Review

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Crosslinking biopolymers for biomedical applications

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Biomaterials made from proteins, polysaccharides, and synthetic biopolymers are preferred but lack the mechanical properties and stability in aqueous environments necessary for medical applications. Crosslinking improves the properties of the biomaterials, but most crosslinkers either cause undesirable changes to the functionality of the biopolymers or result in cytotoxicity. Glutaraldehyde, the most widely used crosslinking agent, is difficult to handle and contradictory views have been presented on the cytotoxicity of glutaraldehyde-crosslinked materials. Recently, poly(carboxylic acids) that can crosslink in both dry and wet conditions have been shown to provide the desired improvements in tensile properties, increase in stability under aqueous conditions, and also promote cell attachment and proliferation. Green chemicals and newer crosslinking approaches are necessary to obtain biopolymeric materials with properties desired for medical applications.

Biomaterials, crosslinkers, and the need for crosslinking

Biomaterials have been used for a plethora of *in vivo* applications [1]. In this review we focus on biomaterials derived from biopolymers such as cellulose, starch, collagen, silk, chitosan, and poly(lactic acid) because of their advantageous features that include cytocompatibility and ability to degrade in the body without releasing harmful substances. Films, fibers, hydrogels, 2D and 3D structures, micro- and nanoparticles made from biopolymers are being used extensively for both *in vitro* and *in vivo* applications (Figure 1) [2–5]. Although several types of biopolymers have been used to fabricate biomaterials, proteins such as albumin, collagen, and silk are preferable for medical applications because of their better biocompatibility [2–5]. In addition, proteins contain abundant functional groups that facilitate the loading and release of drugs, genes, and nutraceuticals [3–5].

Despite the known advantages and wide applicability of biomaterials, there are several limitations that restrict their use for biomedical applications [3]. Primarily, biopolymeric materials lack adequate mechanical properties and in many instances the stability in aqueous and physiological environments required for medical applications [6]. For instance, films and electrospun structures made from proteins disintegrate at high humidities or in aqueous solutions [7,8]. Crosslinking has been the most common approach to overcome the limitations of biomaterials [9,10]. Crosslinkers interconnect molecules, increase molecular weight, and generally provide higher mechanical properties and improved stability. However, crosslinking also leads to decreased degradability, lower availability of functional groups in the crosslinked polymer, and changes the rheology of the polymers, leading to subsequent processing difficulties and potential increase in cytotoxicity [6].

Various types of crosslinkers and crosslinking techniques are used depending on the type of biopolymer to be crosslinked and the extent of improvement in properties desired (Figure 2, Table 1) [9,11,12]. Among the numerous chemical crosslinkers (Table 1) used, glutaraldehyde (Box 1) is predominantly used because it can react with functional groups in both proteins and carbohydrates, and can provide materials with substantial improvement in tensile properties [13,14]. Although glutaraldehyde provides good improvement in mechanical properties, contradictory evidence has been provided on the cytotoxicity of glutaraldehyde-crosslinked materials [7,8,13–15]. Nevertheless, cytotoxicity of glutaraldehyde is dependent on the concentration used, and up to 8% glutaraldehyde was shown to be non-cytotoxic [13]. Apart from glutaraldehyde, several other chemicals including carbodiimide, epichlorohydrin, and sodium metaphosphate have also been used for crosslinking biopolymers but with limited improvement in properties owing to their low crosslinking efficiency [8,16]. Recently, attempts have been made to use carboxylic acids (Box 2) such as citric acid to crosslink and improve the mechanical properties and stability of biomaterials without compromising the cytocompatibility [7,8]. Crosslinking biomaterials with citric acid provides pendant functionality and allows formation of ester bonds leading to better hemocompatibility and increased availability of binding sites for bioconjugation [17]. This review presents an overview of the chemicals and techniques used to crosslink biopolymeric materials intended for medical applications. Particular emphasis has been placed on protein-based biomaterials because they have better biocompatibility than synthetic polymer-based

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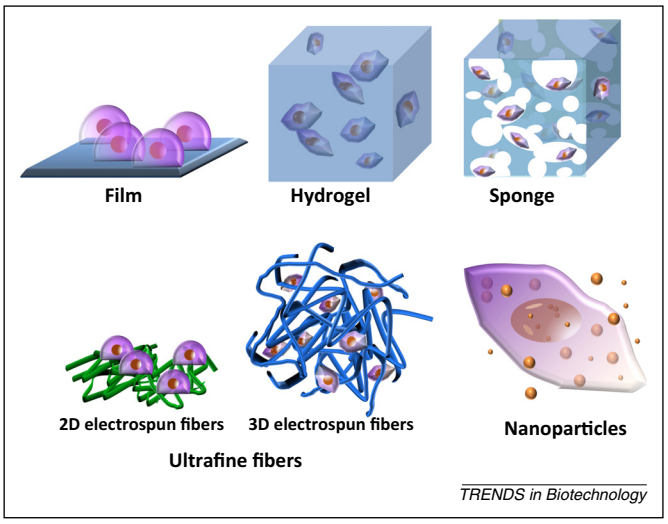

Figure 1. Schematic of the most common forms of scaffolds used for tissue engineering/controlled drug delivery. The relative ease with which cells can penetrate into the scaffolds is shown; the nanoparticles are depicted as being inside the cells.

biomaterials but are less stable in aqueous environments and therefore inevitably need to be crosslinked.

Crosslinking biopolymers to form films and membranes

Films are probably the easiest biomaterial structure to be fabricated and, therefore, most natural and synthetic polymers have been made into films for tissue engineering, controlled release, and other medical applications [18,19]. Films made from a majority of biopolymers including collagen, one of the most widely used proteins for medical applications, have relatively poor mechanical properties and are unstable and dissolve in water or aqueous solutions rapidly [20]. Therefore, primary requirement of crosslinking is the ability to improve mechanical properties and consequently resistance to degradation [21]. Crosslinking collagen films with EDC/NHS [*N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide/*N*-hydroxysuccinimide] or acid chlorides results in an increase in tensile strength up to 57% and an increase in modulus of nearly 17-fold, and the extent of increase could be controlled by varying the crosslinking conditions [21–23]. Considerable decrease in swelling of the films was also observed after crosslinking. Although satisfactory improvement in mechanical properties and aqueous stability was

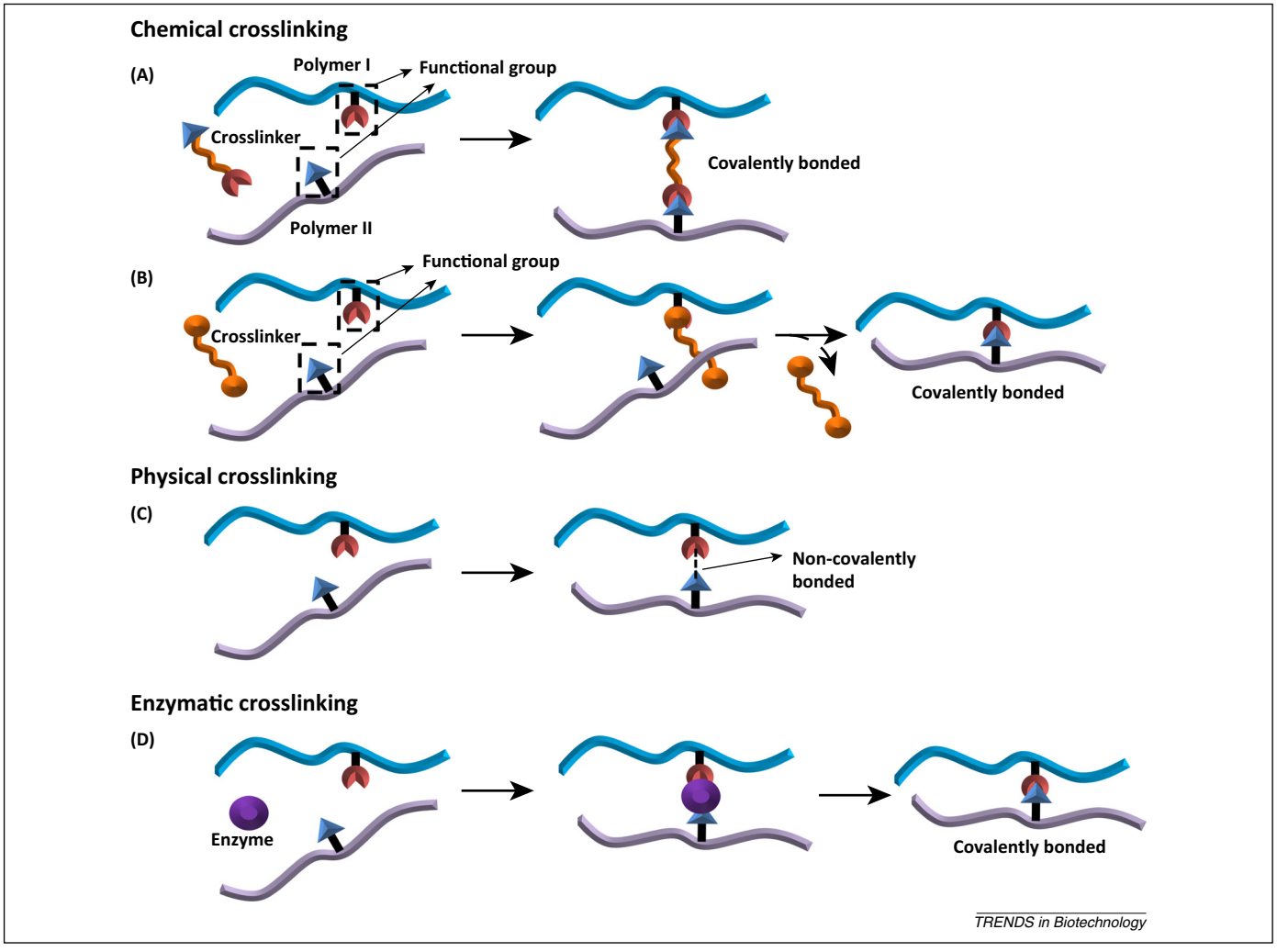

Figure 2. Schematic of the three methods of crosslinking. **(A)** Chemical crosslinking with the crosslinker incorporated into the bond. **(B)** Chemical crosslinking with the crosslinker not incorporated into the bond. **(C)** Physical crosslinking. **(D)** Enzymatic crosslinking.