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REVIEW

Review transglutaminases: part II—industrial applications in food, biotechnology, textiles and leather products

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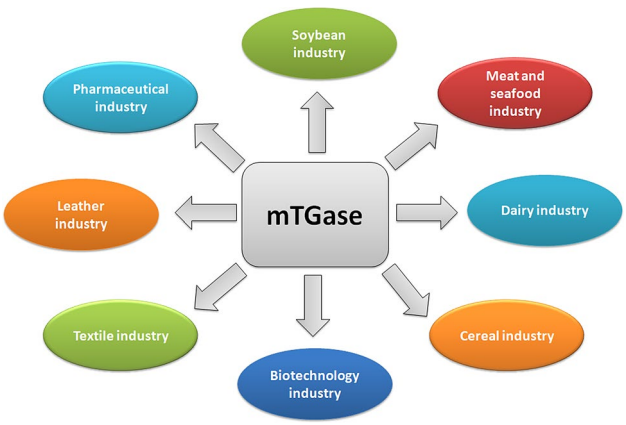
Abstract

Because of their protein cross-linking properties, transglutaminases are widely used in several industrial processes, including the food and pharmaceutical industries. Transglutaminases obtained from animal tissues and organs, the first sources of this enzyme, are being replaced by microbial sources, which are cheaper and easier to produce and purify. Since the discovery of microbial transglutaminase (mTGase), the enzyme has been produced for industrial applications by traditional fermentation process using the bacterium *Streptomyces mobaraensis*. Several studies have been carried out in this field to increase the enzyme industrial productivity. Researches on gene expression encoding transglutaminase biosynthesis were performed in *Streptomyces lividans*, *Escherichia coli*, *Corynebacterium glutamicum*, *Yarrowia lipolytica*, *and Pichia pastoris*. In the first part of this review, we presented an overview of the literature on the origins, types, mediated reactions, and general characterizations of these important enzymes, as well as the studies on recombinant microbial transglutaminases. In this second part, we focus on the application versatility of mTGase in three broad areas: food, pharmacological, and biotechnological industries. The use of mTGase is presented for several food groups, showing possibilities of applications and challenges to further improve the quality of the end-products. Some applications in the textile and leather industries are also reviewed, as well as special applications in the PEGylation reaction, in the production of antibody drug conjugates, and in regenerative medicine.

Graphic abstract



Keywords Transglutaminase · Microbial transglutaminases · Protein cross-linking · Food-enhancing enzymes · *Streptomyces mobaraensis*

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Introduction

Starting in 1989, microbial transglutaminases (mTGase) have been produced for industrial applications through traditional fermentation process using *Streptomyces mobaraensis* bacterium. mTGase is produced by this microorganism as an extracellular enzyme, having a molecular mass of approximately 38 kDa. This enzyme acts in a wide range of pH and temperatures (pH 5.0 to 8.0, and active in between 40 and 70 °C). *S. mobaraensis* mTGase is Ca^{2+} independent, and its activation requires no special cofactors (Ando et al. 1989; Yokoyama et al. 2004).

Enzymatic modifications of proteins mediated by mTGase have been generally used as tools for improving the properties of a given target product. These enzymatic reactions provide high specificity, occurring under gentle reaction conditions, producing no toxic products (Fatima and Khare 2018). In recent years, researchers have intensified their search for application possibilities of the mTGase to obtain methods and products that can alter the technological and functional properties of final products, not only in the food industry, but also in several biochemical reactions.

In the first part of this review, we focused on general aspects of the origins, reactions, and characteristics of transglutaminases of mammals, non-mammals, and microbial sources. Some studies on recombinant transglutaminases were also covered. In this second-part of the review, we highlight the importance of mTGase in four major research areas showing potential of applications of this enzyme: the food, biotechnology, leather, and textile industries.

Food applications

Studies for the application of transglutaminases in food technology began in the nineties, after the discovery of mTGase in microorganisms such as *Streptomyces mobaraensis* (*Streptoverticillium mobaraense*) (Ando et al. 1989), *Streptomyces cinnamoneum* (Duran et al. 1998), and *Bacillus subtilis* (Suzuki et al. 2000). The first application of mTGase in food technology was reported by Gottmann and Sprössler in 1992 (1992), who reported that mTGase could be a cost-effective enzyme to be used in food applications. Two decades later mTGases are mainly used in the processing of meat, fish, dairy, and baking products (Strop 2014). mTGase modifies the functional properties of food proteins by incorporation of amines, crosslinking, deamidation, and bonding surfaces of foods. However, in protein-containing food systems, the cross-linking reaction proceeds prior to other reactions (Santhi et al. 2017). In Table 1 are shown relevant aspects of some investigations on the use of mTGase for the modification of properties in different foods, according to the nature of the protein substrate, the amount of enzyme used, and the conditions of enzymatic reactions. Understanding mTGase mechanisms of action in altering protein properties is of major importance for its industrial use, which has not been completely elucidated (Gaspar and de Góes-Favoni 2015).

The first industrial scale production of mTGase was performed by the Japanese company Ajinomoto Co., in collaboration with Amano Enzyme Co. (Nagoya, Japan). The interest of the scientific community in mTGases is demonstrated by approximately 615 published papers in the last five years (Fig. 1a) investigating their applicability, structural characteristics, and substrate specificities, whereas 346 of these papers deal with applications in the field of Food Science and Technology (Fig. 1b), the area showing the greatest interest in this enzyme, as shown in Fig. 1c (Web of Science: June 2019).

Meat and seafood products

Microbial tranglutaminases attracted initial interests of the food industry due to its ability to mold minced meat into a firm steak. The restructure of meat products ensures greater firmness causing little loss of quality during cooking (Lesiow et al. 2017). The cross-linking of proteins and other compounds of the gel system causes changes in the proteic fraction of food matrices, leading to improved texture and stability in terms of temperature denaturation, emulsifying properties, gelation, and increased water-binding capacity (Dondero et al. 2006). The mTGase yields a final product with retained organoleptic properties similar to conventional meat in terms of flavor, texture, appearance, and taste (Hong et al. 2016).

Several studies are reported on the use of mTGase in meat products. As shown in Table 1, the enzyme can be used in a wide range of temperatures, from 10 to 50 °C. Some of these studies also show that mTGase supplementation could increase the gel strength in meat products and cause positive effects on the development of meat proteins of pork, beef, chicken, and fish (Ahhmed et al. 2009a, b; Canto et al. 2014; Dondero et al. 2006; Feng et al. 2018; Hong and Chin 2010; Hong and Xiong 2012; Jira and Schwagele 2017; Monteiro et al. 2015; Sorapukdee and Tangwatcharin 2018; Wu et al. 2016).

Because meat products are highly proteic, the myofibrillar proteins have marked influence on the textural quality of these products. Actin and myosin, which constitute the majority of myofibrillar proteins, are important substrates of mTGase and can also be polymerized by its addition,

Table 1 Studies with mTGase applied to different protein sources

Group of food	Protein substrate	Microorganism of TGase	Treatment conditions (enzyme concentration, temperature, and incubation time)	References
Meat and seafood products	Pork myofibrillar protein	Activa® TI (*S. mobaraensis*)	0.5% (w/w); 4 °C; 24 h	Hong and Xiong (2012)
	Pork myofibrillar protein	Activa® TI (*S. mobaraensis*)	0.2% (w/w); 4 °C; 24 h	Hong et al. (2012)
	Pork myofibrillar protein	Activa® TI (*S. mobaraensis*)	0.6% (w/w); 4 °C; 24 h	Hong and Chin (2010)
	Pork leg to manufacture dry-cured ham	Activa® EB (*S. mobaraensis*)	0.1% (w/v); 7 °C; 24 h	Romero de Ávila et al. (2010)
	Beef	Activa® TG-K (*S. mobaraensis*)	0.5% (w/w); 60 °C; 2 h	Dondero et al. (2006)
	Steak—beef trimmings	Activa® TG-B (*S. mobaraensis*)	1% (w/w); 8 °C; 4 h	Sorapukdee and Tangwatcharin (2018)
	Chicken and beef myofibrillar proteins	Activa® (*S. mobaraensis*)	5–6.8% (w/w); 40 °C or 78 °C, 0.5 h	Ahhmed et al. (2009a)
	Tilapia fillets	Activa® WM (*S. mobaraensis*)	0.5% (w/w); 4 °C; 24 h	Monteiro et al. (2015)
	Fish myofibrillar protein	NS	0.1%; 4 °C; 2 h	Feng et al. (2018)
	White shrimp	Activa® TG-K (*S. mobaraensis*)	0.8 U/g of protein substrate; 25 °C; 2 h	Tammatinna et al. (2007)
	Caiman steaks	Activa® WM (*S. mobaraensis*)	1% (w/w); 4 °C; 18 h	Canto et al. (2014)
Dairy products	α-Lactalbumin concentrate	Activa® MP (*S. mobaraensis*)	10 U/g of protein substrate; 50 °C; 5 h; pH 5	Sharma et al. (2002)
	Na-caseinate, Ca-caseinate, skim milk powder, condensed milk, whole milk powder, whey, and milk	Activa® (*S. mobaraensis*)	1 U/g of protein substrate; 40 °C, 2 h	Oner et al. (2008)
	Paneer (traditional Indian milk product)	Activa® (*S. mobaraensis*)	1 U/g of protein substrate; 4 °C; 16 h	Prakasan et al. (2015)
	Milk	Activa® TI (*S. mobaraensis*)	0.3% (w/w); 84.5 °C; 1 h	Rodriguez-Nogales (2006)
	Milk	Activa® MP (*S. mobaraensis*)	3 U/g of protein substrate; 40 °C; 2 h	Domagała et al. (2016)
	Milk	Activa® TG-B (*S. mobaraensis*)	7 U/mL of milk proteins; 30 °C; 3 h	Chen and Hsieh (2016)
	Cheese whey protein	NS	40 U/g of whey proteins; 40 °C; 1 h; pH 5	Wen-qiong et al. (2017)
	Ice cream	Activa® (*S. mobaraensis*)	4 U/g of protein substrate; 57 °C; 1.5 h	Rossa et al. (2011)
Cereal based products	Noodle	NS	1% (w/w); 30 °C; 0.5 h	Wang et al. (2011)
	Rice noodle	Activa® (*S. mobaraensis*)	1% (w/w); 40 °C; 2 h	Kim et al. (2014)
	Rice flour	Activa® (*S. mobaraensis*)	1% (w/w); 30 °C; 1 h	Gujral and Rosell (2004)
	Wheat gluten hydrolysate	Activa® TI (*S. mobaraensis*)	0.05% (w/w); 55 °C; 1 h and 5 °C; 18 h	Agyare et al. (2009)
	Bread wheat flour	Activa® WM (*S. mobaraensis*)	8 U/g of protein substrate; 30 °C; 2 h	Mazzeo et al. (2013)
	Damaged wheat flour	Activa® (*S. mobaraensis*)	1.5 U/g of protein substrate; 37 °C; 0.5 h	Bonet et al. (2005)

Table 1 (continued)

Group of food	Protein substrate	Microorganism of TGase	Treatment conditions (enzyme concentration, temperature, and incubation time)	References
Leguminous products	Soy protein	TGase was purified from the culture medium of *Streptoverticillium cinnamoneum subsp. cinnamoneum* IFO12852	0.05% (w/v); 55 °C; 1 h	Babiker (2000)
	Soy protein isolate	Activa® WM (*S. mobaraensis*)	0.08% (w/v); 50 °C; 0.4 h	Song and Zhang (2008)
	Legume protein isolate	NS	0.05% (w/v); 55 °C; 1 h; pH 7.5	Salma et al. (2010)
	Black soybean packed tofu	Activa® (*S. mobaraensis*)	1% (w/w); 55 °C; 0.5 h	Chang et al. (2011)
	Soy-based cream cheese	NS	2.6% (w/w); 50 °C; 24 h	Ting-Jin et al. (2011)
	Soy protein isolate	Activa® (*S. mobaraensis*)	0.5% (w/v); 50 °C; 1 h	Jin et al. (2013)
	Soybean protein	NS	10 U/g of protein substrate; 37 °C; 3 h; pH 7.5	Song and Zhao (2014)

NS not specified

thus improving the textural properties of structured meat products (Uran and Yilmaz 2018). The addition of mTGase also allows for the utilization of raw materials such as collagen and mechanically deboned meat in manufacturing meat products, with enhanced nutritive value by supplementation with amino acids otherwise deficient in these products (e.g. exogenous lysine) (Kieliszek and Misiewicz 2014).

Efforts to reduce the sodium content of meat products is an important issue concerning the health of people and to attend these demands, the meat industry is focusing on the development of techniques to reduce the use of salt in processed meat products, without impacting their quality (Atilgan and Kilic 2017). Strategies such as the use of mTGase can be applied in the manufacture of meat products with low salt content to avoid quality deterioration arising from this reduction, as suggested by Atilgan and Kilic (2017). These authors investigated the effects of mTGase, fibrin/thrombin (fibrimex), alginate, and their combinations on the quality of reduced-salt cooked meat. Their results indicated that the fibrimex/mTGase combination improved the texture properties of minced beef with low salt content.

However, for restructured meat in which fat can interfere with meat binding, it is essential to evaluate the grading level of formulated beef trimmings to enhance product quality or, at least, to avoid the minimum detrimental impact on product quality. The research of Sorapukdee and Tangwatcharin (2018) indicated that the most suitable raw beef for producing restructured steaks without detrimental effect on product quality, was beef trimmings containing up to 17% fat treated with 1% (weight fraction) of mTGase Activa TG-B. At this level of enzyme addition, both the sensory quality and increased tenderness were positively affected.

Dairy products

Improving the quality and functionality of the dairy products is been considered of paramount importance for better appreciation by people in a scenario of competitive dairy market. One of the most promising strategies to promote bio-functionality properties of dairy products is the cross-linking of milk proteins with transglutaminase. The use of mTGase can be a successful strategy to improve dairy products nutritional and technological characteristics, at the same time reducing the costs production by decreasing the amount of fat and stabilizer in the final product (Taghi Gharibzahedi et al. 2018). This enzyme has the ability to form intra- and intermolecular covalent crosslinks between two amino-acid residues in the structure of milk proteins. Both casein and whey α-lactalbumin and β-lactoglobulin are excellent acyl donors and/or acceptors substrates for transglutaminase, although some differences between them apply in relation to the crosslinking reaction (Færgemand and Qvist 1997; Oner et al. 2008; Rodriguez-Nogales 2006; Rossa et al. 2011). According to a study by Chen and Hsieh (2016), in a cascade reaction, mTGase catalyzes the cross-linking of κ-casein (κ-CN) and β-casein (β-CN) before it proceeds to cross-link the serum albumin (AS), α-lactalbumin (α-LA), α_{s1}-casein (α_{s1}-CN), α_{s2}-casein (α_{s2}-CN), and β-lactoglobulin (β-LG) moieties, as shown in Fig. 2. In this particular case, the caseins appear to be readily cross-linked because of their flexible, random-coil structures and the absence of

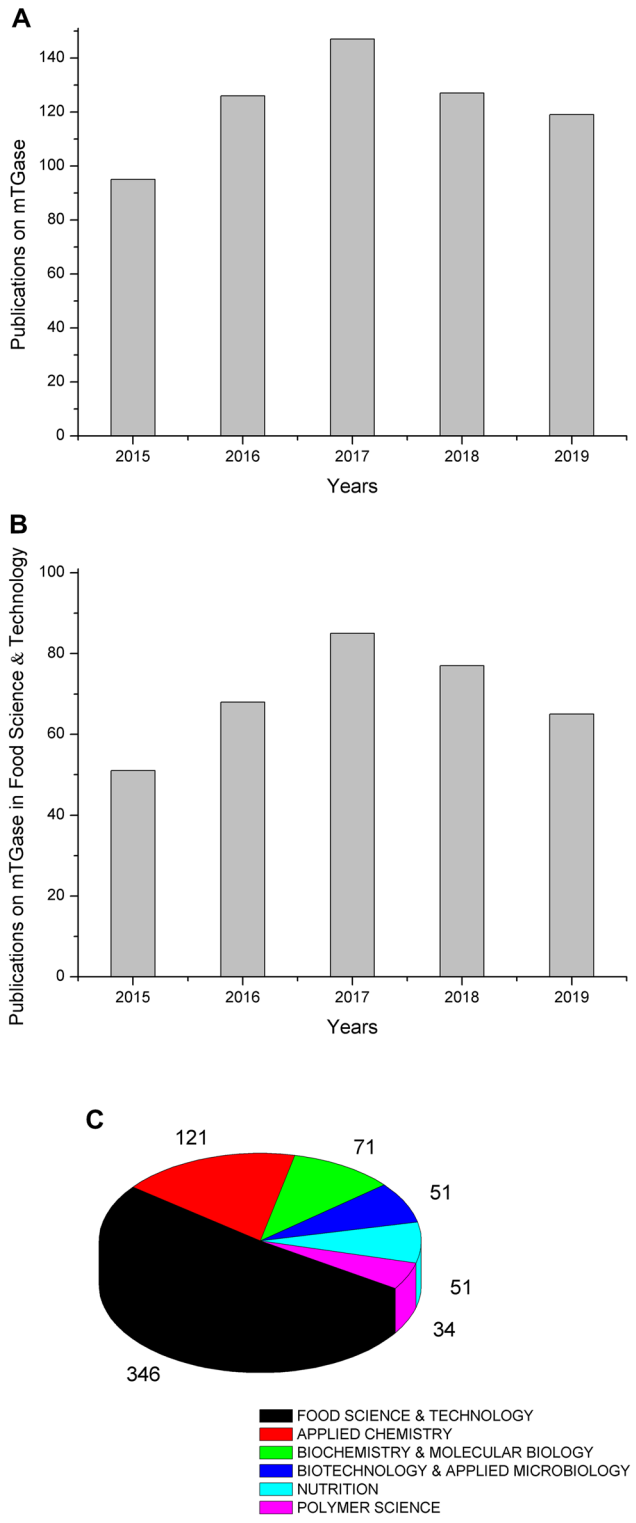

Fig. 1 Publications on microbial tranglutaminases in the last 5 years (2015–2019): **a** the general bulk of publications on mTGase; **b** publications covering applications in Food Science and Technology; **c** study areas of the web of science database that research mTGase (data as November, 2019)

any disulphide bonds in the α_{s1}-CN and β-CN (Færgemand and Qvist 1997; Oner et al. 2008; Rodriguez-Nogales 2006; Rossa et al. 2011). Due to their compact globular structures, whey proteins tend to cross-link less efficiently. The β-LG is more susceptible and show a higher cross-linking rate than α-LA, but the β-LG is able to cross-link with the reduction of its disulphide bonds, whereas α-LA can be cross-linked without the reduction (Rodriguez-Nogales 2006; Rossa et al. 2011).

The benefits brought about by the application of mTGase in dairy products include increased gel strength and improved storage stability and viscosity (Domagała et al. 2016). When mTGase is added to the system, it enhances heat-resistance and firmness of gel. Yogurt, a milk gel formed by acidic fermentation mediated by lactic-acid bacteria, has the disadvantage of serum separation upon change of temperature or physical impact. The addition of mTGase to yogurt can avoid this problem because mTGase improves the water holding capacity of the gel (Yokoyama et al. 2004). Ice creams treated with mTGase result in more consistent end-products, showing better aeration and foam stability. The mTGase also makes it possible to produce ice cream and cheese with low fat contents or reduced content of non-fat solids (Gaspar and de Góes-Favoni 2015; Yokoyama et al. 2004). The addition of mTGase during cheese preparation may increase the moisture content altering the palatability and the yield of different cheese products. In relation to surface texture of curds, ice-creams, milk and cheeses, it is noticed an improvement in the creaminess, homogeneity, smoothness, and consistency after mTGase is used in the production process (Wen-qiong et al. 2017).

Cereal based product

The first positive effects of mTGase application in baking was reported by Gottmann and Sprössler (1992). The applications of mTGase in cereal proteins, particularly wheat proteins (globulins, glutenins, gliadins, and prolamins), have attracted huge interest from the bakery industry (Mazzeo et al. 2013). The cross-links formed between the wheat proteins by the action of mTGase greatly influenced the characteristics of the products, determining the quality, functional and rheological properties of these systems, such as stability, elasticity, resilience, and water adsorption, with proper pore size along with adequate dough volume (Bonet et al. 2005; Gerrard et al. 1998; Gujral and Rosell 2004; Scarnato et al. 2017). The cross-linking reaction of mTGase promotes aggregation and polymerization, leading to the formation of polypeptide networks showing differentiated viscoelastic properties (Bonet et al. 2005; Gujral and Rosell 2004).

Gerrard et al. (1998) were the first researchers who used the mTGase in white bread. These authors suggested that the enzyme could have beneficial effects during the manufacture

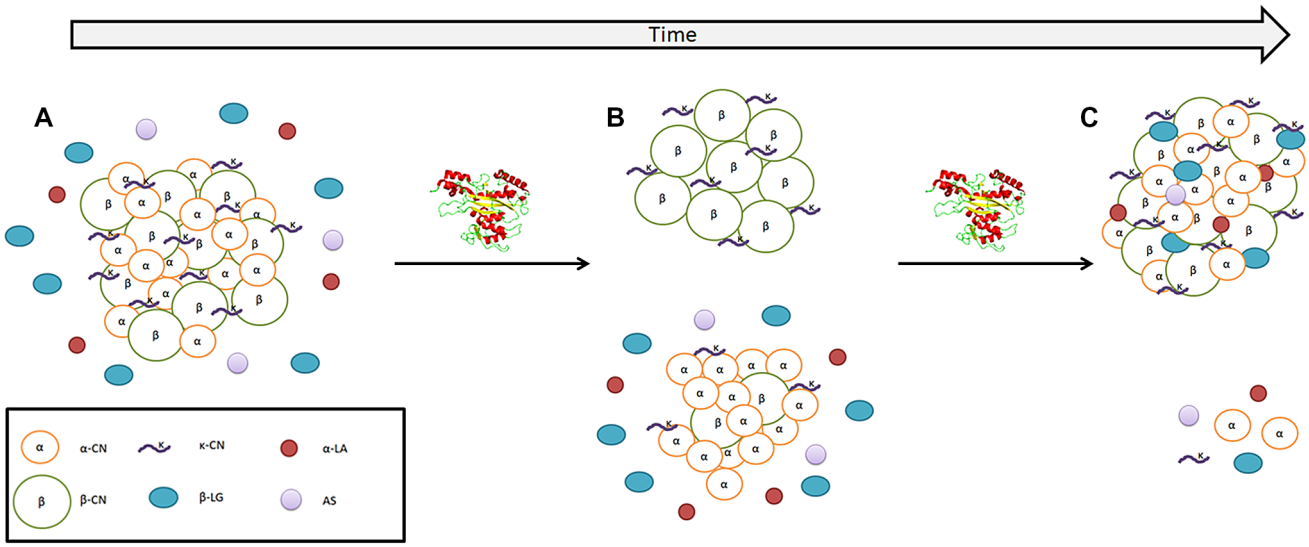

Fig. 2 Representation of the cross-linking reaction of milk proteins induced by mTGase. **a** The milk proteins without mTGase action are schematized; **b** the preference of mTGase for β-CN and κ-CN; **c** cross-linking occurs with all milk proteins with time. Adapted from Chen and Hsieh (2016)

of bread, comparable to those produced by traditional chemical oxidant improvers. Scarnato et al. (2017) showed that using low amounts of mTGase, positive effects were observed on aspects of crumb and crust of bread, as well as in the rheological properties and physico-chemical properties of the dough. The release of some peptides from gluten obtained through the activity of mTGase can also influence the modulation of bread microbiota during the storage and consequently increase the final product shelf-life (Scarnato et al. 2017).

Another application of mTGase in the bakery industry is related to the production of pasta and instant noodles. In 1996, research by Sakamoto et al. (1996) showed that the treatment of noodles and pasta with mTGase prevented the deterioration of texture upon cooking and improved the strength of the products, even when low-grade flours were used in the manufacture, reducing the costs of production (Sakamoto et al. 1996; Yokoyama et al. 2004).

Soybean products

Soy protein isolate (IPS) is widely used as an important ingredient in Asiatic diets and in general processed foods due to its nutritional value and functional properties. IPS consists of glycinin (11S) and β-conglycinin (7S), which account for approximately 70% of its total protein content. These globulins are good substrates for mTGase activity (Qin et al. 2016; Song and Zhao 2014). Tang et al. (2005) investigated the use of mTGase on the properties and microstructures of IPS films molded with various plasticizers (glycerol, sorbitol, and 1:1 mixtures of glycerol and sorbitol). The cross-linking treatment by mTGase produced an effective method to improve the films cast properties of all tested plasticizers.

Tofu, a typical soybean curd product, is prepared by the coagulation of soybean proteins with the addition of Ca^{2+} and Mg^{2+} and/or glucono-δ-lactone. Coagulation or gelation of soymilk is the most important step in the production of tofu. A popular food in many countries, tofu shelf life is generally very short because its softness and smooth texture that prevents its sterilization. The introduction of mTGase in its processing produces an edge of texture control and enhances its quality, yielding a product with better consistency and silky texture and ability to tolerate temperature fluctuations (Chang et al. 2011).

Finally, proteins from sources other than soy can be covalently linked to soy protein by mTGase to produce combinations showing novel functionalities. For instance, conjugation of milk caseins or soybean globulins with ovomucin (an egg white glycoprotein), has shown to improve the emulsifying activity of the combined protein when compared to both isolated proteins (Kato et al. 1991; Yokoyama et al. 2004).

Food coating and edible films

The research to produce protein films as an alternative to petroleum-based polymeric materials has been receiving a great deal of attention in the food industry. Protein films can be used as coatings on fresh fruits and vegetables to increase the shelf life of these products. These films are non-toxic, natural, health safe, biodegradable, and might be edible. Protein edible films produced by the cross-linking action

of mTGase present structural homogeneity, have a smooth surface, are mechanically resistant, and are gas-permeable (Porta et al. 2016). In the work to Rossi Marquez et al. (2017), apple weight losses during storage was significantly reduced, approximately 80%, after 10 days when the samples were coated with whey protein grafted film with pectin and transglutaminase. Similarly, this grafted film was able to prevent weight loss of potato and carrot samples until the 6th day of storage.

The research carried out by Fernandez-Bats et al. (2018) showed that it was possible to obtain mesoporous silica nanocomposite bioplastics prepared by using bitter vetch (*Vicia ervilia*) proteins crosslinked by mTGase, which showed improved gas and water vapor barrier properties. The prepared material showed antimicrobial and antifungal activities, possibly increased by nisin addition to the film-forming solutions, suggesting their potential application as an active bio-preservative packaging to improve the shelf life of a variety of different food products.

Health aspects concerning the use of transglutaminase in food industry

Because of increased applications of mTGase in food, important health concerns appeared, pressuring the need for regulations to inform people on the safety when consuming products containing this enzyme. In 1998, Motoki and Seguro (1998) showed that the only difference between food containing mTGase-modified proteins and native proteins was the number of links between glutamine and lysine residues (G–L). This chemical modification is also present when proteic foods are heated, for example, in cooking, generating the G–L bond. In this respect, humans have been ingesting foods rich in G–L residues since the discovery of fire and cooking. Although not scientifically demonstrated, the safety of the G–L modified linkage can be assumed by the long-term consumption of the G–L moiety in cooked foods (Motoki and Seguro 1998). On the other hand, Bernard et al. (1998) studied the mutagenesis and toxicity risk presented by the addition of mTGase in food preparations, tested in experimental animals. Their results suggest that the acute toxicity of the enzyme seems to be relatively low, since it was not observed any mortality, morbidity, or signs of toxicity at doses of 2 g/kg body weight.

There have been some evidences of increased nutritional properties of foods enzymatically modified by mTGase. According to studies conducted by Xing et al. (2016), the addition of mTGase to soy extract in preparation of tofu lead to modifications of proteins that increased the perception of satiety and reduced the allergenicity towards soy proteins. In another application concerning allergenicity, shrimp products processed using mTGase showed reduced allergenicity due to glycosylation of proteins catalyzed by this enzyme (Yuan et al. 2017).

Concerning bakery products, Zhou et al. (2017a) have shown that mTGase can effectively transamidate gliadin peptides and gluten proteins, thus concluding that mTGase lowered the allergenicity and immunogenicity caused by wheat flours. The resulting peptides are barred to cross intestinal mucosa where they initiate the celiac immunological activity. These results demonstrate a potential strategy to prevent cereal toxicity in celiac disease (Zhou et al. 2017b). However, multiple mTGase linked proteins are immunogenic in celiac disease patients. In the study conducted by Lerner and Matthias (2015), the authors indicate that the use of this enzyme can further increase antigenic load presented to the immune system and increase the risk for gluten-sensitive populations. In a recent research, Matthias et al. (Matthias et al. 2016) have suggested that mTGase increases immunogenicity in children with celiac disease because mTGase antibodies correlates to intestinal damage in the same degree as transglutaminase human tissue antibodies. Authors suggested that further investigation is necessary to elucidate the role of anti-mTGase antibodies in this disease.

Although scientific findings reported in the literature regarding the safety of the use of mTGase in foods can be classified as inconclusive, the FDA has approved the use of mTGase as a "Generally Recognized as Safe—GRAS" for food applications since 1998. This enzyme is considered an adjunct of technology and it is not regarded as an ingredient, and therefore does not need to be listed in the composition of ingredients of the commercial product (Romeih and Walker 2017; Taghi Gharibzahedi et al. 2018).

Biotechnology applications of mTGase

The biotechnological applications of transglutaminases are one of the fastest growing areas on mTGase research. Classical applications of transglutaminases in biomedical research include PEGylation, the production of antibody–drug conjugates, tissue engineering, regenerative medicine, and the production of microparticles for enteric delivery of substances of interest in the food and pharmaceutical industry. Finally, we will be briefly reviewing the use of transglutaminases in the treatment of textiles and leather.

Enzymes immobilization mediated by mTGase-catalyzed bioconjugation

Protein immobilization in solid supports has been used as a technique for biotechnological applications of enzymes, offering several advantages over the use of free forms, such as easing separation from reaction media and the possibility of reuse (Duarte et al. 2017; Mateo et al. 2007; Matte

et al. 2014; Rodrigues et al. 2013). In general, proteins bound to functional groups on supports show high stability due to reduced protein loss to the medium. The immobilization of proteins through covalent bond formation has been routinely carried out using chemically-activated supports or chemical cross-linking reagents (Mateo et al. 2007). However, because the presence of multiple functional groups on protein surfaces, proteins are in general randomly attached onto supports, resulting in the reduction of total enzymatic activity. Several techniques have been devised to preserve the activity of biomolecules upon immobilization, among them the immobilization using mTGase as site-specific binding (Tominaga et al. 2004). The immobilization mediated by transglutaminase-catalyzed bioconjugations offers the advantage of improved selectivity and compatibility with sensitive biological systems relative to traditional chemical methodologies (Wang et al. 2019).

The mTGase is unique in catalyzing the acyl transfer reaction between a primary amine and the γ-carboxyamide group of glutamine (Gln) residues in peptides and proteins. When the ε-amino group of lysine (Lys) residues in protein acts as an acyl acceptor, cross-linking of proteins becomes possible through the ε-(γ-Gln)Lys bond, resulting in the formation of a new γ-glutaminyl covalent link (Kamiya et al. 2003; Li et al. 2018; Motoki and Seguro 1998; Wang et al. 2019). The mTGase displays broad acyl-acceptor substrate specificity, enabling the use of a variety of scaffolds with primary amine groups as solid supports, such as the polysaccharide chitosan and gelatin (Li et al. 2018).

The study of Tominaga et al. (2004) demonstrated site-specific immobilization via covalent attachment of recombinant alkaline phosphatase with a specific peptide linker by mTGase. To allow the mTGase-mediated site-specific immobilization, a solid support of casein-coated polyacrylic resin was designed to display mTGase recognition sites on its surface. It was found that this immobilization exhibited much higher specific activity, with higher stability upon repeated use than the biocatalyst prepared via chemical modification.

Wang et al. (2019) reported the controlled, site-specific and covalent cross-linking of an engineered enterokinase on amine-modified magnetic nanoparticles via mTGase-catalyzed bioconjugation for the development of the oriented-immobilized enzyme. A glutamyl (Gln-donor tag) was genetically incorporated into the C-terminus of enterokinase. An amide linkage was formed between the glutamyl group of Gln tag and the primary amines of the support via the covalent immobilization catalyzed by mTGase. Upon the site-specific immobilization, approximately 90% enterokinase activity was retained, and the biocatalyst exhibited more than 85% of initial enzymatic activity reusable stability over a month (Wang et al. 2019).

In the work of Synowiecki and Wołosowska (2006), a β-glucosidase from *Sulfolobus shibatae* was immobilized on silica gel modified with 3-aminopropyl-triethoxysilane using transglutaminase as a cross-linking factor, and the immobilization process did not influence the optimum pH and temperature of substrate hydrolysis (Synowiecki and Wołosowska 2006). On the other hand, Bechtold et al. reported the preparation of protein G-soybean peroxidase conjugate catalyzed by mTGase with yields calculated to be only about 0.1%, suggesting the difficulty in controlling the reaction when using native proteins (Bechtold et al. 2000).

PEGylation

At the end of the 1960s, Davis proposed the idea of conjugating PEG [poly (ethylene glycol)] to a protein, i.e., to "PEGylate" a protein (Davis 2002; Hoffman 2016). Since then, the PEGylation is considered one of the most successful methods to prolong the circulatory half-life and reduce the in vivo immunogenicity of therapeutic proteins, among many other applications in pharmacology (Pasut and Veronese 2012).

PEG is biocompatible, lacks immunogenicity and antigenicity, is soluble in water and other organic solvents, is readily cleared from the body, and has high mobility in solution and, more important, it is not toxic, making this the polymer of choice for bioconjugations. PEG use was approved by the FDA in the early 1990s (Bhattarai et al. 2005; Harris and Chess 2003; Mariniello et al. 2014; Roberts et al. 2002). Thus, a number of protein/PEG conjugates, are available in the market such as for the treatment of chronic hepatitis C (PEGinterferon α-2a and α-2b), for the treatment of acute lymphoblastic leukaemia (mPEG-L-Asparaginase), to treat severe combined immunodeficiency (SCID) disease (mPEG-Adenosine Deaminase), and to treat acromegaly (PEG-visomant) (Banerjee et al. 2012).

Chemical strategies used for the PEGylation of proteins produces random derivatives of lysine (Lys) residues, leading to heterogeneity and decreased bioactivity of the products (da Silva Freitas et al. 2013). Instead, the use of transglutaminase for the covalent attachment of PEG molecules to pharmaceutical proteins shows stringent substrate specificity, and site specific modification or PEGylation of the Gln residues bound to the proteins on the substrates can be obtained. (Fontana et al. 2008).

Because transglutaminases have partial selectivity to the carboxamide substrate, they are interesting options for the PEGylation of proteins. However, for the reaction to occur, the carboxamide must be in the flexible part of the protein molecule. (Dozier and Distefano 2015; Fontana et al. 2008). Consequently, mTGase has been intensively used to site-specifically incorporate mPEG–NH_2 to the reactive Gln residue of proteins (da Silva Freitas et al. 2013). The reactive

Gln residues modified by mTGase must locate at the disordered protein regions and satisfy its sequence requirement. As many target proteins lack the reactive Gln residues that can satisfy the structural and the sequence requirement of mTGase, its use is limited (da Silva Freitas et al. 2013; Mero et al. 2009). Several researches have been developed in this area and some of them are listed in Table 2.

So far, only a limited number of researches has been carried out on mTGase-mediated protein modification at the level of Lys residues. One of them is the work, of Zhou et al. (2016b), who linked carboxybenzyl-glutaminyl-glycine (CBZ-QG) to mPEG amine to form CBZ-QG-mPEG for the PEGilation of cytochrome C.

The hydroxyethyl starch (HES), which is a biodegradable derivative of starch, can be an alternative to PEG as blood plasma volume expander and in the design of drug delivery systems (Treib et al. 1999). It has been reported the use of HES conjugation using mTGase to produce fully biodegradable polymer–drug and polymer–protein conjugates (Besheer et al. 2009).

Antibody drug conjugates (ADCs)

Another promising technology is the use of mTGase to attach antibodies to diverse compounds in order to produce antibody–drug conjugates (ADC). ADC are emerging therapeutic agents in the treatment of cancer, using antibodies to selectively deliver a cytotoxic compound to tumor cells, thus improving the therapeutic index of chemotherapeutic agents, and showing better safety potential than nontargeted cytotoxics (Anami et al. 2017; Strop et al. 2013). One of the major challenges in the development of ADC is the application of suitable linkers to conjugate drugs to antibodies (Yao et al. 2016). The ADC have been largely manufactured by using chemical conjugation methods, generally resulting in heterogeneous mixtures of ADC having different physical and pharmacokinetic properties of the proposed ones (Axup et al. 2012; Dennler et al. 2014; Junutula et al. 2008; Okeley et al. 2013; Shen et al. 2012; Strop et al. 2013; Xiao et al. 2013; Zuberbühler et al. 2012).

An alternative strategy to the chemical modification of ADC is the use of mTGase because the enzyme will prevent the formation of these heterogeneous mixtures. Moreover, it is possible to introduce appropriate amine containing linkers making the mTGase able to conjugate structurally diverse probes and drugs (Ohtsuka et al. 2000). Strop et al. (2013) investigated how the conjugation site influences the stability, toxicity and efficacy of ADC obtained by mTGase reaction and whether these differences could be directly attributed to the binding position. By designing a "glutamine label", 90 sites were tested to attach several compounds and 12 sites showing a high degree of conjugation were found.

A two-step chemo-enzymatic approach, where mTGase binds a spacer entity that is reactive to the antibody, and subsequently reacts with the antimitotic toxin monomethyl auristatin E (MMAE), produced the highly homogeneous trastuzumab-MMAE conjugate with DAR (Drug-Antibody Ratios) of 2 (Dennler et al. 2014). Some ADC currently in use in clinical development based on target antigens using tranglutaminase are: PF-06664178, Trop-2 ADC, RN927C (Phase I, for treatment of ovarian cancers, non-small cell lung cancer and breast cancer—site-specific transglutaminase tag, AcLys-VC-PABC linker) and PF-06647020, h6M24-vc0101, PTK7-targeted ADC (Phase I, for treatment of non-small-cell lung carcinoma, triple-negative breast cancer and ovarian cancers—transglutaminase tag (LLQGA) located at the C-terminus of the antibody heavy chain, cleavable VC-PABC-linker) (Damelin et al. 2017; Nejadmoghaddam et al. 2019; Sachdev et al. 2016; Strop et al. 2016).

Several other investigations have been reported on the production of monoclonal antibodies using mTGases and are well documented in recent works (Dennler et al. 2014; Farias et al. 2014; Grünberg et al. 2013; Jeger et al. 2010; Lhospice et al. 2015; Siegmund et al. 2015; Spidel et al. 2017; Strop et al. 2013).

Tissue engineering and regenerative medicine

The term Tissue Engineering (TE) was first introduced in 1993 by Langer and Vacanti (1993) to describe an interdisciplinary field encompassing cell biology, material science, chemistry, molecular biology, engineering, and medicine, with the objective of developing advanced biological tissues and organs. These engineered biological materials are intended to maintain, improve, or restore functionalities of natural tissues combining scaffolds, cells and/or bioactive molecules (Griffith and Swartz 2006; Langer and Vacanti 1993; Lee et al. 2014; O'Brien 2011). The potential applications are being investigated in the field of tissue engineering of bones, cartilage, cardiac system, pancreas, and the vascular system, among others (Zhu and Tramper 2008). The main bulk of research in this area has been focused in the development of biomaterials capable of mimicking the structure and composition of the extracellular matrix. Such biomaterials must present biocompatibility and biodegradability and should not be toxic. In addition, the production and processing of biomaterials must be easy and scalable. Because hydrogels have high plasticity and high moisture content they have been the most important biomaterials employed in tissue engineering (Polak 2010; Toh and Loh 2014). Hydrogels can be formed from gelatin, collagen, chitosan, hyaluronic acid, and sodium alginate, as well as synthetic materials such as polylactide, polylactic-co-glycolic acid

Table 2 Summary of PEGylation studies found in literature involving microbial transglutaminases

Protein	Protein size (kDa)	PEG size (kDa)	Functional group on PEG	Use	References
Human growth hormone (hGH)	22	20	Glutamine	Growth hormone deficiency (GHD)	da Silva Freitas et al. (2013), Grigoletto et al. (2015), Khameneh et al. (2015), Zhao et al. (2010)
Human growth hormone (hGH) and salmon calcitonin (sCT)	hGH 22 sCT 34	10 and 0.55	Glutamine	Growth hormone deficiency (GHD) and Paget disease, osteoporosis and hypercalcaemia of malignancy	Mero et al. (2009)
Methionyl human granulocite colony stimulating factor (Filgrastim)	19	20	Glutamine	Neutropenia	Scaramuzza et al. (2012)
Cytochrome C	13.4	CBZ-QG-mPEG (5.3)	Lysine	PEGylation of protein at the level of Lys residues	Zhou et al. (2016b)
Fibronectina (FN)	250–270 kDa	2, 5, or 10 kDa	Lysine	Wound healing	Chen et al. (2014b)
Human glucagon-like peptide-1 (GLP-1)	20	20	Glutamine	Type 2 diabetes	Selis et al. (2012)
α-Lactalbumin and granulocyte colony stimulating factor (G-CSF)	NA	20	Glutamine immobilized mTGase on agarose beads	Enzyme was immobilized (simplifies the purification protocol) and promote the formation of homogeneous mono-conjugates	Grigoletto et al. (2017)
Granulocyte colony-stimulating fator (G-CSF)	NA	20	Glutamine	Computational approach aimed at identifying the glutamines modified by the enzyme (to treat neutropenia)	Maullu et al. (2009)
Cytochrome C	13.4	pNIPAM-mTGase (44)	Immobilized mTGase on carboxylated poly(*N*-isopropylacrylamide) (pNIPAM)	mTGase termo-responsivo	Zhou et al. (2016a)
Human granulocyte colony-stimulating factor, human growth hormone, and horse heart apomyoglobin	NA	Monodisperse Boc-PEG-NH_2	Glutamine	Direct identification of the sites of protein modification by mass spectrometry	Mero et al. (2009)

NA data not available

copolymer, polyethylene glycol, polycaprolactone, and polyacrylamide (El-Sherbiny and Yacoub 2013). Gelatin is a protein derived from the hydrolysis of collagen with characteristics of biodegradability and cell adhesion capacity, considered as GRAS material by the FDA and it has a long history of safe use in food products, pharmaceuticals and cosmetics (Elzoghby et al. 2012). Unfortunately, owing to a lack of mechanical strength and sensitivity to in vivo enzymes, the biomedical applications of gelatin is limited being necessary to increase its physical performance and to strengthen its resistance against enzymes hydrolyses (Zhao et al. 2016). To achieve this goal, crosslinks are usually introduced in biomaterials such as collagen mediated by mTGase, replacing physical methods like dehydrothermal drying (DHT) and UV-irradiation, among others, and chemical crosslinking mediated by glutaraldehyde, formaldehyde, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). The physical methods produce weak bonds that have a high risk of degradation, whereas the chemical methods use often toxic compounds that must be removed from hydrogels before being applied (Stachel et al. 2010; Yang et al. 2018). Therefore, the substitution of these methods by the enzymatic application of mTGases in order to generate hydrogels are among the most promising technologies to obtain biomaterials, since the mTGase-mediated process presents no risk of toxicity and eases the preparation of the materials, showing high mechanical stabilities (Milczek 2018). There is a plethora of research showing the use of transglutaminases to produce hydrogels, some of them listed in Table 3.

In 1986, Charles Hull described for the first time the technique of 3D bioprinting, which has since been used to produce a large variety of scaffolds in different tissue engineering areas. The term 3D bioprinting is used to describe the precise layering of cells, biologic scaffolds, and biologic factors with the goal of building a biological tissue (Bishop et al. 2017). The vascularization of 3D scaffolds is crucial for their functionalities, assuring the delivery of nutrients and oxygen to tissues, promoting cell proliferation and subsequent development of the new tissue (Castells-Sala et al. 2013). The main techniques used in tissue engineering by 3D bioprinting are stereolithography, extrusion-based, laser-assisted and inkjet-based printing (Derakhshanfar et al. 2018), and a sample of the investigations using 3D printing for tissue engineering with the use of transglutaminase are listed in the Table 3. In this technique, a great variety of polymers, hydrogels, bioceramics, among other biomaterials, have been used. Natural biological materials, such as cells, can also be employed in 3D bioprinting (Tappa and Jammalamadaka 2018).

Transglutaminase-crosslinked microparticles for enteric delivery

The microencapsulation technique is widely used in the fields of food, pharmaceuticals, and biotechnology in order to preserve physicochemical and sensorial attributes and to produce control-released compounds. Microencapsulation is obtained by the use of various techniques, such as spray drying, cooling, extrusion coating, fluidized bed coating, liposome retention, inclusion complexation, centrifugal extrusion, and rotational suspension separation, techniques that are chosen based on final product characteristics and costs (Desai and Park 2005). Recently, another technique has been described, the complex coacervation, which produces high encapsulation efficiencies, and has since been widely used in food and pharmaceutical industries, because it does not require harsh production conditions (temperature, pressure, pH, etc.) (Tello et al. 2016). This technique uses a combination of encapsulating agents of opposing charges to create electrostatic attraction between two molecules, and other interactions, such as hydrogen bonds and hydrophilic interactions, that also contribute to the formation of complexes (Ach et al. 2014). As the nature of these bonds is weak, there is the need to strengthen the interactions between polymers. For this purpose, mTGase has been used as a cross-linking agent showing promising results (Sanchez and Renard 2002). In Table 4 is presented a summary the microencapsulation and complex coacervation techniques found in literature involving the use of microbial transglutaminase.

Transglutaminase applications in textile industry

The textile finishing industry has been the focus of considerable criticism because it uses traditional chemical treatments in wool processing, which is perceived as highly damaging to the environment. Unfortunately, the alternative enzymatic processes using proteases can cause an excessive loss of fabric weight and yarn strength. Therefore, the use of transglutaminases in treatments of wool and leather fabrics has become extensively explored in order to develop appropriate technologies based on the use of this enzyme. It has been found that mTGase is capable of recovering properties of wool and silk treated with chemicals and enzymes used at different processing stages, such as cleaning, carding, bleaching, combing, drawing, spinning, and twisting (Tesfaw and Assefa 2014). Wool fabrics treated with *Streptomyces hygroscopicus* mTGase showed recovered fiber structures that were damaged during protease treatments (Du et al. 2007). The application of Guinea pig liver transglutaminase or the mTGase isolated from *Streptoverticilium mobaraense* in wool processing

Table 3 Summary of tissue engineering studies (hydrogels and 3D matrix) studies found in literature involving microbial transglutaminases

Matrix	3D bioprinting	Uses	References
Hyaluronic acid (HA) carboxylated chitosan recombinant human-like collagen (HLC)	Not used	Wound dressing	Zhu et al. (2018)
Gelatin	Human mesenchymal stem cell (hMSCs) (3D cultures)	Promote myocardial lineage commitment and improve the organization and rhythmic beating of cardiomyocytes	Ajay et al. (2018)
Gelatin	HRP to crosslink hyaluronic acid grafted with tyramine (HA-Ty)	Skin defects stemming from trauma, burns and skin infections	Fan et al. (2015)
Collagen peptide molecules amino group of chitosan (CS-COP)	Schiff-base reaction CS-COP and the aldehyde of oxidized konjac glucomannan	Wound dressing	Liu et al. (2018)
Gelatin (porcine skin)	Entrapment of Human adipose-derived stem cells (hASCs) inside the hydrogels (3D cultures)	Injectable hydrogels for the engineering of musculoskeletal and other types of connective tissues	Alarake et al. (2017)
Collagen nano-hydroxyapatite Chondroitin sulphate	Human mesenchymal stem cells (hMSC)	Bone grafting	Sharma et al. (2017)
Gelatin	Adipose tissue-derived stromal cells (ADSCs) cultured on the 2D gel surface and 3D hydrogel encapsulation	Wound healing, and soft and hard tissue repair, or as a drug delivery carrier for drug screening	Yang et al. (2016)
Gelatin	Not used	An alternative adhesive that is analogous to the fibrin sealant for stop bleeding, seal leaks, bind tissue, and/or ease healing	McDermott et al. (2004)
Gelatin	3T3 fibroblasts	Protein-based scaffolds for use in soft tissue regeneration	Broderick et al. (2004)
Fish gelatin	Not used	Investigates diverse types of formed networks in physical gels, chemical gels, chemical-physical gels and physical-co-chemical gels	Bode et al. (2011, 2013)
Gelatin	MC3T3-E1 pre-osteoblastic cells (3D)	How 3D matrix and cell density regulating osteocyte differentiation and the formation of the osteocyte network in vitro	Garrigle et al. (2016)
Gelatin	Human mammary fibroblasts (HMFs)	How 3D matrix stiffness affects breast cancer associated fibroblasts morphology and activation	Woods et al. (2017)
Human-like collagen (HLC)	Not used	Injectable soft-tissue filling hydrogel	Zhao et al. (2016)
Chitosan tilapia fish skin gelatin	Not used	Applying small-angle neutron scattering (SANS) was investigated the nanoscale architecture of biopolymer gels conducted in two different microenvironments: an enzymatic process and a hybrid physical-co-chemical process	da Silva et al. (2015)
Gelatin (with/without polyethylene oxide)	Human umbilical vein endothelial cells (HUVECs) and human embryonic kidney cells (HEK 293)	Optimization two gelatin bioink systems for bioengineering (2D and 3D) for cardiovascular, skin and other soft tissue bioengineering	Irvine et al. (2015)
Gelatin and chitosan	Not used	Gelation kinetics and equilibrium rheological properties of mixed gels of chitosan and gelatin for tissue repair applications	da Silva et al. (2014)

Table 3 (continued)

Matrix	3D bioprinting	Uses	References
Gelatin	Mouse embryonic fibroblast cells (NIH 3T3), adipose-derived stem cells (ADSC) and Human hepatoma Huh7 cells	Fabricating large, perfusable, macroporous and cell-laden hydrogel scaffolds	Chen et al. (2014a)
Gelatin	*E. coli* BL21/pTrcHisBGFPuv-plasmid pTrcHisB that had been modified to express a hexahistidine-tagged green fluorescent protein (GFP)	Entrapment of cells within a biopolymeric hydrogel matrix especially useful for microfluidic biosensor systems	Chen et al. (2003)

resulted in the reduction of the propensity of wool yarn or fabric to shrink, and to improve yarn resistance, suggesting that transglutaminases can remediate the negative effects of proteolytic processing of the wool (Cortez et al. 2004). Mojsov (2017) showed that the characteristics of wool fabric pretreated with proteolytic enzymes and transglutaminase is comparable to untreated wool fabric. The author points to the following benefits of treating wool with mTGase: improvement in fabric softness, increased absorption characteristics, and resistance to pilling and retraction of the felting (Mojsov 2017).

Wool garments industrialized using fabrics treated with mTGase are likely to have increased resistance to domestic washing. Biological detergents containing proteases can cause irreversible damage to the fiber, leading to loss of fabric strength, shape, and color fading (Cortez et al. 2005). However, combining the advantages of using both proteases and transglutaminases in a simultaneous enzymatic treatment of wool, resulted in the development of a bioprocess for machine washable wool with insignificant fiber damage (Gaffar Hossain et al. 2008). Casein incorporated to wool mediated by mTGase was used as a surface coating material for smoothing the texture of the wool fiber by coating or filling the damaged scales in wool yarn (Cui et al. 2011).

Finally, excellent antibacterial properties were obtained when Ɛ-Poly-L-lysine (Ɛ-PL), which is a natural biomacromolecule having a broad spectrum of antibacterial activity, was grafted onto the wool fiber via mTGase, showing 97% bacteriostasis to *Escherichia coli* (Wang et al. 2010).

Transglutaminase applications in leather processing

The process of *filling*, which is the introduction of materials into the voids between leather fibers in order to smooth surface irregularities is considered one of the most important steps in leather processing, used to increase material quality. Common materials used as fillers are glucose, flour, and gum, as well as enzyme-modified gelatin and casein, the last two being cross-linked with leather proteins by the action of mTGase (Zhu and Tramper 2008). Experimental results showed that fillers incorporated by mTGase were firmly bound to the leather and would not be easily removed during further processing (Taylor et al. 2006).

Finally, the use of gelatin-sodium caseinate modified by mTGase was investigated regarding subjective aspects of leather (visual aspects, touch, etc.), as well as for its mechanical and structural properties. The application of mTGase improved the subjective aspects, without significantly affecting the mechanical properties such as tensile strength and elongation at break (Liu et al. 2011).

Table 4 Summary the microencapsulation and studies of complex coacervation technique found in literature involving mTGases

Microcapsules	Cells	Results	References
Sodium caseinate (Na-Cs), skim milk powder (SMP) and Extra virgin grape seed oil	*Lactobacillus paracasei* cells	Probiotic remained alive during storage time (above 8 log CFU/g) Incorporation of *L. paracasei* resulted in an incremented antioxidative activity of cheese	Moghaddas Kia et al. (2018)
Whey proteins isolates (WPI), acacia gum and sea buckthorn (SBT) supercritical CO2 extract	Not use	A satisfactory antioxidant activity Antifungal activity against *Penicillium expansum*	Mihalcea et al. (2018)
Gelatin and gum Arabic	Not use	Effective in maintaining the integrity of the microcapsule wall under simulated gastric conditions Dissolved under simulated intestinal conditions	Tello et al. (2016)
Gelatin–maltodextrin (G-MD) and oil	*Lactobacillus* spp.	Survival of *Lactobacillus* spp. in gastrointestinal tract under simulated conditions and released in the intestinal under simulated conditions	Nawong et al. (2016)
Soy protein isolate (SPI)	*Lactobacillus rhamnosus*	Survival the *L. rhamnosus* in the simulated gastrointestinal juice and during storage of probiotic yoghurt	Li et al. (2016)
Gelatin-sodium hexametaphosphate (SHMP) and tuna oil	Not use	Oil was successfully microencapsulated and the stability of omega-3 oils was more than double that of non-encapsulated oil	Wang et al. (2014)
Whey protein microcapsules (WPMs) and soy oil	*Bifidobacterium bifidum* F-35	Increased survival of the encapsulated cells at room temperature and at temperature of 4 °C	Zou et al. (2012)

Conclusion

We addressed the several uses of microbial transglutaminases in the food, pharmaceutical, and biotechnology industries. The applications of mTGase have important implications for the development of these industries, producing new products at low cost, improving the application and quality of food, pharmaceuticals, and other goods such as wool and leather, designed for improving human life in a more sustainable way. mTGases became crucial to produce processed meat and seafood products, dairy products, bread, noodle, soybean products, and to produce coating and edible films. In more sophisticated fields, mTGase has become relevant in PEGylation, antibody drug conjugates, tissue engineering, regenerative medicine, production of microparticles for enteric delivery, directly impacting health products and services. Due to its importance and value aggregation to final products, research on the applications of mTGases is ever growing, showing many possibilities to produce new materials and improving the quality of the existing ones. Further research should focus on the bioprocess technology to reduce production costs of mTGases and enhance their biochemical properties.

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Compliance with ethical standards

Conflicts of interest All authors of this research declare to have no conflicts of interest.

Ethical approval Research was conducted without using human or animal experimentation.

Informed consent No informed consent was necessary to conduct this research.

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