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Interferon-γ-loaded collagen scaffolds reduce myofibroblast numbers in rat palatal mucosa

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SUMMARY Wound contraction and scar formation after cleft palate repair lead to growth impairment of the maxilla and midface. Myofibroblasts play a key role in these processes. The application of an interferon-γ (IFN-γ)-loaded collagen scaffold after surgery might reduce the differentiation of myofibroblasts. In this study, the tissue response to IFN-γ-loaded collagen scaffolds was evaluated after implantation in the palate of rats.

Scaffolds, with or without IFN-γ, were implanted submucoperiosteally in the palate of two groups of 25 five-week-old male Wistar rats. Groups of five rats were sacrificed at 1, 2, 4, 8, and 16 weeks post-implantation and processed for histological analyses. On haematoxylin and eosin-stained sections, the cell density and number of giant cells within the scaffolds were determined. Blood vessels, inflammatory cells, and myofibroblasts were detected by immunohistochemistry. The data for cell density, blood vessels, and giant cells were compared with a two-way analysis of variance. The scores for myofibroblasts and inflammation were compared by a rank sum test.

A mild and rapidly subsiding inflammatory and foreign body response was found in both groups. Angiogenesis had already begun after 1 week, showed a peak after 4 weeks, and declined thereafter. IFN-γ induced a faster influx of host cells and a major reduction in myofibroblast numbers. The scaffolds might be suitable for future applications in oral surgery.

Introduction

A cleft lip and/or palate is a frequently occurring congenital malformation (Murray, 1995). Surgical closure of these clefts is indicated to overcome feeding and speech problems. However, the existing surgical procedures lead to wound contraction and subsequent scar formation, which impair the growth of the maxilla and the development of the dentoalveolar complex (Ross, 1987; Wijdeveld *et al.*, 1988; Liao and Mars, 2005). This usually requires additional orthodontic treatment.

Myofibroblasts expressing alpha-smooth muscle actin (α-SMA) are generally recognized to play a key role in wound contraction by producing high contractile forces (Tomasek *et al.*, 2002). A direct correlation between α-SMA expression and contraction has been shown both *in vitro* and *in vivo* (Arora and McCulloch, 1994; Hinz *et al.*, 2001). The prolonged persistence of myofibroblasts in a wound leads to hypertrophic scarring (Desmouliere *et al.*, 2005).

A promising approach to reduce these problems may be the application of growth factors that inhibit myofibroblast differentiation and hence wound contraction and scar formation. Interferon-γ (IFN-γ) inhibits myofibroblast differentiation *in vitro* (Mallat *et al.*, 1995; Yokozeki *et al.*, 1999). The contraction of collagen gels *in vitro* is also decreased by IFN-γ (Dans and Isseroff, 1994; Mallat *et al.*, 1995; Moulin *et al.*, 1998; Yokozeki *et al.*, 1999). Clinical studies of skin have shown that IFN-γ also decreases the incidence and size of hypertrophic scars, keloids, and Dupuytren's nodules by reducing the number of myofibroblasts (Granstein *et al.*, 1990; Pittet *et al.*, 1994). IFN-γ injected in rat palatal wounds also decreases the number of myofibroblasts and collagen content *in vivo* (Cornelissen *et al.*, 2000). However, repeated injections into the mucosa also delays the wound healing process. Therefore, an alternative application method is required.

A possible solution is the implantation of IFN-γ-loaded scaffolds. Collagen scaffolds are widely used for tissue engineering because of their biocompatibility and biodegradability in skin (Klopper, 1986). Subcutaneous implantation of crosslinked collagen scaffolds in rats showed that they are biocompatible. Furthermore, loading of the scaffolds with fibroblast growth factor 2 improved cell ingrowth and angiogenesis (Pieper *et al.*, 2002; Nillesen *et al.*, 2007).

A previous study has shown that collagen scaffolds are biocompatible after submucoperiosteal implantation in the palate (Jansen *et al.*, 2008a). So far, no experimental data are available on IFN-γ-loaded collagen scaffolds implanted in the oral tissues. Before clinical application of these scaffolds can be developed, the tissue reactions to these IFN-γ-loaded collagen scaffolds need to be determined.

In this study, collagen scaffolds, with and without IFN-γ were implanted submucoperiosteally in the palate of rats. The tissue reactions were (immuno)histochemically evaluated and quantified. The hypothesis was that scaffolds loaded with IFN-γ reduce the number of myofibroblasts.

Materials and methods

The experiment was approved by the Board for Animal Experiments of Radboud University Nijmegen (no. 2003-108).

Animals

Fifty, 5-week-old, male Wistar rats (Harlan, Zeist, The Netherlands), weighing between 106 and 172 g, were used. All animals were kept under normal laboratory conditions and fed standard rat chow and water *ad libitum*. The rats had been acclimatized to the animal housing facility for 1 week before the start of the experiment. The experiment was approved by the Board for Animal Experiments of Radboud University Nijmegen (no. 2003-108).

Collagen scaffolds

Type I collagen was purified from bovine Achilles tendon (Pieper *et al.*, 1999; Geutjes *et al.*, 2006). To prepare collagen scaffolds, 0.8 per cent (w/v) type I collagen suspension in diluted acetic acid was shaken overnight at 4°C and homogenized on ice using a Teflon glass Potter-Elvehjem homogenizer (Louwers Glass and Ceramic Technologies, Hapert, The Netherlands). Air bubbles were removed by centrifugation at 250 *g* for 10 minutes at 4°C. The suspension was then slowly poured into a plastic mould (10 ml per 25 cm^2), frozen in a bath of ethanol and solid CO_2 (−80°C), and lyophilized. Scaffolds were cross-linked using 33 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and 6 mM *N*-hydroxysuccinimide (NHS) in 50 mM 2-morpholinoethane sulphonic acid, pH 5.5, containing 40 per cent ethanol for 4 hours at 22°C. Scaffolds were then washed with 0.1 M Na_2HPO_4, 1 M NaCl, 2 M NaCl, and MilliQ water, frozen in ethanol/CO_2 again, and lyophilized.

IFN-γ-loaded scaffolds

Recombinant rat IFN-γ (PeproTech, Rocky Hill, New Jersey, USA) was loaded onto the EDC/NHS-crosslinked collagen scaffolds by incubating them in 7 μg/ml cytokine in phosphate-buffered saline (PBS, pH 7.2) per 8 mg of scaffold for 30 minutes, followed by washings with PBS. The amount of IFN-γ bound to the scaffolds was determined with sodium dodecyl sulphate polyacrylamide gel electrophoresis. Scaffolds with bound IFN-γ and a standard curve of 0–10 ng recombinant IFN-γ were incubated for 15 minutes in a bath of boiling water under reducing conditions with 5 per cent (v/v) 2-mercaptoethanol. The samples were loaded on a 15 per cent (w/v) gel, followed by silver staining using a 0.1 per cent (w/v) $AgNO_3$ solution. The loading efficiency was about 0.2 μg IFN-γ per milligram of dry scaffold.

Surgical procedures

The rats were anaesthetized with an intraperitoneal injection of 1 ml/kg body weight of ketamine (Nimatek; Eurovet, Bladel, The Netherlands) combined with an intraperitoneal injection of 0.25 ml/kg body weight of xylazine (Sedamun; Eurovet). A standardized transverse incision of 3 mm was made in the palatal mucoperiosteum at the level of the contact points between the first and second molar. The mucoperiosteum was then elevated caudally for 3 mm with a periodontal probe to create a submucoperiosteal envelope. A circular collagen scaffold with a diameter of 3 mm was placed in the envelope and a 10 × 0 vicryl suture was used to close it. The rats were medicated post-operatively with 0.02 mg/kg body weight of buprenorfine (Temgesic®; Schering Plough, Brussels, Belgium) subcutaneously as an analgesic.

Study design

Twenty-five rats received a crosslinked scaffold without IFN-γ and the other 25 rats an IFN-γ-loaded collagen scaffold. Groups of five rats were sacrificed at 1, 2, 4, 8, and 16 weeks post-implantation and processed for histological analyses of the wound tissue. Sham-operated animals without a scaffold did not show any difference from the unoperated animals (data not shown).

Histology

The rats were perfused using a solution of freshly prepared 4 per cent paraformaldehyde in PBS. The rats were then decapitated, and the palate was dissected from the skull. The palate samples were fixed in 4 per cent paraformaldehyde solution for 24 hours. Thereafter, the samples were decalcified in 10 per cent ethylenediaminetetraacetic acid in PBS at 4°C. Decalcification was checked radiographically. All samples were embedded in paraffin. Serial paraffin sections of 6 μm were cut in the transverse plane and stained with haematoxylin and eosin (H&E). Histomorphometric analyses were performed blindly using a microscope equipped with an ocular micrometer (Carl Zeiss Imager Z.1 system; Carl Zeiss Microimaging Gmbh, Jena, Germany). The total number of giant cells and the cell density in the scaffolds were determined on three frontal sections of the maxilla, including the centre of the scaffold. These sections had an interval of 150 μm. The cell density in the scaffolds was determined with a microscopic grid and expressed as the number of cells per square millimetre.

Immunohistochemistry

Paraffin sections were collected on Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany), deparaffinized, and rehydrated. Before staining, the slides were rinsed in PBS for 10 minutes. α-SMA staining was performed to

detect myofibroblasts, ED-1 staining to detect inflammatory cells, and type IV collagen staining to detect blood vessels. All measurements were performed on three frontal sections of the maxilla, including the centre of the scaffold. These sections had an interval of 150 μm.

α-*SMA staining*

Sections were treated with 3 per cent H_2O_2 in methanol for 10 minutes to block endogenous peroxidase and rinsed in PBS. The sections were then pre-incubated in 10 per cent normal donkey serum (Chemicon Europe, Chandlers Ford, Hampshire, UK) in PBS. After pre-incubation, the sections were incubated with monoclonal mouse anti-α-SMA (clone A-2547; Sigma Chemical Co, St Louis, Missouri, USA) 1:6400 for 60 minutes. After washing with PBS, detection was carried out using biotinylated donkey anti-mouse IgG (Jackson Labs, West Grove, Pennsylvania, USA) 1:100 for 60 minutes and the avidin–biotin complex method (Vectastain ABC-Elite kit; Vector Laboratories, Burlingame, California, USA). The presence of myofibroblasts was scored on a scale from 0 to 3 on three sections (150 μm apart) for every sample.

0: No, or only a few myofibroblasts present
1: Groups of myofibroblasts around the scaffold; no or only a few myofibroblasts inside the scaffold
2: Groups of myofibroblasts around and inside the scaffold
3: Myofibroblasts throughout the scaffolds and the surrounding tissue.

ED-1 staining

ED-1 staining was performed similar to α-SMA staining, but the sections were incubated with monoclonal mouse anti-rat anti-CD-68 (Clone ED-1; Serotec, DPC, Breda, The Netherlands) 1:400 for 60 minutes. This antibody recognizes a single-chain glycoprotein of 90–110 kDa that is expressed predominantly on the lysosomal membrane of myeloid cells (Damoiseaux *et al.*, 1994). It mainly stains macrophages and monocytes. The inflammatory response was scored on a scale from 0 to 3 on three sections (150 μm apart) for every sample, similar to that for myofibroblasts (Figure 1).

Type IV collagen staining

Type IV collagen staining was performed similar to α-SMA staining, but the sections were incubated with rabbit anti-type IV collagen (Euro-diagnostica B.V., Arnhem, The Netherlands) 1:200 for 60 minutes. The total number of blood vessels present in the scaffolds was counted on three sections (150 μm apart) for every sample.

Statistical analysis

All measurements were performed on triplicate sections from each sample. The data for cell density, the number of blood vessels, and the number of giant cells were compared between the two groups and at the different time points with a two-way analysis of variance (ANOVA). Significant differences were further analyzed by the Holm–Sidak method (Sidak, 1967). A *P*-value of less than 0.05 was considered significant. Differences in the scores for myofibroblasts and the degree of inflammation were compared at each time point by a Mann–Whitney rank sum test. Differences in time of myofibroblast proliferation and the degree of inflammation were compared at each time point by a Kruskal–Wallis one-way ANOVA on ranks. Significant differences were further analyzed by the Dunn's method. To correct for the multiple use of ANOVA, a Bonferroni correction was applied, and a *P*-value of less than 0.01 was considered significant.

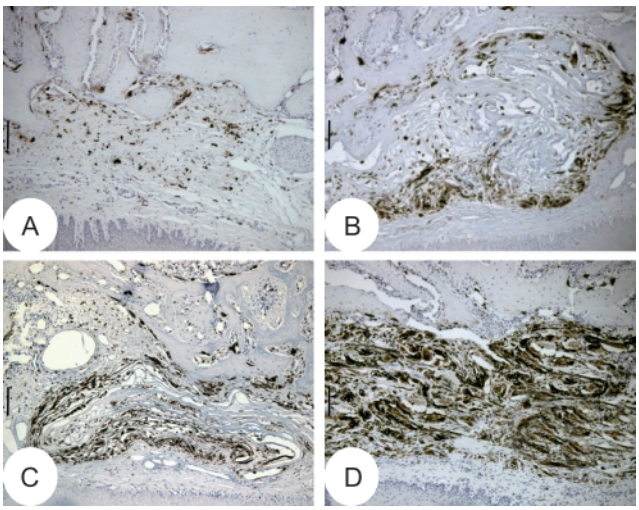

Figure 1 Reference photomicrographs. Representative ED-1-stained sections for the different inflammation scores. (A) Score 0. (B) Score 1. (C) Score 2. (D) Score 3 (bar = 100 μm).

Results

General histology

The scaffolds were clearly visible in the histological sections of both groups at 1 and 2 weeks. They were located on the palatal bone between the second molars and were covered with the palatal mucoperiosteum (Figure 2a). In the collagen group, all scaffolds were still visible at 8 and 16 weeks. Only at 4 weeks, were two scaffolds in the collagen group lost. At 8 weeks, only three scaffolds were detected in the IFN-γ group, while at 16 weeks all IFN-γ-loaded scaffolds had completely degraded.

Cell density

Cell density within the scaffolds was determined on H&E-stained sections (Figure 2). In both groups, cells had infiltrated the scaffolds after the first week. However, significantly more cells were counted in the IFN-γ group

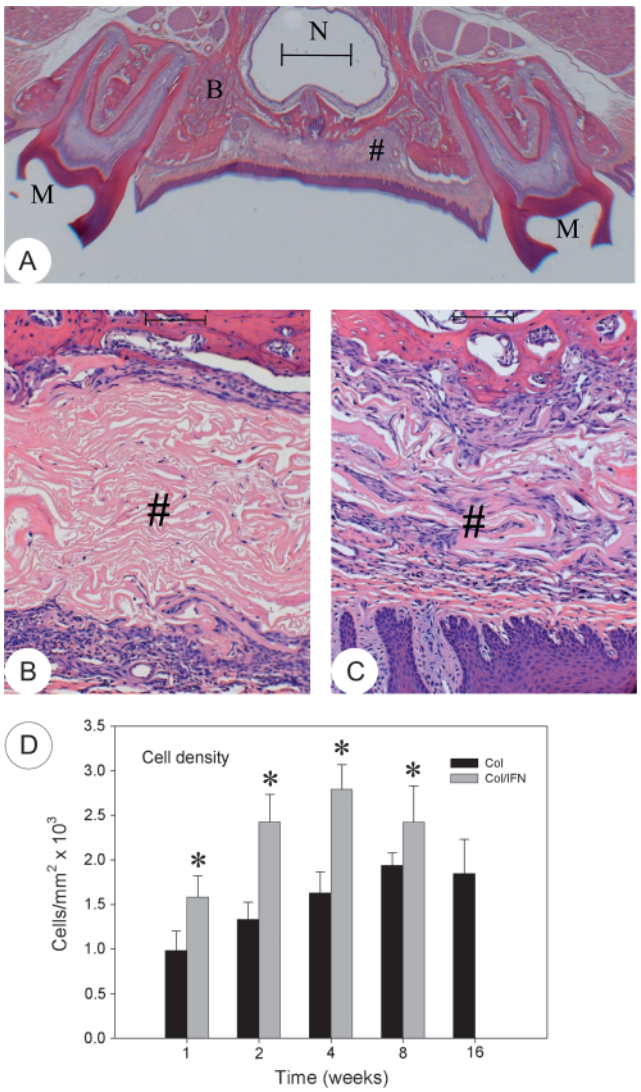

Figure 2 Representative haematoxylin and eosin-stained sections of 1 week samples. (A) Overview: collagen sample 1 week after implantation. Indicated are the nasal cavity (N), palatal bone (B), and molars (M); bar = 1000 μm. (B) Collagen sample 1 week after implantation (bar = 100 μm). (C) Collagen-interferon-γ (IFN-γ) sample 1 week after implantation (bar = 100 μm). The scaffolds (hash) are indicated. (D) Quantification of cell density within the scaffolds. Asterisk denotes a significant difference between the collagen-IFN-γ group and the collagen group.

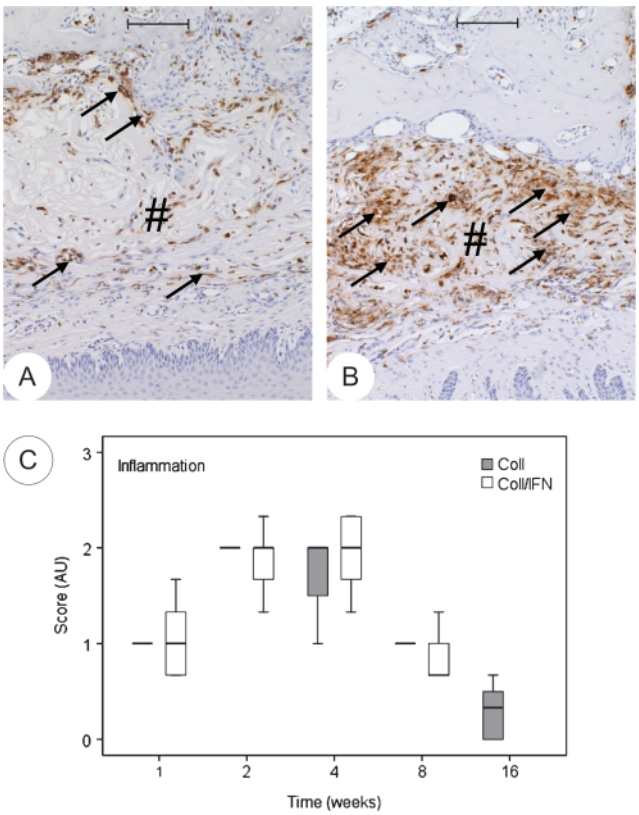

Figure 3 Inflammatory cells were detected with a ED-1 staining. Representative ED-1-stained sections of 1 and 4 week samples are shown. Collagen-interferon-γ (IFN-γ) sample 1 week (A) and 4 weeks (B) after implantation (bar = 100 μm). The scaffolds (hash) are indicated. (C) Inflammation scores. The box plots display the 25th, 50th, and 75th percentiles, if available. Arrows indicate clusters of inflammatory cells. No significant differences were found between the collagen-IFN-γ group and the collagen group.

compared with the collagen group (Figure 2B and 2C). A significantly higher cell density was also observed in the IFN-γ group at all other time points, except 16 weeks, when the IFN-γ-loaded scaffolds were no longer detectable (Figure 2D). Cell density increased gradually over time up to 8 weeks in the collagen group and was significantly higher at 4 and 8 weeks than at 1 week. Cell density at 8 weeks was also significantly higher than at 2 weeks. Within the IFN-γ group, cell density increased over time up to 4 weeks and was significantly greater at all time points compared with the first week.

Inflammation

Inflammation was scored on a scale from 0 to 3 on ED-1-stained sections (Figure 3). At 1 week, inflammatory cells were mainly found at the edges of the scaffolds (Figure 3A). At 2 and 4 weeks, inflammatory cells had infiltrated throughout the scaffolds (Figure 3B). At 16 weeks, almost all inflammatory cells had disappeared from the collagen group, while the IFN-γ-loaded scaffolds could not be scored because they were degraded. No significant differences were found between the collagen group and the IFN-γ group (Figure 3C). Although there was a significant general effect of time ($P < 0.001$) for both groups, no significant differences were found between any two time points.

Giant cells

The total number of giant cells in the scaffolds was counted on H&E-stained sections (Figure 4). Their number increased from 1 to 4 weeks (Figure 4A and 4B), while at 16 weeks, almost no giant cells remained in the collagen group. At that time, the IFN-γ-loaded scaffolds were no longer detectable. No

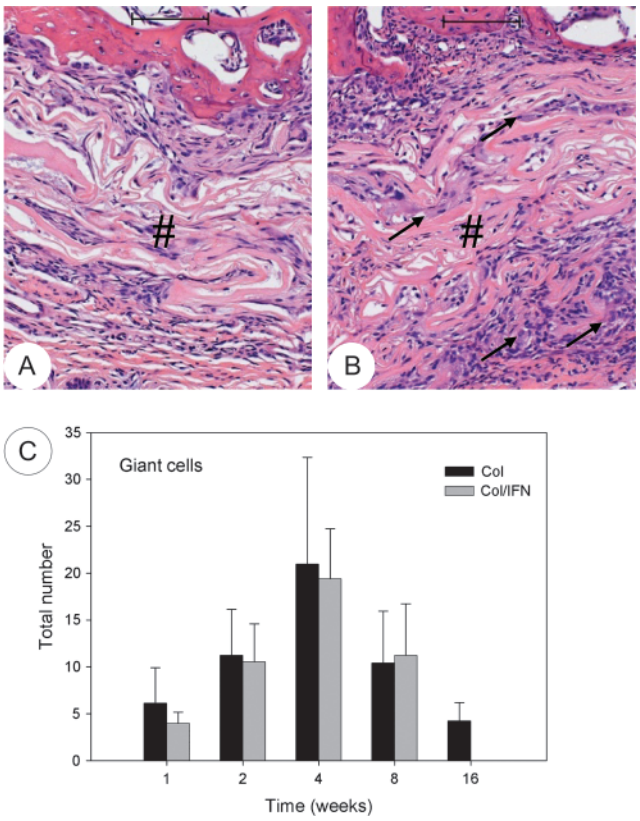

Figure 4 The total number of giant cells in the scaffolds was determined on haematoxylin and eosin-stained sections. Representative 1 and 4 week samples are shown. (A) Collagen samples 1 week and (B) 4 weeks after implantation (bar = 100 μm). Scaffolds (hash) and giant cells (arrows) are indicated. (C) Number of giant cells within the scaffolds. No significant differences were found between the collagen-interferon-γ group and the collagen group.

significant differences were found between the collagen and the IFN-γ group (Figure 4C). There was a significant general effect of time within both groups ($P<0.001$), but a significant difference was found only between 1 and 4 weeks.

Myofibroblasts

α-SMA was detected in blood vessels and in myofibroblasts (Figure 5). At 1 week, myofibroblasts were observed both at the edges and inside the scaffolds in the collagen group (Figure 5A), while in the IFN-γ group only a few myofibroblasts were present at the edges (Figure 5B). At 4 weeks, some myofibroblasts were still present in the collagen group, while in the IFN-γ group hardly any myofibroblasts remained (Figure 5C and 5D). At 1 and 2 weeks, significantly fewer myofibroblasts were present in the IFN-γ group compared with the collagen group (Figure 5E). At later time points, myofibroblasts were no longer present in either group. Within the collagen group, a significant difference was noted between 2 and 8 weeks, while in the IFN-γ group only a significant general effect of time was found ($P<0.001$).

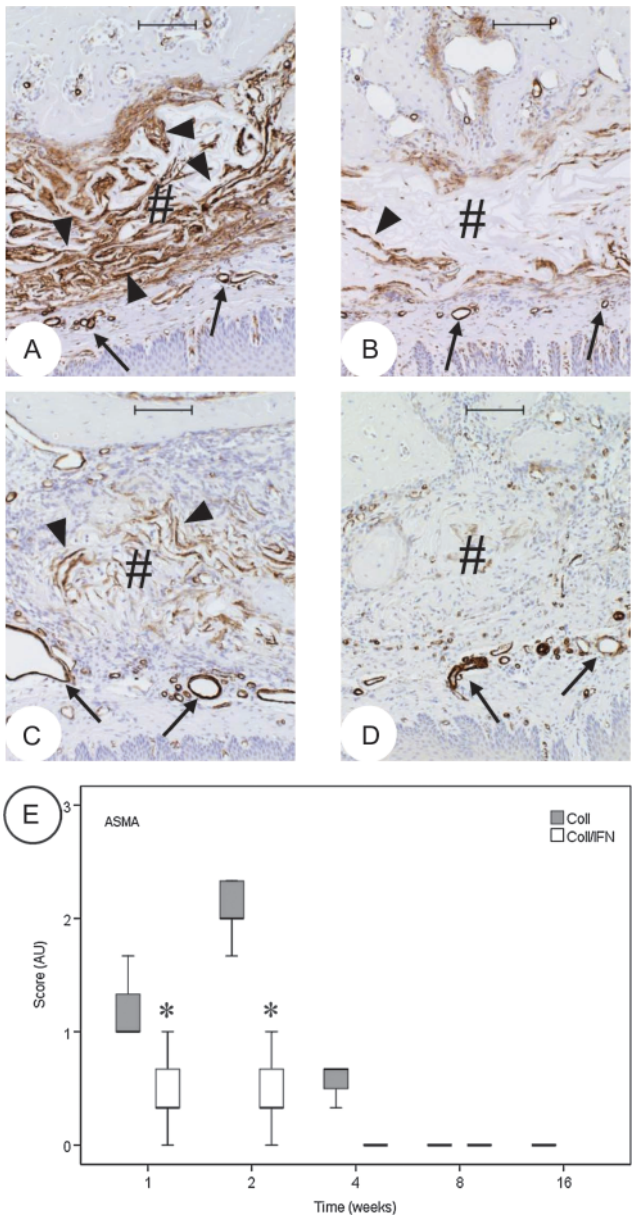

Figure 5 Myofibroblasts stained with an antibody against alpha-smooth muscle actin (α-SMA), which also stains the blood vessel walls. Representative α-SMA-stained sections of 1 and 4 week samples are shown. (A) Collagen sample 1 week after implantation. (B) Collagen-interferon-γ (IFN-γ) sample 1 week after implantation. (C) Collagen sample 4 weeks after implantation. (D) Collagen-IFN-γ sample 4 weeks after implantation. Scaffolds (hash), blood vessels (arrows), and myofibroblasts (arrowhead) are indicated (bar = 100 μm). (E) Myofibroblast scores. Box plots display the 25th, 50th, and 75th percentiles, if available. Asterisk denotes a significant difference between the collagen-IFN-γ group and the collagen group.

Vascularization

The number of newly formed blood vessels within the scaffolds was counted on sections stained for type IV collagen (Figure 6). In both groups, a gradual increase in the number of blood vessels occurred from 1 week (Figure 6A) to 4 weeks (Figure 6B). After 4 weeks, the

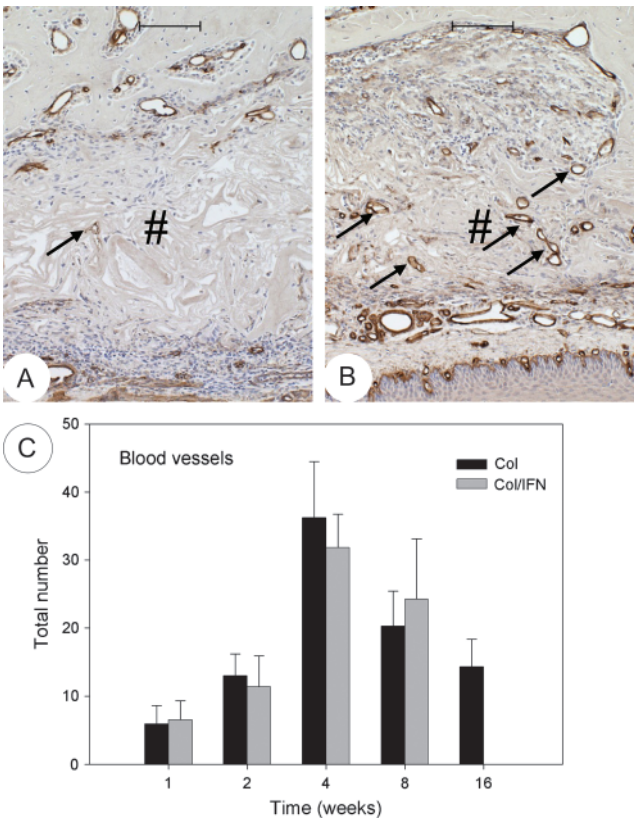

Figure 6 Blood vessels stained with an antibody against collagen type IV, which stains all basement membranes. Representative collagen type IV-stained sections of 1 and 4 week samples are shown. Collagen samples 1 week (A) and 4 weeks (B) after implantation (bar = 100 μm). Scaffolds (hash) and blood vessels within the scaffolds (arrows) are indicated. (C) Number of blood vessels within the scaffolds. No significant differences were found between the collagen-interferon-γ group and the collagen group.

number of blood vessels declined. No significant differences were found between the collagen and the IFN-γ group (Figure 6C). Within the collagen group, the number of blood vessels was significantly greater at 4 weeks than at all other time points, except 16 weeks. A significant difference was also found between 1 week compared with 2 and 8 weeks. Within the IFN-γ group, a significant effect of time occurred between 1 week and 4 and 8 weeks. A significant effect was also found between 2 weeks and 4 and 8 weeks.

Discussion

The final aim of this research is to develop a growth factor or cytokine delivery system based on collagen scaffolds, which can diminish wound contraction and scar formation after cleft palate surgery. This might be achieved by reducing myofibroblast numbers with growth factor-loaded collagen scaffolds. Before biomaterials can be used for tissue engineering, the tissue response to these materials has to be evaluated. Although subcutaneous implantation studies have been performed with growth factor-loaded collagen scaffolds, no studies have been carried out on the subcutaneous or submucoperiosteal implantation of IFN-γ-loaded collagen scaffolds. The tissue reaction to collagen scaffolds in the skin and in the palate can be quite different (Jansen *et al.*, 2008b). In the present study, the tissue response to crosslinked porous collagen type I scaffolds loaded with IFN-γ was evaluated up to 16 weeks following submucoperiosteal implantation in the palate of rats.

The scaffolds induced only a mild and rapidly subsiding inflammatory response and no extensive foreign body reaction occurred after implantation. IFN-γ did not affect these parameters, which is in accordance with results after subcutaneous administration of IFN-γ alone in clinical studies (de Metz *et al.*, 2002; Marciano *et al.*, 2004). IFN-γ plays an important role in the regulation of inflammatory processes (Schroder *et al.*, 2004) and is associated with an increase in pro-inflammatory cytokine release and leucocyte infiltration *in vitro* (Rubbiabrandt *et al.*, 1991; Trindade *et al.*, 1999). However, evidence in recent years has shown a dual role for IFN-γ in inflammation. IFN-γ is able to act both pro-inflammatory by activating cytokines such as interleukin (IL)-12, IL-15, and tumour necrosis factor-α, and anti-inflammatory by inhibiting IL-1 and IL-8 and inducing apoptosis in leukocytes (Muhl and Pfeilschifter, 2003). Injection of IFN-γ in palatal wounds in rats does not lead to more intense inflammation (Cornelissen *et al.*, 2000).

IFN-γ is able to increase the formation of giant cells from macrophages *in vitro* (Fais *et al.*, 1994), and anti-IFN-γ therapy delays the formation of giant cells after subcutaneous implantation of collagen scaffolds in rats (Khouw *et al.*, 1998). The present study, however, showed no effect of IFN-γ on the number of giant cells. There is a clear difference between the oral and dermal tissue environment, and generally lower levels of macrophages, neutrophils, and T cells are present in oral compared with skin wounds (Szpaderska *et al.*, 2003). This might have decreased the effect of IFN-γ on the foreign body response in the current study.

The biological activity of IFN-γ was evident from the increased ingrowth of cells into the scaffolds. This might be caused by increased expression of matrix metalloproteinases (MMPs) by IFN-γ (Tamai *et al.*, 1995). MMPs are important mediators of cellular migration as they pave the way by degrading the extracellular matrix (VanSaun and Matrisian, 2006). The higher levels of MMPs might also have caused a more rapid degradation of the IFN-γ-loaded scaffolds, which were no longer present at 16 weeks (Lee *et al.*, 2007).

Since myofibroblasts cause contraction, the effect of IFN-γ on the expression of α-SMA, a marker for myofibroblasts, was investigated. IFN-γ is known to inhibit the differentiation of skin, gingival, and palatal fibroblasts into myofibroblasts *in vitro* (Yokozeki *et al.*, 1999; Sobral *et al.*, 2007; Tanaka *et al.*, 2007). Also *in vivo*, IFN-γ is able to reduce myofibroblast differentiation in the palate (Cornelissen *et al.*, 2000) and in the skin (Pittet *et al.*, 1994).

This effect is partly caused by the antagonistic effect of IFN-γ on transforming growth factor-β1 that stimulates differentiation of myofibroblasts (Tredget *et al.*, 2000; Sobral *et al.*, 2007). Furthermore, IFN-γ stimulates the production of nitric oxide, which also suppresses α-SMA expression (Kawada *et al.*, 1996). The present results show that loading IFN-γ onto collagen scaffolds leads to a significant reduction in myofibroblast numbers after implantation. Myofibroblasts are responsible for wound contraction and deposit large amounts of extracellular matrix, which is remodelled into scar tissue (Gabbiani, 2003). Therefore, IFN-γ is likely to reduce these processes. Furthermore, IFN-γ reduces collagen synthesis of wound fibroblasts *in vitro* (Cornelissen *et al*, 1999) and stimulates collagen breakdown by inducing collagenase production of wound fibroblasts (Tamai *et al.*, 1995).

Vascularization is essential for the integration of an implant into the host tissue. In both groups in the present study, neovascularization was observed after 1 week, reached a maximum after 4 weeks, and decreased thereafter. This sequence is similar to neovascularization during wound healing (Singer and Clark, 1999). IFN-γ is able to inhibit the growth and migration of endothelial cells *in vitro*, thus decreasing angiogenesis (Broutyboye and Zetter, 1980; Maheshwari *et al.*, 1991; Trompezinski *et al.*, 2002). However, no significant effect of IFN-γ on vascularization was found in this study. This might be due to the short half-life of IFN-γ *in vivo*. It has a half-life in the bloodstream of 25–35 minutes (Kurzrock *et al.*, 1985; Wills, 1990) and 4.5 hours after subcutaneous administration (Foon *et al.*, 1985). However, the incorporation of IFN-γ into the collagen scaffold might prolong its half-life. Furthermore, it is known that the cross-linking of collagen scaffolds increases their angiogenic properties (Yao *et al.*, 2008), which might counteract the anti-angiogenic effects of IFN-γ.

Despite the suspected short half-life of IFN-γ, the effects on cell ingrowth and myofibroblasts were evident until at least 2 weeks after implantation. This supports a sustained release of the cytokine from the collagen scaffolds. Alternatively, an initial burst of IFN-γ might lead to an increased invasion of host precursor cells into the scaffolds. Following this invasion, the host cells release additional growth factors, which may further enhance cellular influx.

Conclusion

Cross-linked collagen scaffolds are highly biocompatible after submucoperiosteal implantation in the palate of rats. The scaffolds elicited only a mild and transient inflammatory response. Loading with IFN-γ induced a faster influx of cells and a reduced differentiation of myofibroblasts in palatal mucosa, which confirms the initial hypothesis. Therefore, IFN-γ-loaded collagen scaffolds might be suitable for tissue engineering in cleft palate repair or other intra-oral reconstructions.

Funding

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