Journal of Dental Research
2016, Vol. 95(9) 1049–1056

DOI: 10.1177/0022034516648604
jdr.sagepub.com

PDL Progenitor–Mediated PDL Recovery Contributes to Orthodontic Relapse

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Abstract

Periodontal ligament (PDL) is subjected to mechanical force during physiologic activities. PDL stem/progenitor cells are the main mesenchymal stem cells in PDL. However, how PDL progenitors participate in PDL homeostasis upon and after mechanical force is largely unknown. In this study, force-triggered orthodontic tooth movement and the following relapse were used as models to demonstrate the response of PDL progenitors and their role in PDL remodeling upon and after mechanical force. Upon orthodontic force, PDL collagen on the compression side significantly degraded, showing a broken and disorganized pattern. After force withdrawal, the degraded PDL collagen recovered during the early stage of relapse. Correspondingly, increased $CD90^+$ PDL progenitors with suppressed expression of type I collagen (Col-I) were observed upon orthodontic force, whereas these cells accumulated at the degradation regions and regained Col-I expression after force withdrawal during early relapse. Our results further showed that compressive force altered cell morphology and repressed collagen expression in cultured PDL progenitors, which both recovered after force withdrawal. Force withdrawal–induced recovery of collagen expression in cultured PDL progenitors could be regulated by transforming growth factor–β (TGF-β), a key molecule for tissue homeostasis and extracellular matrix remodeling. More interesting, inhibiting the regained Col-I expression in $CD90^+$ PDL progenitors by blocking TGF-β interrupted PDL collagen recovery and partially inhibited the early relapse. These data suggest that PDL progenitors can respond to mechanical force and may process intrinsic stability to recover to original status after force withdrawal. PDL progenitors with intrinsic stability are required for PDL recovery and consequently contribute to early orthodontic relapse, which can be regulated by TGF-β signaling.

Keywords: periodontal ligament, stem cells, orthodontic tooth movement, stress, collagen, growth factor

Introduction

Periodontal ligament (PDL) is a specialized connective tissue that facilitates attachment of teeth onto the alveolar bone. Heterogeneous cell populations in collagen-rich PDL are subjected to mechanical stimulation caused by occlusion and mastication, and the homeostasis of PDL is maintained continuously. As the main mesenchymal stem/progenitor cells (MSCs) in PDL, PDL stem/progenitor cells (PDLSCs) have the capacity to differentiate into periodontal cell types in vitro and generate PDL complex structure in vivo (Seo et al. 2004), playing an important function in PDL homeostasis and regeneration. However, how PDL progenitors contribute to PDL homeostasis upon and after mechanical force has not been clearly elucidated.

Force-triggered orthodontic tooth movement (OTM) rebuilds periodontal tissue. Along with osteoclastic and osteoblastic activities of alveolar bone, PDL undergoes remodeling during OTM (Krishnan and Davidovitch 2006). Collagen is the main structural component of extracellular matrix (ECM) of PDL and absorbs mechanical load during physiologic activities (Butler et al. 1975). Upon application of orthodontic force, PDL collagen demonstrates degradation in the compression region, accompanied by altered expression of type I collagen (Col-I; Rygh 1973; Bumann et al. 1997). When orthodontic force is withdrawn, relapse occurs, and PDL has the potential to regain original structure during relapse (Yoshida et al. 1999). However, the response of PDL progenitors upon and after orthodontic force and their role in the recovery of PDL and the process of relapse remain unknown.

Transforming growth factor–β (TGF-β) performs an important function in tissue homeostasis and repair (Crowe et al. 2000; Guasch et al. 2007). TGF-β mediates ECM synthesis and

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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plays an indispensable role in maintenance of articular cartilage (Yang et al. 2001). During wound healing, TGF-β activates fibroblasts, regulates ECM deposition, and is required for healing process (Sporn et al. 1983). Furthermore, under mechanical loading, TGF-β signaling can induce Col-I production of tendon fibroblasts to remodel tendon matrix (Yang et al. 2004). Therefore, we speculated that TGF-β might participate in PDL recovery after mechanical force withdrawal and relapse.

This study aimed to investigate 1) the responses of PDL collagen and PDL progenitors upon and after mechanical force and 2) the role of PDL progenitors in the recovery of PDL collagen and the process of orthodontic relapse.

Materials and Methods

Animals

Sprague-Dawley rats (male, 6 to 7 wk old) were used in this study. Experimental protocols were approved by the Animal Use and Care Committee of Peking University (LA2013-92).

Application of Orthodontic Devices and Relapse

Orthodontic force was applied on rats as previously described (Cao et al. 2014). Briefly, maxillary right first molars were ligated to incisors by coiled springs (Smart Technology) and moved mesially. The springs delivered approximately 60 cN of constant force measured by a dynamometer, as 40 to 60 cN of force was used to induce OTM in rat models (Han et al. 2010; He et al. 2015). The contralateral side served as control. Upon orthodontic force for 14 d, rats were randomly divided into 4 groups ($n = 7$), and springs were removed to let the first molars relapse for 0 d (F14d), 1 d (R1d), 5 d (R5d), and 14 d (R14d). At each time point, rats were sacrificed, and silicone impressions of maxillary dentitions were obtained (Appendix Fig. 1A). The distance between the midpoint of the distal-edge ridge of the first molars and the midpoint of the mesio-edge ridge of the second molars was measured from the occlusal view of impressions with stereoscope, and the ratio of relapse was calculated (distance of relapse / distance of original tooth movement × 100%).

TGF-β Receptor I Inhibitor Administration during Relapse

Another batch of rats was used to explore the participation of TGF-β in relapse. The first molars of rats also experienced orthodontic force for 14 d. One day prior to relapse, rats were randomly divided into 2 groups: a group that received local injection of vehicle and a group that received TGF-β receptor I (TGF-β RI) inhibitor (SB 431542 [SB], 10 μg/rat; S4317, Sigma) every day ($n = 7$; Sugiyama et al. 2008). The injection of TGF-β RI inhibitor was performed at the subperiosteum area around the moved first molars. All rats were harvested at 5 d of relapse. Silicone impressions were obtained, and relapse ratio was calculated as previously described.

Tissue Preparation

Maxillae were harvested for fixing in 4% paraformaldehyde, demineralized in 15% ethylenediaminetetraacetic acid, and embedded in paraffin. Consecutive horizontal sections (4 μm) were obtained from mid- to cervical third of each sample for consistent observations. The middle buccal roots of first molars were observed, and the contralateral side was defined as the control (Hirate et al. 2012).

Cell Culture

The protocol used to obtain human tissue samples was approved by the Ethical Guidelines of Peking University (PKUSSI RB-201311103) and was performed with appropriate informed consents. Human PDL progenitors were isolated and cultured as previously described (Seo et al. 2004). The cultured PDL progenitors were identified and used at passage 4 (Appendix Fig. 2).

Compressive force was applied on cultured cells as previously described (Cao et al. 2014). Briefly, a layer of glass cover and additional metal weights on top were placed over an 80% confluent cell layer. PDL progenitors were cultured upon compressive force (1 g/cm^2) for 24 h or were followed by another 12- or 24-h culture without or with SB (10 μM) treatment after force withdrawal.

Methods

The following are described in detail in the Appendix:

- Masson trichrome staining
- Picrosirius red staining
- Immunofluorescence staining
- Toluidine blue staining
- Quantitative real-time polymerase chain reaction
- Western blot

Statistical Analysis

Statistical analysis was performed with SPSS 13.0. Comparison among groups was statistically analyzed by 1-way analysis of variance, followed by the least significant difference multiple-comparison test. Comparison between groups was statistically analyzed by 2-sample t tests. All data were presented as mean ± SD. Statistical significance was considered at $P < 0.05$.

Results

PDL Collagen Significantly Recovered from Orthodontic Force–Induced Degradation during Early Relapse

Upon orthodontic force for 14 d, significant tooth movement was observed. Relapse occurred after orthodontic force withdrawal. At 5 d of relapse, the relapse ratio was up to 53.42% ± 6.59%, which increased to 83.25% ± 0.43% at 14 d of relapse (Fig. 1A, B). These data indicate that relapse ratio rapidly increased in the

early stage (F14d to R5d). Meanwhile, the number of osteoclasts on the former tension side showed no significant increase until 5 d of relapse (Appendix Fig. 1B), which suggests an osteoclast nondependent pattern in the early relapse.

Collagen plays a pivotal role in anchoring the tooth into the alveolar bone. PDL collagen fibers were continuous and well organized in the control group, assessed by Masson trichrome staining. Upon orthodontic force for 14 d, the density of PDL collagen on the compression side remarkably decreased, showing a broken and disorganized pattern. After force withdrawal, degraded PDL collagen recovered during early relapse. Several tiny and discontinuous fiber fragments were observed in the R1d group. Relatively continuous and organized collagen fibers were detected in the R5d group. In the R14d group, collagen fibers returned to a continuous and well-organized pattern similar to those in the control group (Fig. 1C, D).

Picrosirius red staining with polarizing images was done to further investigate the alteration of PDL collagen. PDL collagen fibers from the control group showed yellowish-red predominated polarization color with well-organized feature. Upon orthodontic force for 14 d, PDL collagen fibers on the compression side mostly disappeared, and no evident polarization color could be observed. During the relapse, polarization color–indicated collagen fibers gradually recovered. Small discontinuous fibers with green polarization color were observed in the R1d group, and relatively continuous greenish-yellow collagen fibers were detected in the R5d group. These fibers returned to yellowish-red in the R14d group (Fig. 1E, F). These results demonstrate that PDL collagen significantly recovered from orthodontic force–induced degradation after force withdrawal during early relapse.

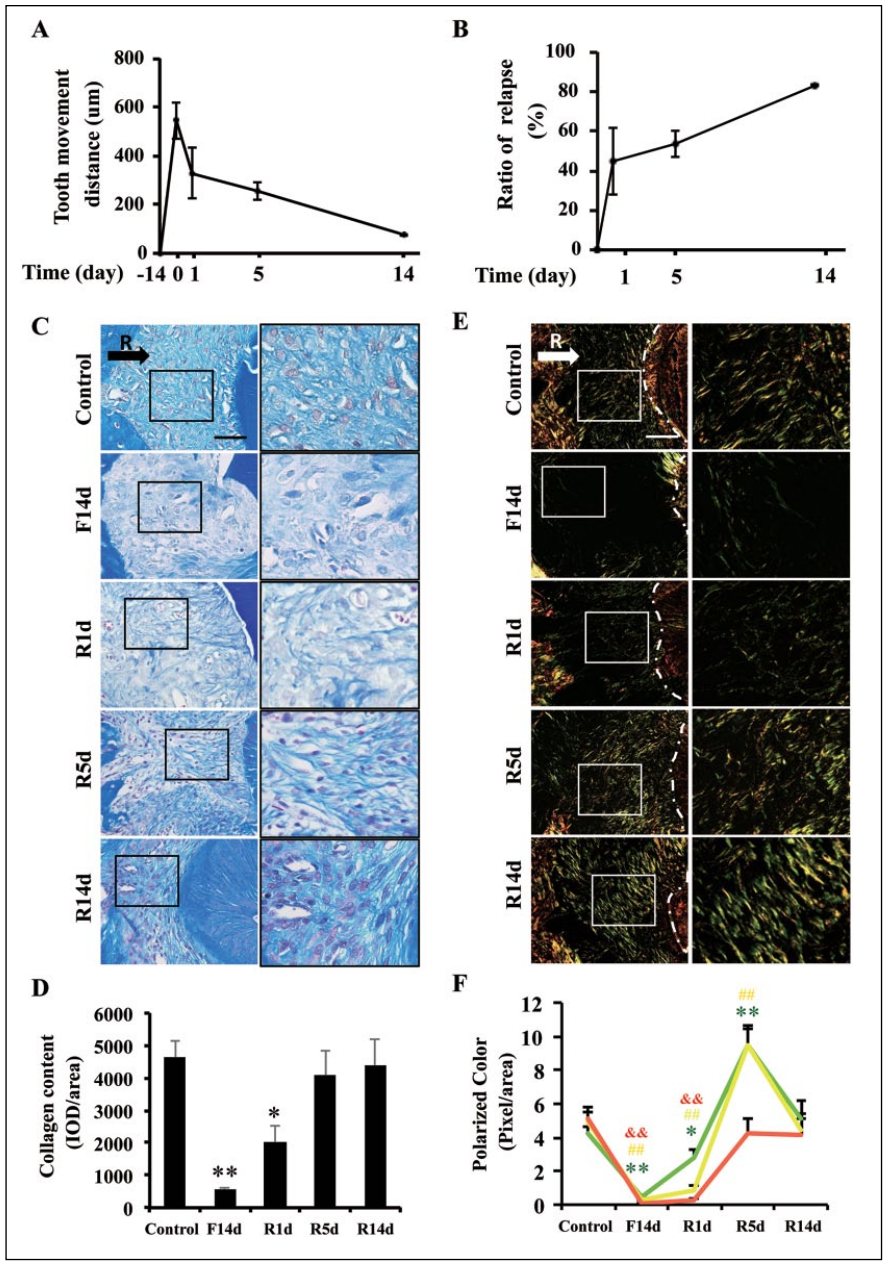

Figure 1. Periodontal ligament (PDL) collagen significantly recovered from orthodontic force–induced degradation after force withdrawal during early relapse. (**A**, **B**) Distance of tooth movement (A) and relapse ratio (B) at each time point. $n = 7$. (**C**) Masson trichrome staining results exhibited the remodeling process of PDL collagen upon orthodontic force and the following relapse. F14d, upon orthodontic force for 14 d, relapse for 0 d; R1d, relapse for 1 d; R5d, relapse for 5 d; R14d, relapse for 14 d. (**D**) Semiquantitative analysis of Masson trichrome staining showed that collagen content of PDL was significantly decreased in the F14d group and recovered during early relapse (R1d and R5d groups). $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group. IOD, integral optical density. (**E**) Representative images of PDL collagen with picrosirius-polarization method upon orthodontic force and the following relapse. (**F**) Semiquantitative analysis of green, yellow, and red of polarized images of PDL collagen upon orthodontic force and the following relapse. $n = 3$; green, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. green of control group; yellow, $^{\#\#}P < 0.01$ vs. yellow of control group; red, $^{\&\&}P < 0.01$ vs. red of control group. Small and large frames indicate the original and magnified areas, respectively. The large arrow represents the direction of relapse. Dashed lines mark the outline of the roots. Original scale bar: 50 μm.

CD90^{+} PDL Progenitors Regained Col-I Expression during Early Relapse

CD90 is expressed in stem/progenitor cells (Dennis et al. 2007) and can be used as a marker for characterizing progenitors in rat (Kon et al. 2009; Hosoya et al. 2012). Col-I is the main component of PDL collagen (Butler et al. 1975). We double stained CD90 and Col-I to investigate the response of PDL progenitors upon and after orthodontic force and their contribution to the recovery of PDL collagen

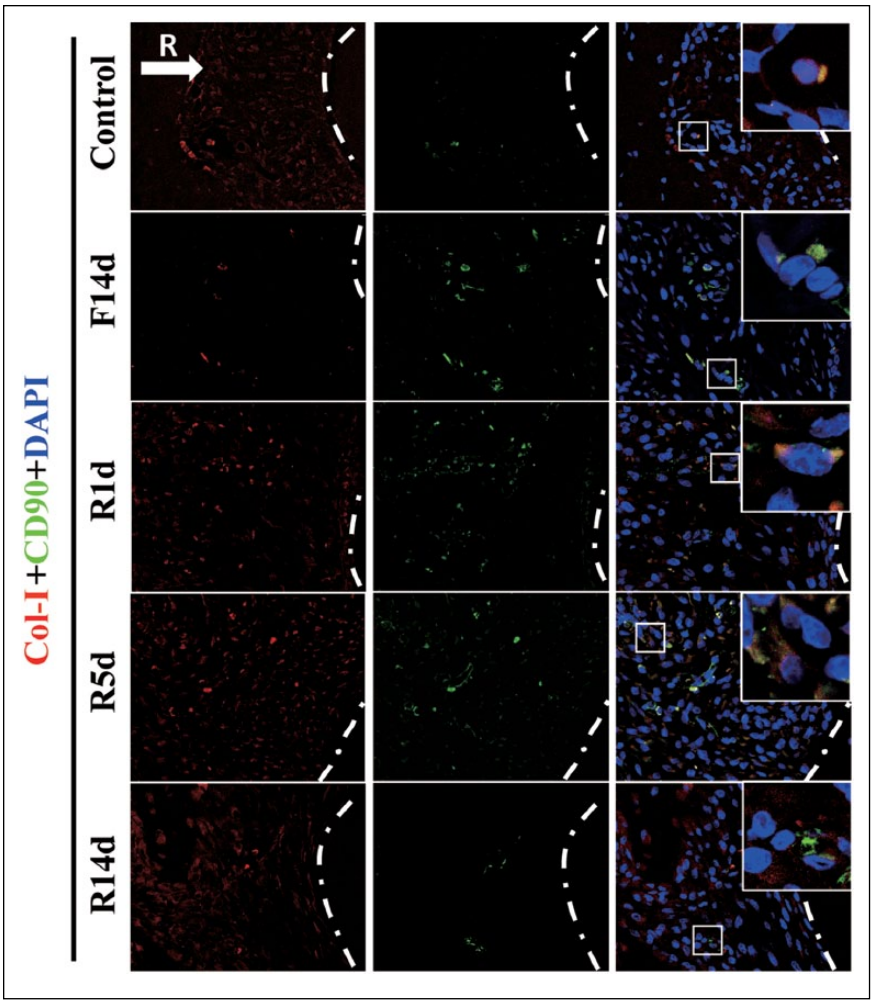

Figure 2. CD90$^+$ periodontal ligament (PDL) progenitors regained type I collagen (Col-I) expression after orthodontic force withdrawal during early relapse. Immunofluorescence staining demonstrated that a few CD90$^+$ PDL cells (green) with Col-I expression (red) were observed in the control group. CD90$^+$ PDL cells (green) increased with suppressed Col-I expression (red) on the compression side of PDL in the F14d group. Accumulated CD90$^+$ PDL cells (green) regained Col-I expression (red) in the R1d and R5d groups. Then the number of CD90$^+$ PDL cells (green) with Col-I expression (red) returned to similar level to the control group in the R14d group. The large arrow represents the direction of relapse. Dashed lines mark the outline of the roots. Scale bars: 50 μm. F14d, upon orthodontic force for 14 d; R1d, relapse for 1 d; R5d, relapse for 5 d; R14d, relapse for 14 d.

during relapse. In the control group, a few CD90$^+$ cells with Col-I expression were observed in PDL. Upon orthodontic force for 14 d, CD90$^+$ cells increased, while their Col-I expression was suppressed. After force withdrawal, these CD90$^+$ cells accumulated at the degradation regions and regained Col-I expression during early relapse. Afterward, the number of CD90$^+$ cells in the R14d group returned to a level similar to that of the control group (Fig. 2). These data indicate that PDL progenitors could respond to orthodontic force with suppressed collagen expression and regain collagen expression after force withdrawal, which may contribute to the recovery of PDL collagen during early relapse.

Compressive Force Repressed Collagen Expression in Cultured PDL Progenitors, Which Recovered after Force Withdrawal Regulated by TGF-β Signaling

To confirm whether PDL progenitors had the potential to recover after mechanical force withdrawal, we investigated the response of cultured PDL progenitors upon and after compressive force. Extracellular strain can induce conformational changes in cytoskeletal elements, which in turn change and activate signaling pathways (Sen et al. 2014). We found that cultured PDL progenitors acquired significantly elongated morphology and denser actin distribution upon compressive force, which recovered after force withdrawal (Fig. 3A). Concomitantly, collagen matrix and Col-I expression in cultured PDL progenitors were repressed upon compressive force and also gradually recovered after force withdrawal (Fig. 3B–E). By contrast, the repressed Col-I expression upon compressive force in PDL fibroblasts showed delayed recovery after force withdrawal (Appendix Fig. 3). These data are consistent with the in vivo results and suggest that PDL progenitors could respond to mechanical force and have the potential to recover after force withdrawal, which may be defined as intrinsic stability. The intrinsic stability of PDL progenitors is required for the regained collagen expression after force withdrawal.

TGF-β plays an important role in cell homeostasis and ECM remodeling (Creely et al. 1992; Yang, Zhou, et al. 2014). We investigated whether TGF-β could regulate the intrinsic stability of collagen expression in cultured PDL progenitors. TGF-β1 and TGF-β3 expressions in cultured PDL progenitors were repressed upon compressive force and recovered after force withdrawal, which was consistent with the alteration of collagen expression (Appendix Fig. 4). Moreover, the force withdrawal–induced recovery of collagen expression in cultured PDL progenitors was partially inhibited by TGF-β RI inhibitor, accompanied with repression of TGF-β/Smad pathway (Fig. 4). In addition, the repressed Col-I expression upon compressive force was partially rescued by TGF-β1 and TGF-β3 treatment (Appendix

Fig. 5). These data suggest that the intrinsic stability of collagen expression in PDL progenitors could be regulated by TGF-β signaling.

Inhibiting Regained Col-I Expression in PDL Progenitors Interrupted PDL Collagen Recovery and Alleviated Early Relapse

To further demonstrate the contributions of PDL progenitors and their intrinsic stability of collagen expression to the recovery of PDL collagen and the process of early relapse, we locally administered TGF-β RI inhibitor SB during early relapse. Immunofluorescence staining confirmed that the regained Col-I expression in $CD90^+$ cells was significantly suppressed by SB treatment when compared with the vehicle group at 5 d of relapse (Fig. 5A, B). Correspondingly, Masson trichrome staining showed that relatively organized and continuous PDL collagen fibers were observed at 5 d of relapse in the vehicle group, which remained broken and disordered in the SB group (Fig. 5C). Semiquantitative analysis confirmed that the recovery of PDL collagen content was statistically repressed by SB treatment ($P < 0.05$; Fig. 5D). With the picrosirius-polarization method, PDL collagen fibers appeared organized and continuous with greenish-yellow polarization color in the vehicle group, whereas a discontinuous pattern with green polarization color was presented in the SB group (Fig. 5E, F).

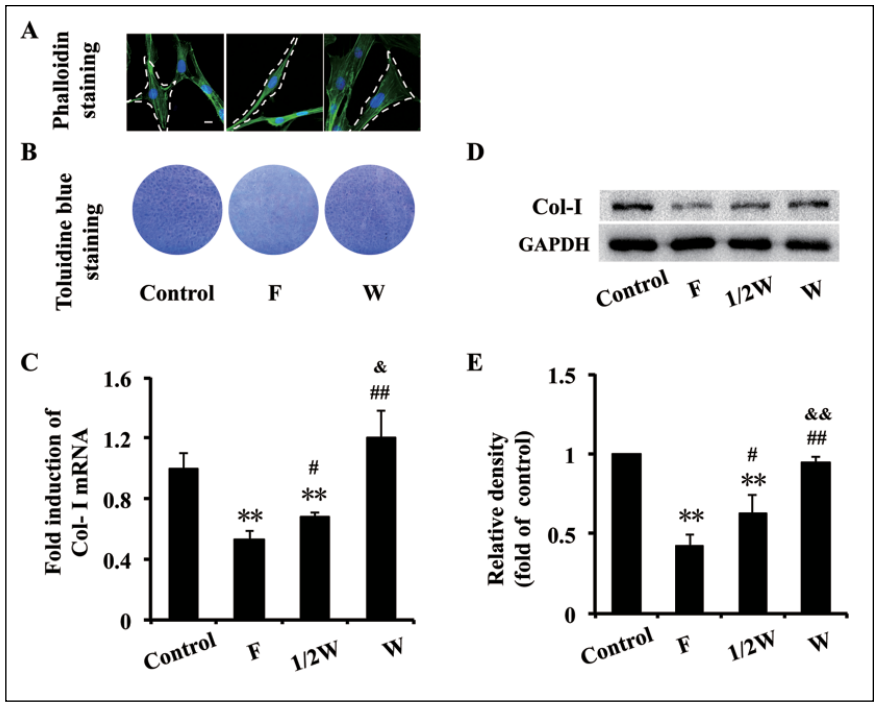

Figure 3. Compressive force repressed collagen expression in cultured periodontal ligament (PDL) progenitors, which recovered after force withdrawal. (**A**) Phalloidin staining showed that cultured PDL progenitors acquired significantly elongated morphology and denser actin distribution upon compressive force, which recovered after force withdrawal. Dashed lines mark the outline of the cells. Scale bars: 20 μm. (**B**) Toluidine blue staining demonstrated that compressive force repressed collagen matrix in cultured PDL progenitors, which significantly recovered after force withdrawal. (**C–E**) Compressive force repressed type I collagen (Col-I) expression in cultured PDL progenitors, which partially recovered after force withdrawal for 12 h and fully recovered after force withdrawal for 24 h, as examined by real-time polymerase chain reaction (C) and Western blot (D, E). F, group with force alone; 1/2W, group with force application, then force withdrawal for 12 h; W, group with force application, then force withdrawal for 24 h. $n = 3$; $^{**}P < 0.01$ vs. control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. F group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ vs. 1/2W group. GAPDH served as internal control for equal loading. These data represent 3 independent experiments.

Furthermore, the ratio of early relapse was significantly reduced by SB treatment as compared with the vehicle group ($P < 0.05$; Fig. 5G, H). In addition, no statistical difference in the number of osteoclasts on the former tension side was observed between the vehicle and experimental groups at 5 d of relapse (Appendix Fig. 6). These data suggest that PDL progenitors with intrinsic stability play an important role in the recovery of PDL collagen and consequently contribute to early relapse, which can be regulated by TGF-β.

Discussion

In this study, we demonstrated that PDL progenitors with intrinsic stability contributed to the recovery of PDL collagen and the process of orthodontic relapse. First, orthodontic force prominently altered the pattern and density of PDL collagen, which recovered after force withdrawal during early relapse. Concomitantly, Col-I expression in $CD90^+$ PDL progenitors was suppressed upon orthodontic force and recovered after force withdrawal during early relapse. Second, cultured PDL progenitors responded to compressive force and recovered to original status after force withdrawal. The intrinsic stability–induced recovery of collagen expression in cultured PDL progenitors could be regulated by TGF-β signaling. Moreover, inhibiting the intrinsic stability of Col-I expression in PDL progenitors by blocking TGF-β interrupted PDL collagen recovery and alleviated early relapse. These results indicate that PDL progenitors with intrinsic stability to regain collagen expression are required for the recovery of PDL and consequently contribute to early relapse, which can be regulated by TGF-β signaling.

Tissue-specific MSCs can generate corresponding ECM to participate in tissue repair, healing, and regeneration (Prockop 2009; Lee et al. 2015). Transplanted PDLSCs can form a typical cementum/PDL-like structure with a dense Col-I-positive tissue (Seo et al. 2004) and are used to regenerate periodontal tissues for periodontitis (Liu et al. 2008). In this study, we focused on the participation of PDL progenitors in homeostasis

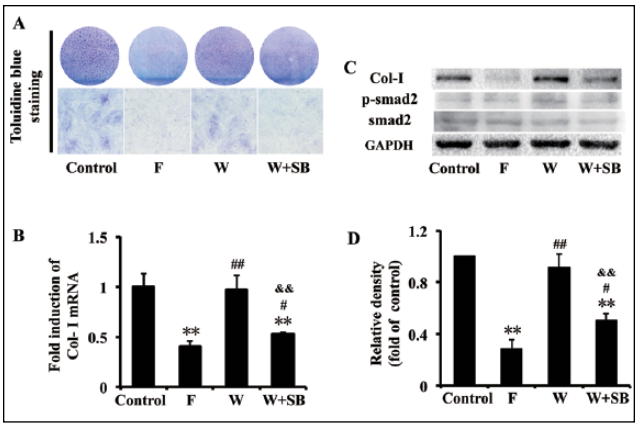

Figure 4. Regained collagen expression in cultured periodontal ligament (PDL) progenitors after compressive force withdrawal was regulated with transforming growth factor–β (TGF-β) signaling. (**A**) Toluidine blue staining demonstrated that force withdrawal–induced recovery of collagen matrix in cultured PDL progenitors was partially inhibited by SB 431542 (SB) treatment. (**B–D**) Force withdrawal–induced recovery of type I collagen (Col-I) expression in cultured PDL progenitors was partially inhibited by SB treatment accompanied by the repression of the TGF-β/Smad pathway, as examined by real-time polymerase chain reaction (B) and Western blot (C, D). F, group with force alone; W, group with force application, then withdrawal force for 24 h; W+SB, group with force application, then force withdrawal for 24 h with SB treatment. $n = 3$; $^{**}P < 0.01$ vs. control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. F group; $^{\&\&}P < 0.01$ vs. W group. GAPDH served as internal control for equal loading. These data represent 3 independent experiments.

of PDL collagen upon and after mechanical force. Upon orthodontic force for 14 d, PDL collagen on the compression side significantly degraded, and $CD90^+$ PDL progenitors increased with suppressed Col-I expression. After force withdrawal, $CD90^+$ PDL progenitors accumulated at the degradation regions and regained Col-I expression during early relapse, and PDL collagen regained its pattern and density correspondingly. Moreover, inhibiting the regained Col-I expression in $CD90^+$ PDL progenitors significantly interrupted the recovery of PDL collagen during early relapse. These results indicate that PDL progenitors with regained collagen expression could contribute to the recovery of PDL collagen during force withdrawal–related relapse, especially in the early stage. Additionally, PDL fibroblasts responded to mechanical force but showed relatively delayed recovery after force withdrawal, which could contribute to relapse in a relatively later stage. However, the distinctive roles of PDL progenitors and PDL fibroblasts in the recovery of PDL collagen during relapse need further studies.

Mechanical stimulation can affect phenotype and function of MSCs (Luu et al. 2009; Ruan et al. 2015). Proliferation and differentiation of PDLSCs can be influenced by mechanical stimulation (Zhang et al. 2015). However, the response of PDL progenitors after force withdrawal is unknown. In this study, PDL progenitors responded to compressive force with altered cell morphology, suppressed collagen, and TGF-β expression, which recovered after force withdrawal. Previous studies showed that MSCs responded to environmental stimulation (e.g., stiffness of substrate and proinflammatory factors), retained the environmental signals for a short period, and finally regained their original status (Yang, Tibbitt, et al. 2014; Liu et al. 2016); our findings are consistent with these results. To our knowledge, this study is the first to demonstrate that PDL progenitors have the potential to recover to original status after mechanical force withdrawal, which can be defined as intrinsic stability and is required for PDL homeostasis to withstand mechanical stimulation. Nevertheless, mechanical force application and force withdrawal in vitro may not perfectly reproduce the situation of cells upon and after stimulation in vivo, and evidences of cell morphology, collagen, and TGF-β expression only demonstrated several aspects of responses of PDL progenitors upon and after mechanical force. Our results provided extended understandings on the property of PDL progenitors.

Upon mechanical force, TGF-β can regulate ECM remodeling to adapt tissues to force condition (Yasuda et al. 1996). TGF-β pathway increases α1 (II) collagen expression in chondrocytic cells under tensile strain (Furumatsu et al. 2013) and modulates Col-I production in tendon fibroblasts under mechanical stretching (Yang et al. 2004). In our study, collagen expression in cultured PDL progenitors upon and after compressive force could be regulated by the TGF-β/Smad pathway, which is consistent with these previous studies and extends the regulatory role of TGF-β. Three isoforms of TGF-β—namely, TGF-β1, TGF-β2, and TGF-β3—activate the same intracellular signaling pathway, demonstrate overlapping biological functions in vitro, and exhibit unique expression patterns in vivo (Massagué 1990). We found that TGF-β1 and TGF-β3 expressions were consistent with collagen expression upon and after compressive force and could partially rescue the repressed Col-I expression upon compressive force, which indicates the possible involvement of these 2 isoforms in collagen expression upon and after mechanical stimulation in PDL progenitors. Moreover, blocking TGF-β inhibited PDL progenitors regaining Col-I expression and interrupted PDL collagen recovery during early relapse. Previous study showed that interruption of TGF-β signaling in chondrocytes resulted in degeneration of cartilage ECM and pathologic phenotype of articular cartilage (Shen et al. 2013), which supports our results. Taken together, TGF-β signaling plays an important role in intrinsic stability of collagen expression in PDL progenitors and homeostasis of PDL upon and after mechanical stimulation.

Relapse is a major clinical challenge in orthodontic treatment. In this study, significantly increased osteoclasts on the former tension side were detected in the later stage of relapse, which is consistent with previous observations (Adachi et al. 1994; Franzen et al. 2013). These results suggest that osteoclasts could participate in the later stage of relapse. Meanwhile, PDL collagen recovered from force-induced degradation during early relapse correspondingly with rapid relapse ratio. Inhibiting the recovery of PDL collagen significantly alleviated the early relapse. These data indicate the important role of PDL collagen recovery in early relapse. Previous studies showed that relaxin, which is related to collagen deposition, significantly prevented relapse tendency (Stewart et al. 2005;

Hirate et al. 2012), which supports our findings. Nevertheless, the remodeling process of other ECM components, such as fibronectin and elastin, needs further studies, and the observations of tooth movement and relapse based on rat models may have limitations to demonstrate orthodontic clinical works. Our results suggest that the recovery of PDL collagen could contribute to the early relapse, which extends the mechanism of relapse and provides a future approach to alleviate relapse.

In conclusion, PDL progenitors may possess intrinsic stability to recover after mechanical force withdrawal. PDL progenitors with intrinsic stability to regain collagen expression are required for PDL recovery and consequently contribute to the early orthodontic relapse.

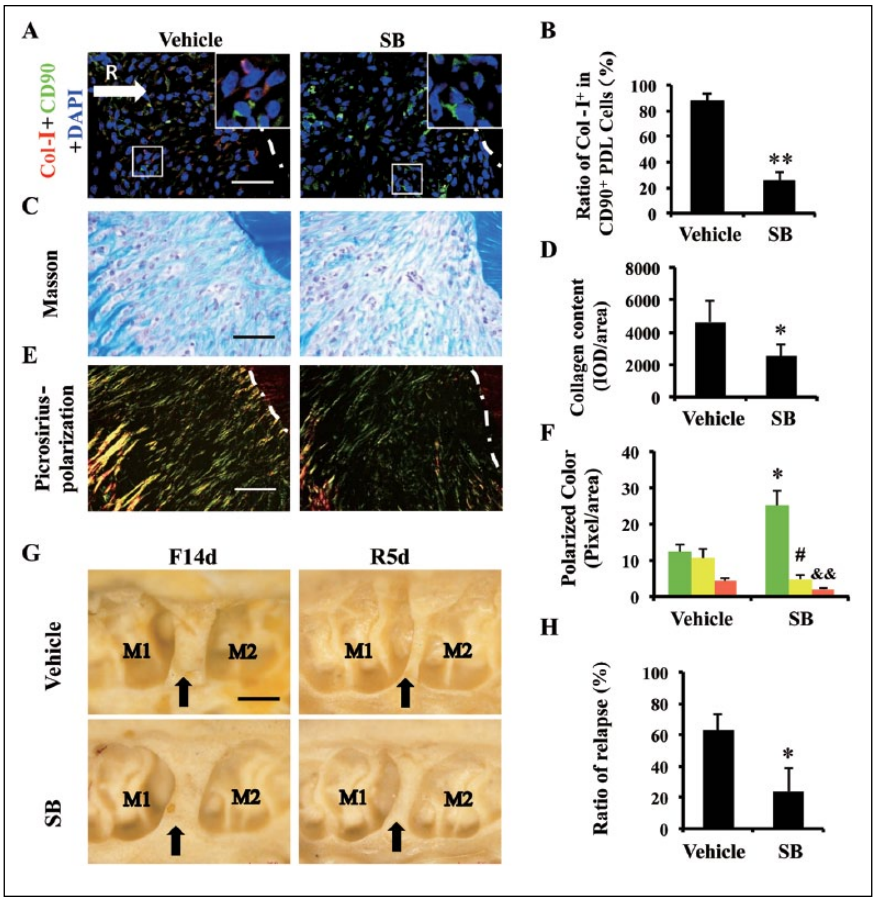

Figure 5. Inhibiting regained type I collagen (Col-I) expression in periodontal ligament (PDL) progenitors by blocking transforming growth factor–β (TGF-β) interrupted PDL collagen recovery and alleviated early orthodontic relapse. (**A**, **B**) Immunofluorescence staining demonstrated that Col-I expression (red) in $CD90^+$ PDL cells (green) was significantly suppressed in the SB 431542 (SB) group as compared with the vehicle group at 5 d of relapse. $n = 3$; $^{**}P < 0.01$ vs. vehicle group. (**C**, **D**) Recovery of PDL collagen was inhibited in the SB group versus the vehicle group at 5 d of relapse, as examined by Masson trichrome staining. $n = 3$; $^{*}P < 0.05$ vs. vehicle group. (**E**, **F**) Picrosirius-polarization method showed that recovery of PDL collagen was inhibited in the SB group versus the vehicle group at 5 d of relapse. $n = 3$; $^{*}P < 0.05$ vs. green color of vehicle group; $^{\#}P < 0.05$ vs. yellow color of vehicle group; $^{\&\&}P < 0.01$ vs. red color of vehicle group. Small and large frames indicate the original and magnified areas, respectively. The large arrow represents the direction of relapse. Dashed lines mark the outline of the roots. Original bar: 50 μm. (**G**, **H**) Relapse ratio of 5 d was significantly decreased in the SB group when compared with the vehicle group. Scale bars: 1,000 μm. M1 and M2 indicate the maxillary first and second molars of rats, respectively. $n = 7$; $^{*}P < 0.05$ vs. vehicle group.

Author Contributions

L. Feng, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; R. Yang, D. Liu, X. Wang, Y. Gan, contributed to data analysis and interpretation, critically revised the manuscript; S. Yang, H. Cao, D. He, contributed to data analysis, critically revised the manuscript; K. Kou, Y. Zhou, conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This study was supported by grants from the International S&T Cooperation program of China (2013DFB30360) and the National Natural Science Foundation of China (81470717, 81300897, 81571815). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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