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Chemical modification strategies to prepare advanced protein-based biomaterials

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A B S T R A C T

Nature is a superb source of inspiration when it comes to the development of biomaterials. Proteins, an exquisite asset virtually involved in all biological functions, are envisioned as a biomaterial due to their ability to be chemically modified. Owing to the rich chemical repertoire provided by the side chains and C-/N-terminus present in their backbone, scientists are pursuing chemical ways to upgrade isolated proteins, while maintaining their biological activity or relevant structural properties. By inserting chemical motifs, the crosslinking capability of proteins and capability to attach biochemical and molecular groups can be controlled yielding nano to macro constructs and hydrogels with improved physicochemical and mechanical properties. These cutting-edge approaches elevate the potential use of proteins as promising biomaterials for biotechnology and biomedicine.

Chemical modification of proteins

Throughout evolution, nature has dictated the survival of the fittest, providing sophisticated and highly functional structures like proteins. These macromolecules play vital roles in the growth and maintenance of the entire body (from cells to organs) by participating as structural support and energy providers, catalysts for biochemical reactions (e.g. digestion, energy production, blood clotting, muscle contraction), messengers (hormones) or transporters. Being naturally synthesized by cells across an endogenous process known as translation, proteins hold a complex 3D structure composed by a backbone of amino acids. These building blocks are linked together through peptide bonds, C-/N-terminus, and side chains able to interact with the environment. After biosynthesis, proteins can be subjected to some modifications through distinct processes being the post-translational the most acknowledged one. During this, proteins experience chemical changes at the amino acid side chains or C-/N-terminus, increasing the prevailing chemical repertoire of the 20 canonical amino acids prescribed by the genetic code. Phosphorylation, acetylation and glycosylation are some of the well-known examples within this process. Attempting to mimic these processes, researchers created new powerful tools to tailor-made proteins with different chemical and biochemical motifs, empowering their use as biomaterials in fields such as biomedicine and biotechnology [1].

Up to date, chemical modification of proteins has been explored directly in cells for labelling and surface engineering purposes, or after isolation from tissues or cells. By taking advantage from the well-organized and sophisticated chemical machinery provided by cells, scientists discovered different ways to modify proteins chemically: i) by hijacking the translational process, ii) through the expansion of the native genetic code or, iii) using the native side chains residues. In the first approach, aldehydes or azides are tailor in conjugated proteins (e.g. glycoproteins) via a metabolic pathway and resorting to unnatural monosaccharide substrates (e.g. mannosamine analogues). In opposite, the second attaches non-canonical amino acids to the growing polypeptide chain in the ribosomes with the virtual insertion of any chemical motif [2]. Finally, the third approach and the simplest one, take advantage of the chemical motifs provided by the amino acid side chains, tailoring any chemical motif (Fig. 1). When targeting isolated proteins, the first two methodologies lack feasibility, as it requires the control of the cellular machinery differing from the latter, that only relies on the available side chains present in proteins.

With specific chemical reactivities, proteins side chains bearing sulfhydryl (-SH) groups from cysteine, amines (-NH_2) from lysine, histidine, arginine or *N*-terminus, and oxygen-containing groups, hydroxyl (-OH) from tyrosine or carboxyl (-COOH) from aspartic or glutamic acids and C-terminus. Some fashionable chemistries as tyrosine residues coupling, cysteine-ene chemistry, lysine crosslinking, aldehyde-amino reactions can be used to tailor-made protein under physiologically relevant conditions (both pH and temperatures). However, amines are obvious top-choice targets due to its abundance and accessibility in the native form, as well as their versatile reactivity with the formation of amine/imine linkages using aldehydes, carbodiimide systems or NHS-bearing molecules. When targeting other side chains, it is crucial to acknowledge the chemistry and structure of the target protein as polarity and quantity of the side chains differ among them. Take the example of keratin known by their richness in cysteine residues (usually limited in most proteins), or silk fibroin that is rich in hydrophobic sides chains (e.g. glycine, serine, alanine). Like these proteins, others also have different chemical side chains residues, expanding the repertoire of chemical modification. Nevertheless, it is needed to take into consideration that targeting side chains available in large quantities may

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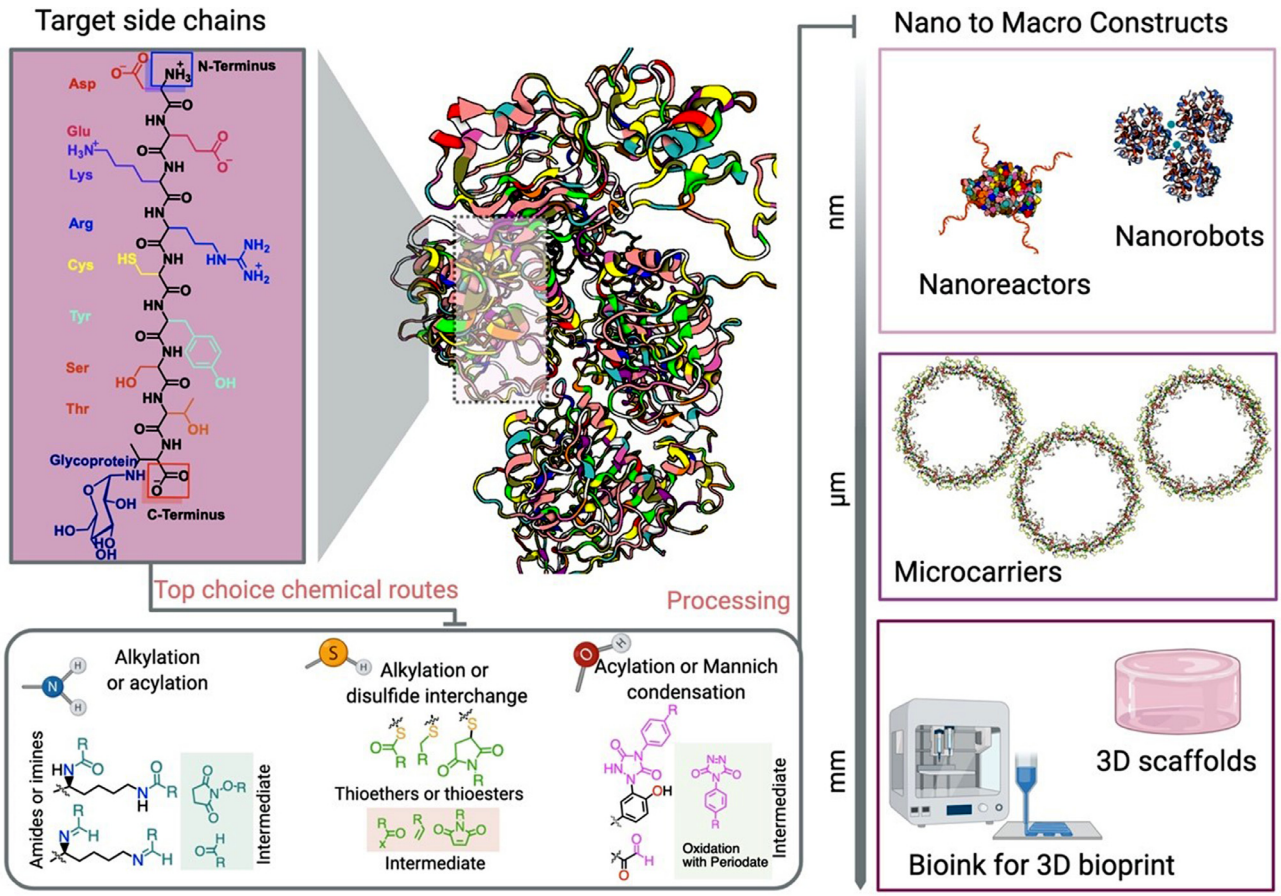

Fig. 1. Top choice chemical routes to modify proteins and their application as biomaterials.

hinder control over the extent and site-specific modifications. As a result, the protein function may be impaired, or for the specific case of amines obstruct the number of cell recognition motifs. For the vast majority of proteins, targeting cysteine or tyrosine side chains is pursued as a work-around to control the modification as both residues have limited occurrence.

Tailoring proteins as biomaterials

Some of the work developed within this scope is directed towards tailored proteins with tunable functionalities as building blocks from nano to macro constructs. As nano building blocks, chemical modifications allow tailoring nano- or micro-constructs via conjugation with hydrophobic linkers, oligonucleotide or to nucleic acids [3]. Explored as microcarriers, nanoreactors or nanorobots, these constructs found applications in fields such as biotechnology or biomedicine. Upscaling, chemical modifications provided tools to explore proteins as building blocks on the fabrication of hydrogels or 3D scaffolds. When designing a hydrogel, features such as biocompatibility, suitable chemical and mechanical properties, water content or degradation kinetics are often envisioned to mimic the native cellular niche in a tissue-dependent manner [4]. Although proteins are equipped with a rich repertoire of amino acids supporting cellular attachment, first attempts resorted on gelation processes that were not compatible with the maintenance of cell viability and generated structures with poor mechanical/rheological properties. The rational design behind hydrogels fabricated with other macromolecules, such as polysaccharides (e.g. chitosan, hyaluronic acid, laminarin) inspired the use of chemical strategies towards the fabrication of protein-crosslinked hydrogels. In here, proteins can overcome the lack of cellular recognition motifs present by most polysaccharides that can be overcome with the addition of external functionalities such as growth factors, RGD peptides or even native proteins.

The first hydrogels fabricated by the crosslink of proteins resorted in simple molecules (e.g. genipin, glutaraldehyde) able to react with the amine-bearing sides chains, without previous protein modification. From here, a broad library of bifunctional linkers has emerged, bearing suitable chemical motifs (e.g. NHS, carboxylic acid, alkoxyamines) able to promote the crosslink and fabricate hydrogels [5]. Alternative methods may include preceding chemical modifications with small molecules able to crosslink proteins through external stimuli (such as light) or by the simple mixture with proteins bearing complementary chemical motif (e.g. ketone/amines, azides/alkynes or cyclooctyne). One of the conventional modifications is the insertion of the methacryloyl groups, fashioning photocrosslinkable hydrogels mediated by a photoinitiator under UV or visible light. With impressive constant kinetics, this modification ensures interfacial optimization between the crosslinked proteins while improving the mechanical properties of the final construct [6]. The crosslink via stimuli-responsive chemical motifs (by temperature, pH or magnetic field), dynamic covalent bonds or supramolecular/coordination interactions, can also offer control and synchronized dissociation over the hydrogel, exhibiting properties such as self-healing capability or injectability [5]. Less explored but with increasing importance in recent years, is the ability to crosslink through Staudinger ligation, Copper-free click reactions and the unmatchable inverse electron demand Diels-Alder [7].

To date, several animal-based (e.g. collagen, gelatin, elastin, keratin, albumin, silk fibroin, resilin) and plant-based (e.g. zein or soy) proteins have found their use as crosslinked hydrogels. In fact, some of them already shown promising results in the biofabrication of artificial blood vessels, skin replacement, cell delivery and wound healing devices [8].

One of the most successfully modified protein is gelatin, a derivative resulted from a hydrolytic degradation of collagen. The modification with methacryloyl groups generated GelMA, a biomaterial widely used in diverse areas such as in 3D cell-printing technology. Interestingly, hydrogels made of proteins with human origin are still undervalued, despite the fact that they can help reducing possible adverse responses of the immune system [6]. Despite involving strict isolation and purification steps, human-based proteins can be further processed as other animal-based ones, unveiling the same aptitude to be fashioned with the exquisite library of chemical strategies already established.

When designing a new bioink, in addition to biocompatibility and adequate physico-chemical properties, it must exhibit suitable printability exhibited by the ability to create stable and well-defined 3D constructs [9]. The success of GelMA as a bioink rely on the combination of its gelling functionality provided by the gelatin at lower temperatures (<20 °C) with the radical photocrosslink provided by the modification. However, the printability exhibited by other proteins, without the same gelling ability, hamper their use as an effective bioink. Changing the position of the UV light source within the bioprinting apparatus, or other pre-crosslinking strategies, may increase the range of printable proteins modified with the same chemical motifs [10]. However, expanding the chemical toolkit to other modifications that do not require the external help of UV light is long-awaited. Fashioning multifaceted crosslinking systems (like double chemical modifications) or the use of external helpers such as viscoelastic supporting matrix or the combination with polymers as sacrificial support can open a new range of printable proteins [10].

Final remarks

The use of advanced chemical modification strategies in proteins enhances their use as biomaterials, refining the current and next generation of nano to macro constructs, including nano/microparticles and fibers, membranes up to 3D scaffolds and hydrogels. The set of available proteins to be used, from animal to vegetal origin, is also noteworthy, although the use of modified proteins from human-origin can be further explored. In the particular case of 3D bioprinting, the chemical toolkit can expand the fabrication of new tailor-made proteins with printable capabilities. Such advances will lead to upgraded biomaterials with proper biological and mechanical properties to address the growing fields of biomedicine and biotechnology.

Declaration of Competing Interests

No potential conflict of interest relevant to this article was reported.

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