents D-protein characters replaced considerable benefit for biomedicine design (36,37). Furthermore, an ensemble regarding radical structures representative of protein has bene utilizing full antirational space indicating that the number of beneficial mutations that are predicted for renew modelling have been implemented with the use of Python based interface(38).

B. Designed Whole-cell Biocatalysts

The whole-cell biocatalysts requiring that several or single enzymes to be built in the host cells for constructing synthetic pathways with regard to conversion of required feedstocks to targeted products. Also, the metabolic engineering in addition to the synthetic biology are enabling rational organizing, also constructing biosynthetic pathways which are optimizing continued pathway to the products through offering pre-optimized mastership cells which simulate the production of required compounds. The approaches which are utilized in selection and design of whole-cell biocatalysts (39).

The steered evolution methodology is the ability to co-evolve enzymes in biosynthetic pathways. In one experiment was *E. coli*. Showed how whole-cell biocatalysts can be developed for realization of valuable chemicals for carotenoid to enable to expulsion of L-methionine.

The greatest challenges in many catalytic processes were indicated in enantioselective synthesis of organic compounds, like reactions with transition metal catalysts. The researches suggest that bio-catalysis might be using isolated enzymes or whole cells (plants, microalgae, bacteria, and fungi) as catalysts in the organic reactions (40). Often, such artificial strategy is providing high enantioselectivity transformations; also, other benefits like the potential of production and recycling of eco-friendly wastes, reduced toxicity, and benign reaction conditions are making the bio-catalysis a tool of high importance.

III. STEM CELLS MODIFICATION AND BIOCATALYSTS

The scientists are using stem cells — which have the potential to become many types of cells — to improve the discovery process for small molecules (41,42).

Researchers are converting patient stem cells into specific cell types affected by disease — for instance, neurons for neurodegenerative diseases — so that they have a model of the disease in a petri dish. Then, researchers use the cell models to rapidly screen thousands of small molecules, searching for drugs that improve the cells' condition (43).

All investigated facts explicative regarding tissue engineering has been developing materials which are retaining or better tissue function. Selecting adequate cells, 3-D substrates, also the induction of appropriate signal for re-generation tissues have been of high importance in the tissue engineering. Therefore, the stem cells have been the

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initial step for researches in such field (44). Fetal as well as mature stem cells, that have been changed from the adult diverted somatic cells in a lot of researches, making them adequate stem cells source with regard to cellular therapies and tissue proofing.

In last years, it was created over possible of human embryonic stem cells in the medical transplantation for therapeutic effects. For example, hearing loss occurs when certain cells in the inner ear get damaged because they cannot build themselves. Researchers are developing small-molecule drugs that encourage those cells to regenerate (45).

It´s can be used for human embryonic stem cells as "catalysts" for promoting biological repair as well as regeneration in the transplantation therapy (46). Yet, the immunological hold back against allogenic transplantation, in addition to the teratogenic potential of human embryonic stem cells posing major technical challenge (fig.4).

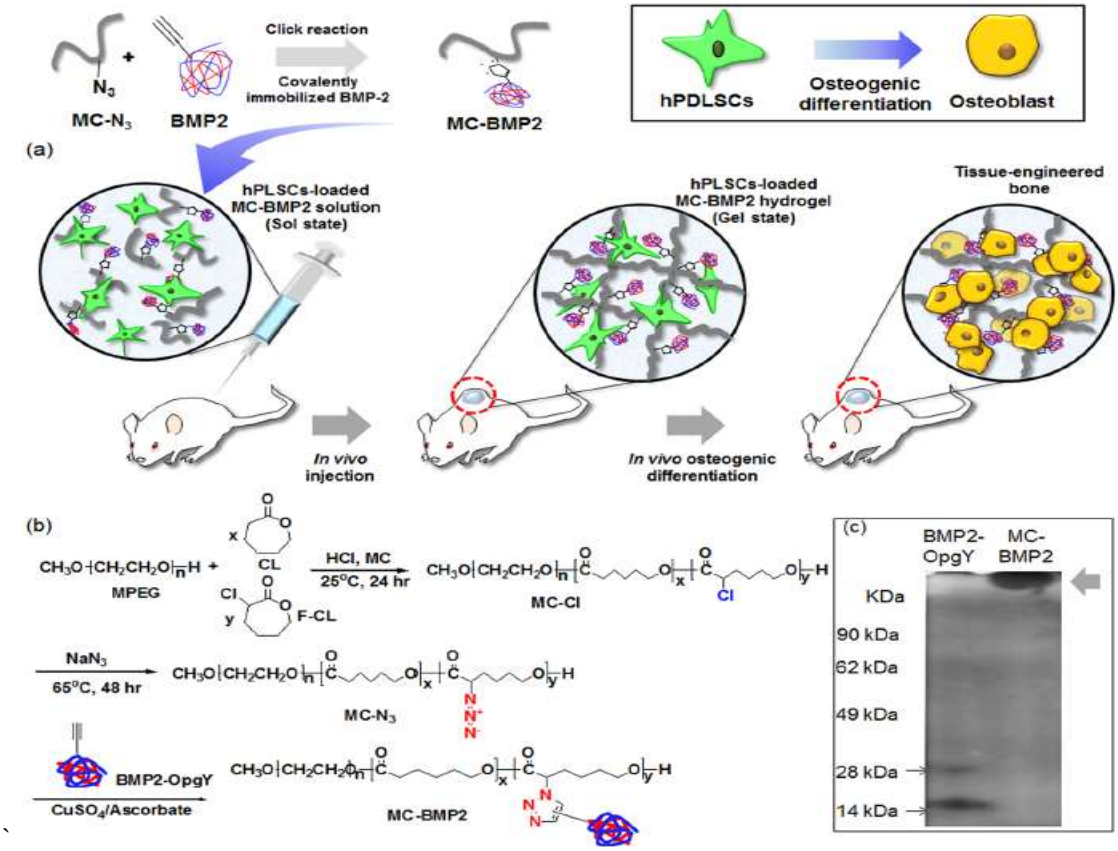

Figure 4: (a) Schematic of osteogenic differentiation of human periodontal ligament stem cells in an in vivo formed MC-BMP2 hydrogel, (b) synthesis of MC-Cl, MC-N3, and MC-BMP2 and (c) sodium dodecyl sulfatepolyacrylamide gel electrophoresis of BMP2-OpgY and MC-BMP2. Sulfate-polyacrylamide gel was cropped and full-length gel included in Supplementary

IV. CONCLUSION

Several selection strategies have been set up directed advancement has become an important means of improving

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an enzyme or altering its substrate specificity, has always resulted linking phenotype to genotype. To can analyses libraries of at least 1010 variants at a time, whatever, selection for catalysis needs to [1]) enzymatic activity can to be specifically tailored for each enzyme,[2]) reaction and substrate. All system have own advantages and disadvantages. [3]) The vivo applications can be limited in their use because of the parochial range of reactions can be utilize in selection. [4]) Part of techniques in vitro have been advancement to overcome these limitations, also they still involve a transformation step. Cell-free systems combined with hold enormous potential analysis, allowing rapid analysis of enzyme variants. This review, the paper systems will prove the most promising for the future of directed enzyme engineering.

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