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Review Article

Scaffolding Biomaterials for Cartilage Regeneration

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Completely repairing of damaged cartilage is a difficult procedure. In recent years, the use of tissue engineering approach in which scaffolds play a vital role to regenerate cartilage has become a new research field. Investigating the advances in biological cartilage scaffolds has been regarded as the main research direction and has great significance for the construction of artificial cartilage. Native biological materials and synthetic polymeric materials have their advantages and disadvantages. The disadvantages can be overcome through either physical modification or biochemical modification. Additionally, developing composite materials, biomimetic materials, and nanomaterials can make scaffolds acquire better biocompatibility and mechanical adaptability.

1. Introduction

Articular cartilage belongs to hyaline cartilage which is avascular and low metabolic. So repairing of cartilage damage resulting from trauma or degeneration has been a thorny clinical issue [1]. Current treatments used in small cartilage defects repairing include multiple drilling, abrasion arthroplasty, mosaicplasty, and autogenous and allogeneic chondrocyte transplantation. Several disadvantages of allograft use include disease transmission, immune reaction, and slower remodeling. Likewise, autograft also has its disadvantages for its requirements of the patient to undergo many surgeries [2]. The rise of tissue engineering in which three basic elements are cells, biodegradable scaffolds, and growth factors provides a new choice for the repair of articular cartilage [3].

In cartilage tissue engineering, scaffolds can provide three-dimensional structure for cartilage cells and be in favor of cell adhesion and proliferation [4]. More importantly, they mediate cell-cell signaling and interaction. However, the physical and biochemical properties are crucial for the scaffolds on the entire cartilage repair process [5].

2. Current Classification and Basic Requirements of Scaffolds

Currently matrix materials suitable for cells can be divided into native biological materials and synthetic polymeric materials [6]. Collagen is a kind of native biological materials with excellent tissue compatibility, little toxicity, and facile biodegradation; meanwhile its degradation products are absorbed facilely without inflammation. Fibrin originates from blood without immunogenicity. So it is widely applied in clinical treatment. Besides its excellent biocompatibility, fibrin can effectively promote the adhesion of chondrocytes. But their common drawbacks, such as weak mechanical properties and unstable degradation rate, limit its application in tissue engineering. Synthetic polymeric material can be molded easily whose microstructure, mechanical properties and degradation can be designed. With its fine biocompatible property, poly (lactic-co-glycolic acid) (PLGA) and polymer of lactic acid (PLA) are widely used in tissue engineering for cartilage. However, as synthetic materials, they are expensive and have weak cell adhesive ability. Polycaprolactone

(PCL) can maintain phenotype and promote chondrocytes proliferation. The most significant advantages of PCL are slow degradation rate and high drug permeability. But it also has drawbacks such as poor hydrophilicity and acidic degradation products which may cause inflammation [7].

Ideal scaffolds for cartilage tissue engineering should be satisfied with the following basic requirements: biocompatible, biodegradable, highly porous, suitable for cell attachment, proliferation and differentiation, osteoconductive, noncytotoxic, flexible and elastic, and nonantigenic [8].

3. Material Modification and Process

The characteristics of native biological materials and synthetic polymeric materials have been described above. Composite materials are currently applied to overcome the disadvantages of single materials [9]. The materials can also be modified by physical and biochemical methods to retain their advantages and overcome their shortcomings [10, 11]. According to recent studies, the scaffold processed into biomimetic materials and nanomaterials is the new trend [12–15] as shown in Figure 1.

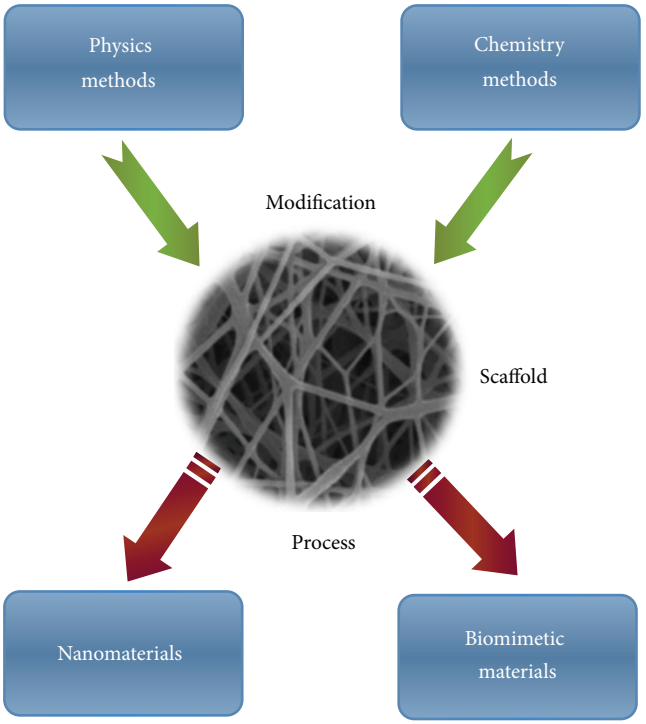

Figure 1: The materials can be modified by physical and biochemical methods and processed into biomimetic materials and nanomaterials to retain their advantages and overcome their shortcomings.

3.1. Physical Modification. Physical modification refers to modification of scaffolds by physical methods such as compression, filtration, and ultraviolet light irradiation to improve the porosity and biomechanical property of materials and ultimately contribute to cartilage repair. Cartilage-derived matrix (CDM) scaffold that mimics chondroinductive environment is a type of acellular matrix material [21]. But it is disappointing to find the scaffolds contract during *in vitro* culture, thus affecting the results of tissue engineering cartilage repair. After treated with dehydrothermal (DHT) or ultraviolet light irradiation (UV), CDM scaffold not only can prevent cell-mediated contraction but also can support cell attachment [16]. Collagen gel as matrix scaffold has become a clinically applicable treatment for focal defects of articular cartilage. However, its biomechanical property is still not satisfying [22]. Compression and filtration make it acquire a higher force carrying capacity. Meanwhile, condensed collagen gel is also suitable for three-dimensional autologous chondrocyte implantation [17]. Another study found that different collagen scaffold structures may provide different immunogenicity. And hydrogels that can avoid severe immune rejection were found to be a promising scaffold structure [23]. Due to the excellent biocompatibility and suitability for cell attachment, alginate scaffold has been applied in cartilage tissue engineering. Recently, Wang et al. [18] produced a highly organized alginate scaffold to improve interconnected porous structure and porosity by microfluidic device. They seeded chondrocytes in the scaffold and found that cells can maintain normal phenotypes, highly express aggrecan and type II collagen, and secrete a great deal of extracellular matrix. The structure of a cartilage scaffold is required to mimic native articular cartilage, which has an oriented structure associated with its mechanical function. Oriented extracellular matrix- (ECM-) derived scaffolds enhance the biomechanical property of tissue engineering cartilage and oriented poly PLGA scaffolds efficiently promotes cell migration thus probably contributes to improving tissue regeneration [19, 20]. An overview of the physical modification on scaffolds is shown in Table 1.

3.2. Biochemical Modification. The weak mechanical property is the most serious problem of native biological materials. As for synthetic polymeric material, its drawbacks are poor hydrophilicity and weak cell adhesive ability [37]. However, scaffolds can be combined with biological modifier which is called biochemical modification to overcome the problems above. In other words, biochemical modification is introduced in the original material to make scaffolds have better tissue compatibility and provide appropriate microenvironment for cell growth and proliferation as shown in Table 2.

3.2.1. Surface Peptide. Peptide is a promising bioactive molecule to improve chondrogenesis in porous biomaterials. Mesenchymal stem cells- (MSCs-) affinity EPLQLKM peptide (E7) was covalently conjugated onto PCL which is implanted into a cartilage defect site of rat knee joints with endogenous MSCs. After 7 d of implantation, the results suggested that the E7 peptide sequence has a high specific affinity to MSCs and enhances the MSCs recruitment of PCL *in vivo* [24]. Another study investigated the generation of tissue engineering cartilage in TATVHL peptide-grafted polyethylene oxide/chitin/chitosan scaffold in which bovine knee chondrocytes were seeded. The results demonstrated that TATVHL peptide-grafted construct improved the proliferation of chondrocytes in constructs, the secretion of glycosaminoglycans, and the production of collagen [25].

Table 1: Physical modification on scaffolds.

Scaffolds	Methods	Effect	References
Cartilage-derived matrix scaffolds	Dehydrothermal treatment ultraviolet light irradiation	Make chondrocytes to produce higher glycosaminoglycan and collagen contents and support cell attachment	Rowland et al., 2013 [16]
Collagen type-I gel	Compression and filtration	Improve the biomechanical and biochemical properties of scaffold	Mueller-Rath et al., 2010 [17]
Alginate scaffold	Microfluidic technology	Enable the scaffold to have a regular interconnected porous structure and high porosity	Wang et al., 2011 [18]
PLGA	Scaffold orientation	Promote cell migration and improve the mechanical property of engineered cartilage	Zhang et al., 2012 [19]
Cartilage extracellular matrix	Scaffold orientation	Enhance the biomechanical property of engineered cartilage	Jia, et al., 2012 [20]

PLGA: poly(lactic-co-glycolic acid).

Table 2: Biochemical modification on scaffolds.

Modifier	Scaffolds	Effect	References
Surface peptide	PCL	Enhance the MSCs recruitment and provide a high specific affinity to MSCs	Shao et al., 2012 [24]
	Polyethylene oxide/chitin/chitosan	Enhance the adhesion and proliferation of bovine knee chondrocytes	Kuo and Wang, 2012 [25]
	PEO/chitosan scaffolds	Stimulate chondrogenesis with enhanced quantities of BKCs, glycosaminoglycans (GAGs), and collagen	Kuo and Wang, 2011 [26]
Bioglass	Agarose scaffolds	Improve the biochemical and mechanical properties of a tissue-engineered cartilage layer	Jayabalan et al., 2011 [27]
	PHBV	Improve the hydrophilicity of scaffolds and promote cell migration into the inner part of the constructs	Wu et al., 2013 [28]
Hyaluronic acid	Silk fibroin scaffolds	Protect the chondral phenotype and improve the structural and physical properties of scaffolds	Foss et al., 2013 [29]
	Gelatin-methacrylamide	Enhance the natural functions of scaffolds in cartilage mechanical and geometrical properties	Schuurman et al., 2013 [30]
	PLGA	Provide appropriate mechanical and structural properties of scaffolds for cells	Chang et al., 2013 [31]
	Collagen scaffolds	Improve cellular infiltration and promotes early-stage chondrogenesis	Matsiko et al., 2012 [32]
Chitosan	PLCL	Improve cells compatibility and form better quality cartilage tissue	Yang et al., 2012 [33]
	PLCL	Promote cell adhesion and proliferation and enhance excretion of aggrecan and type-II collagen	Li et al., 2012 [34]
	Silk fibroin scaffolds	Serve as excellent carrier for stem cells to repair cartilage defects	Deng et al., 2013 [35]
	Gelatin scaffolds	Enhance the cartilage regeneration in vitro and in vivo	Whu et al., 2013 [36]

PCL: polycaprolactone; MSCs: mesenchymal stem cells; PEO: polyethylene oxide; PHBV: polyhydroxybutyrate valerate; PLGA: poly(lactic-co-glycolic acid); PLCL: poly l-lactide-co-ε-caprolactone.

Surface CDPGYIGSR was grafted via cross-linking onto polyethylene oxide (PEO) and chitosan scaffold. After seeding of bovine knee chondrocytes (BKCs) in the scaffolds, the constructs were cultured in a spinner system, indicating that the adhesion of BKCs and the maintenance of phenotypic chondrocytes were more efficient [26].

3.2.2. Bioglass. Bioglass is a sort of glass which possesses particular biological and physiological functions. After implanted into osteochondral defects, bioglass directly combines with the host tissue, playing the role of tissue repairing and restoring. When used as a subchondral substrate, bioactive glass (BG) 13–93 did not improve biochemical properties of scaffolds. However, as a culture medium supplement, BG 13–93 improved the biochemical and mechanical properties of a tissue-engineered cartilage layer. BG 13–93 may be suitable as a medium supplement for neocartilage formation [27]. Another research compared the effects of PHBV scaffolds and PHBV/BG composite scaffolds on the properties of engineered cartilage *in vivo*. The results showed that the incorporation of BG into PHBV efficiently improved both the hydrophilicity of the composites and the percentage of adhered cells and promoted cell migration into the inner part of the constructs [28].

3.2.3. Hyaluronic Acid. As a sort of acidic mucopolysaccharides, hyaluronic acid displayed a variety of important physiological functions due to its unique molecular structure and physicochemical properties such as lubricating joints, regulating vascular permeability, and promoting repair in trauma. More importantly, hyaluronic acid called natural moisturizing factor has such special role of water retention that it has important applications in cartilage tissue engineering scaffolds. Among biomaterials proposed for cartilage repair, silk fibroin (SF) has been recently proposed as a material template for porous scaffolds cultured with chondrocytes and investigated under static and dynamic conditions. The combination of hyaluronic acid (HA) with silk fibroin scaffolds can protect the chondral phenotype and improve the structural and physical properties of scaffolds [29]. Gelatin-methacrylamide (gelMA) hydrogels were shown to support chondrocyte viability and differentiation. However, incorporation of HA allows gelMA to match the natural functions of scaffolds in cartilage mechanical and geometrical properties [30]. Another group fabricated gelatin/hyaluronic acid-treated PLGA (PLGA-GH) sponge scaffolds for articular cartilage tissue engineering. The results showed that cells attachment ratio, proliferation, and extracellular matrix secretion on PLGA-GH scaffolds were superior to those of PLGA scaffolds [31]. Collagen-glycosaminoglycan (CG) scaffolds have been extensively applied in a range of tissue engineering successfully. It is well known that there are two types of glycosaminoglycan: chondroitin sulphate (CS) and hyaluronic acid. Compared to collagen-CS scaffolds, collagen-HA scaffolds showed significant acceleration of early-stage gene expression of SOX-9 and collagen type II as well as cartilage matrix production. The results demonstrated that collagen-HA scaffolds own great potential as appropriate matrices for promoting cartilage tissue repair [32].

3.2.4. Chitosan. Because of the excellent biocompatibility, chitosans have been widely utilized in the field of biomedical materials, such as artificial skin [38], absorbable sutures, hemostatic sponge, and antiadhesion agent. Moreover, chitosans not only can provide appropriate microenvironment for cartilage regeneration but can also stimulate cell proliferation and promote tissue repairing through varieties of ways. A porous elastomeric poly l-lactide-co-ε-caprolactone (PLCL) was generated and cross-linked at the surface to chitosan to improve its wettability. Bone marrow-derived mesenchymal stem cells (BMSCs) were seeded in the constructs to evaluate attachment, morphological change, and proliferation. The results showed that chitosan modification of the PLCL scaffold improved cell compatibility without significant alteration of the physical elastomeric properties of PLCL and resulted in formation of cartilage tissue with better quality [33]. Coincidentally, another study fabricated chitosan-modified poly PLCL scaffolds to simulate the main biochemical components of cartilage, which revealed that the chitosan-modified PLCL scaffolds not only could promote cell adhesion and proliferation, but also could significantly enhance excretion of aggrecan and type-II collagen [34]. In addition to synthetic scaffolds, chitosans can also improve natural materials such as gelatin and silk fibroin scaffolds. BMSCs were seeded in a three-dimensional scaffold of SF and chitosan to repair cartilage defects in the rabbit knee, which indicated that SF/chitosan scaffold can serve as excellent carriers for stem cells to repair cartilage defects [35]. In addition, chitosan-gelatin (1 : 1) complex scaffolds cross-linked by water-soluble carbodiimide (WSC) may enhance cartilage regeneration [36].

3.3. Nanomaterials. Nanomaterials have recently attracted considerable attention because of its high surface-to-volume ratio. Nanomaterials provide a new space for seed cells with a wide range of applications in cartilage tissue engineering. The annulus fibrosus comprises concentric lamellae that can be damaged due to intervertebral disc degeneration. Electrospun nanofibrous scaffolds of polycaprolactone are fabricated in random, aligned, and round-ended configurations to support the growth of annulus fibrosus cells. Primary porcine annulus fibrosus cells are grown on the scaffolds and the results demonstrated that the scaffolds are favorable to attachment, proliferation, and production of extracellular matrix of cells. In addition, the scaffold consisting of round-ended nanofibers substantially outperforms the random and aligned scaffolds on cell adhesion while aligned nanofibers strongly effect the orientation of cells [43]. The menisci are crescent-shaped fibrocartilaginous tissues whose structural organization consists of dense collagen bundles that are locally aligned but show a continuous change in macroscopic directionality. A novel electrospinning method to produce scaffolds composed of circumferentially aligned (CircAl) nanofibers was developed. The results showed these novel scaffolds, with spatially varying local orientations and mechanics, enabled the formation of functional anatomic meniscus constructs [44]. Aligned nanofibrous scaffolds can dictate cell and matrix organization. However, their widespread application has been hindered by poor cell infiltration due to the

Table 3: Proteins, drugs, or cytokine embedded in biomimetic scaffolds.

Embedding	Scaffolds	Effect	References
Antiangiogenic drug	Fibrin/hyaluronan	Block host vessels ingrowth and enhance constructs survival rate	Centola et al., 2013 [39]
Parathyroid hormone-related protein	Collagen-silk	Inhibit terminal differentiation of chondrocytes and enhance chondrogenesis	Zhang et al., 2013 [40]
Transforming growth factor-β1	Poly(L-lactide)	Induce an anabolic stimulus on AFCs and mimic the ECM three-dimensional environment of AF tissue	Vadalà et al., 2012 [41]
Cadherin-11	Collagen type II sponges	Promote adhesion of chondrocytes and stimulate chondrogenic differentiation	Dong et al., 2013 [42]

AFCs: annulus fibrosus cells; ECM: extracellular matrix.

tight packing of fibers during fabrication. Containing two distinct fiber fractions: slow-degrading poly (ε-caprolactone) and water-soluble, sacrificial poly (ethylene oxide) can be selectively removed to increase pore size; tunable composite nanofibrous scaffolds are produced. It is found that increasing the initial fraction of sacrificial poly (ethylene oxide) fibers enhanced cell infiltration and improved matrix distribution [45].

Biochemical modification of nanomaterials will make scaffolds more biocompatible and bioactive. A new functionalized peptide RLN was designed containing the bioactive motif link N and the amino terminal peptide of link protein. A link N nanofiber scaffold (LN-NS) was self-assembled by mixing peptide solution of RLN. This designer functionalized nanofiber scaffold exhibited little cytotoxicity and promoted nucleus pulposus cells (NPCs) adhesion. Besides, it also stimulated the biosynthesis of ECM by NPCs [46]. Biodegradable nanofibrous membrane was prepared from poly-L-lactic acid by electrospinning and used as a scaffold for cartilage tissue engineering. In order to improve cell attachment and growth, nanofibrous membrane was subject to direct current- (DC-) pulsed oxygen plasma treatment, followed by acrylic acid grafting and collagen coating by covalent binding of collagen to carboxylic moieties of the polyacrylic acid. Primary chondrocytes seeding into the membrane proliferated well and maintained high viability according to previous study [47].

3.4. Biomimetic Materials. Biomimetics refers to the structure and function of tissue-engineered cartilage similar to the cartilage extracellular matrix which provides an ideal microenvironment for chondrocytes. Fibrous scaffolds offer a template for cartilage extracellular matrix production. However, the utilization of homogeneous scaffolds is limited by their inability to mimic the cartilage's zone-specific organization and properties. Trilaminar scaffolds were fabricated by sequential electrospinning and varying fiber size and orientation in a continuous construct, to create scaffolds that can mimic the structural organization and mechanical properties of cartilage's collagen fibrillar network on which bovine chondrocytes proliferated and produced a type II collagen and a sulfated glycosaminoglycan-rich extracellular matrix. The results demonstrated that trilaminar composite scaffolds mimicked key organizational characteristics of native cartilage, supported cartilage formation *in vitro*, and had superior mechanical properties [48]. Tissue engineering strategies for the intervertebral disc (IVD) have traditionally focused either on the annulus fibrosus (AF) or the nucleus pulposus (NP) in isolation or have simply compared AF cells (AFCs) and NP cells (NPCs) under identical culture conditions. One group developed biomimetic circumferentially orientated polycaprolactone fibres (AF analogue) and seeded them with cells (porcine chondrocytes) and then coagulated a cell-agarose solution in the centre (NP analogue). The results demonstrated that the composite IVD scaffolds had higher modulus and cells were viable and well-distributed around the interface between the NP and AF regions [49]. Besides, a three-layered wedge shaped silk meniscal scaffold system was engineered to mimic native meniscus architecture, which were seeded with human fibroblasts and chondrocytes in a spatial separated mode similar to native tissue in order to generate meniscus-like tissue *in vitro*. This multiporous silk construct is a useful micropatterned template for directed tissue growth with respect to form and function of meniscus-like tissue [50].

Besides mimicking the structure of extracellular matrix, embedding proteins, drugs, or cytokine in scaffolds to mimic the function of ECM is also a biomimetic method as suggested in Table 3. In the process of cartilage repaired with tissue engineering, blood vessel ingrowth and macrophage migration may endanger graft stability of immature constructs. So, control of angiogenesis was proposed as an adjuvant for the treatment of cartilage defects. A clinically compatible fibrin/hyaluronan scaffold with nasal chondrocytes (NC) and functionalized with an FDA-approved antiangiogenic drug (bevacizumab) sequestrates vascular endothelial growth factor from the surrounding environment. The proposed pharmacological control of angiogenesis by a therapeutic drug released from a scaffold might enhance constructs survival rate and cartilage regeneration [39]. The repair of cartilage defects can be enhanced with scaffolds but is often accompanied with undesirable terminal differentiation of bone marrow-derived mesenchymal stem cells. Parathyroid hormone-related protein (PTHrP) has been shown to inhibit

aberrant differentiation [51]. Combining PTHrP administration with collagen-silk scaffold is an effective strategy for inhibiting terminal differentiation and enhancing chondrogenesis, thus improving cartilage repair and regeneration [40]. Transforming growth factor- (TGF-) β1 plays an important role in chondrogenesis [51]. A bioactive microfibrous poly (L-lactide) scaffold was synthesized by electrospinning, with a direct incorporation of TGF-β1 into the polymeric solution, on which bovine AFCs were cultured up to 3 weeks. Results demonstrated that AFCs cultured on PLLA/TGF deposited a significantly greater amount of glycosaminoglycans and total collagen with higher neo-ECM thickness [41]. In cartilage tissue engineering, cell adhesion is commonly promoted through the use of polypeptides; however, due to their lack of complementary or modulatory domains, polypeptides must be modified to improve their ability to promote adhesion. According to the principle of matrix-based biomimetic modification, our team utilized a recombinant protein, which spans fragments 7–10 of fibronectin module III (heterophilic motif) and extracellular domains 1-2 of cadherin-11 (rFN/Cad-11) (homophilic motif), to modify the interface of collagen type II (Col II) sponges. The results suggested that the rFN/Cad-11-modified collagen type II biomimetic interface has dual biological functions of promoting adhesion and can stimulate chondrogenic differentiation [42].

4. Challenges and Perspectives

Currently, the main research directions of biomaterials are as follows: the first one is modifying the surface of scaffolds though physical and chemical methods improve the bioactivity of materials for seed cells adhesion and distribution. The second one is making use of new technology to modify the morphology and spatial structure of the materials to compensate the insufficiency in order to build ideal scaffolds. The last one is combining natural materials with synthetic materials to fabricate composite materials with good biocompatibility and mechanical adaptability. With the emergence of new preparation techniques such as 3D fibre deposition and three-dimensional printing, research on cartilage tissue engineering scaffolds has made considerable progress. In addition, as an advanced detecting and monitoring method, biosensors are essential to the development of biotechnology. Electrochemical biosensors can be used for the detection of microRNAs [52]. And electrochemical immunoassays could be used in cancer diagnosis, prognosis, and therapy monitoring [53]. So, the combination of biosensing techniques with biomaterials would vigorously promote the development of tissue engineering. However, with either natural or synthetic materials there exists some problems, such as degradation rate and poor biocompatibility. There is still a great gap to the clinical application of tissue engineering cartilage. Notably, besides scaffolds, other elements of cartilage tissue engineering, cells, and growth factors cannot be ignored. Future research priorities of tissue engineering scaffolds are to improve existing materials and fabrication techniques and to further develop the composite materials, biomimetic materials, nanomaterials, and modified materials. In the near future, artificial cartilage might show its full potential for the treatment for cartilage injury.

Conflict of Interests

The authors declare that they have no financial or personal relationship with any people or organization that may inappropriately influence their work; there is no professional or commercial interests of any kind in all the commercial identities mentioned in their paper.

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