Mucosal Wound Healing

The Roles of Age and Sex

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Hypothesis: It remains unclear whether aging delays wound healing, as past human studies have not adequately controlled for confounding factors such as morbidity and medications. Furthermore, although dermal wounds heal more quickly in women than in men, clinical observations suggest that the opposite may be true for mucosal healing. We assessed age and sex differences in mucosal wound healing, and we hypothesized that aging delays healing and sex modulates healing independent of age.

Design and Setting: Clinical experimental study performed from June 2000 to August 2003 involving younger and older adult volunteers from the general community.

Participants: Two hundred twelve male and female volunteers aged 18 to 35 years (n=119) or 50 to 88 years (n=93).

Intervention: Standardized 3.5-mm circular wounds were placed on the oral hard palates of volunteers.

Main Outcome Measure: Wound videographs were taken daily for 7 days after wounding to assess wound closure.

Results: Wounds healed significantly more slowly in older adults compared with younger adults (P<.001) regardless of sex. This remained true even when individuals receiving medication and/or having a coexisting medical condition were excluded. Mucosal wounds healed more slowly in women than in men (P=.008) regardless of age. These effects were independent of demographic factors such as ethnicity, alcohol or nicotine use, or body mass index.

Conclusions: Wound closure in older individuals was clearly delayed even when eliminating potential age-related confounds, indicating that aging does slow wound healing. Wound closure in women was also delayed, suggesting that wound healing is modulated by different mechanisms depending on tissue type. These findings may help target patients with increased surgical risks and greater need for postsurgical care.

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See Invited Critique at end of article
older skin has been associated with reductions in vascularization,\textsuperscript{10} collagen density or production,\textsuperscript{10-12} granulation tissue,\textsuperscript{10} and elastin.\textsuperscript{9,13} However, many of these changes that occur in aged skin are due to extrinsic aging factors such as sun exposure.\textsuperscript{9,12} Mucosal tissue is not exposed to the sun and thus may provide a better assessment of the effects of intrinsic aging on wound healing. Mucosal wounds occur frequently, and the healing of the mucosa is important in most surgical outcomes. Oral wounds are a common type of mucosal wound and are comparable to other mucosal tissues in both repair rates and susceptibility to infection. However, little is currently known regarding the effects of aging on the healing of mucosal tissue.

Sex has been implicated as a factor in wound healing, and a number of studies\textsuperscript{14-16} clearly show a female advantage in the healing of nondermal wounds. However, these findings are mainly derived from dermal wound studies, and a female advantage in the healing of nondermal wounds is unapparent. A number of clinical studies\textsuperscript{17-19} observed no sex difference in the healing of the oral mucosa. Other studies that examined the healing of mucosal tissues after third-molar surgery found that women healed significantly more slowly\textsuperscript{20,21} and needed additional postsurgery treatment\textsuperscript{21,22} compared with men. Taken together, aging and sex may additively or synergistically affect the healing of mucosal wounds.

This study examined age and sex differences in the healing of standardized mucosal wounds created on the oral hard palate. Potentially confounding effects, such as individuals reporting morbidity or medication usage, were eliminated by the exclusion of these subjects from specific follow-up analyses. Using this rigorous approach, our results indicate the following: (1) older adults heal mucosal wounds more slowly than young adults, even when factors such as medication use and morbidity are eliminated; and (2) women heal mucosal wounds more slowly than men, independent of age. These findings further indicate that older women are at the highest risk for delayed healing of mucosal tissue.

STATISTICAL ANALYSIS

Data were analyzed using a mixed-design analysis of variance that treated the wound sizes for 7 days after wounding as a within-subject factor and sex and age as between-subject factors. Separate analyses of variance were used for post hoc comparisons. Correlations were determined using Pearson product moment correlations ($r$), and $\chi^2$ tests were used to assess between-group differences in healing rates. Demographic analyses consisted of analyses of variance and correlations. Age was treated as a covariate when determining sex differences within younger or older groups. All of the hypothesis tests were 2-tailed and used $\alpha = .05$ to determine significance. Data were analyzed using SPSS version 11.5 for Windows (SPSS, Inc, Chicago, Ill).

RESULTS

Healing rates were unrelated to ethnicity, body mass index, exercise, alcohol consumption, or nicotine use (although only 31 subjects smoked).

AGE EFFECTS

Younger participants displayed a different pattern of wound healing than older participants ($F_{1,205} = 31.67; P < .001$). Although all of the wounds were initially the same size, younger individuals had smaller wounds by day 2; this difference continued to the end of the study ($P < .001$ for each day) (Figure 1A). In addition, the proportion of individuals who were considered healed (wounds $>90\%$ closed) was significantly higher in younger subjects than in older subjects on days 5 through 7 (Figure 1B).

Studies that have assessed healing in aged persons have been criticized because analyses have not accounted for the higher occurrences of medication use and morbidity in elderly individuals. In this study, 4 sets of analyses that excluded the following individuals were performed: (1) those receiving any type of medication...
Men exhibited a different pattern of wound healing than women. Older individuals had larger wounds on all days, even when excluding individuals receiving medication (younger group, n=104; older group, n=41). Error bars indicate SEM. *P<.05 (older group receiving medications [n=51] vs older group not receiving medications [n=41]). †P<.001 (younger group vs older group). B, Percentage of subjects considered healed (wounds <10% of original size) on days 4 through 7. Compared with the younger group, a lower proportion of the older group was considered healed on days 5 through 7, even when excluding individuals receiving medication. For the younger group vs the older group: *P<.01; †P<.001. For the younger group vs the older group not receiving medications: ‡P<.05; §P<.01; ||P<.001.

Table 1. Age-related changes in wound healing parameters. Statistical significance was determined with 2-tailed t tests and χ2 tests. A, Wound sizes for 7 days after wounding. Compared with younger individuals, older individuals had initially the same size, at 24 hours after wounding, men had significantly smaller wounds; this difference was apparent until day 5 (Figure 2A). In addition, the proportion of individuals considered healed was significantly higher for men than for women on days 5 and 6 (Figure 2B).

AGE AND SEX

The effects of age occurred regardless of sex, as older subjects healed significantly more slowly than younger subjects for both women (F1,125=24.13; P<.001) and men (F1,131=10.79; P<.001). Wounds were significantly larger in older women compared with younger women on all 7 days (P≤.002). Within men, a significant day × age interaction occurred (P<.001), as wound sizes were similar on day 1 but significantly larger in older men than in younger men on days 2 through 7 (P≤.02) (data not shown). Compared with the older subjects, the proportion of individuals considered healed was significantly higher in younger women on days 5 through 7 (P≤.007) and in younger men on days 6 and 7 (P=.009 each day) (data not shown).

The effects of sex occurred regardless of age, as there were significant day × sex interactions within both younger (F1,600=3.16; P=.003) and older (F1,34=2.95; P=.008) subjects. Women had significantly larger wounds (ie, slower healing) than men on days 3 through 5 in the younger group (P=.02) and on day 1 in the older group (P=.002) (data not shown). Also, within each age group, a higher proportion of men than women were considered healed. In younger subjects, this effect approached significance on day 5 (P=.08) and was significant on day 6 (P=.02); in older subjects, this effect approached significance on days 5 and 6 (P=.05 and .09, respectively) (data not shown).

The magnitudes of these age and sex effects on wound healing were substantial. For example, observing values obtained 5 days after wounding, wounds were 56% larger in older subjects as compared with younger subjects, and younger individuals were 3.7 times more likely to be considered healed than older individuals. Similarly, the wounds of women were 27% larger than those of men, and men were 2.5 times more likely than women to be...
considered healed. Older women were the slowest to heal, and their wounds as compared with those of younger men were 95% larger by 5 days after wounding. Thus, older women appear to be at the greatest risk for delayed wound closure.

A chief strength of this study was that all of the wounds were created under the same experimental conditions and were standardized for size, depth, site, and time of placement. The results were also independent of common demographic factors (eg, ethnicity), allowing for a more clear determination of the roles of age and sex in wound healing.

In this study, older adults (aged ≥50 years) healed oral mucosal wounds significantly more slowly than younger adults (aged 18-35 years). This remained true even when individuals who were receiving medication and/or had a coexisting medical condition were removed from the analyses. A common criticism of past studies is that the inclusion of such individuals may exaggerate or even account for age-associated healing impairments. Surprisingly, the exclusion of individuals receiving medication strengthened our findings (Figure 1), and within the older group, those receiving medication had an improved pattern of healing. This suggests that age-associated delays in wound healing are not generally exaggerated by medication use. Furthermore, the deleterious effects of age on healing may be stronger than previously suspected.

Much of the current literature on wound healing stems from dermal wounds, and a clear sex difference in favor of women is evident. However, the current study indicates that men heal oral mucosal wounds more quickly than women. This male advantage in mucosal healing is a robust effect, and we have recently replicated this finding in another clinical study using young adults (n=193; mean±SD age, 20.14±0.15 years; age range, 18-31 years).

These results suggest that there are fundamental differences between the healing of mucosal vs dermal wounds. Compared with skin, mucosal epithelial turnover is more rapid and its tissue is more vascularized. As a result, it takes less time to recruit inflammatory cells to a mucosal wound site. Furthermore, immunomodulating compounds such as growth factors are more readily supplied to the site via mucosal secretions (ie, saliva), and lower levels of neutrophils, macrophages, and their associated cytokines have been reported in mucosal wounds as compared with dermal wounds. These differences all likely contribute to the divergent healing rates seen in these tissues and may help to explain why the sexual dimorphism in these rates varies between tissue types.

Sex hormones likely modulate oral mucosal wound healing, as they have been shown to play a role in both dermal wound healing and periodontal disease. We hypothesize that sex hormones modulate both oral mucosal and dermal wound healing but do so differentially, perhaps driving healing in opposite directions. Lower inflammatory responses have been associated with faster wound healing and inflammation appears to be substantially reduced in mucosal wounds as compared with dermal wounds, possibly explaining why mucosal wounds heal more rapidly. Given that testosterone has potent anti-inflammatory qualities and is abundant in saliva and other mucosal fluids, testosterone is a putative mechanism for explaining the faster healing of mucosal wounds observed in men in this study.

Our results indicate that women heal oral mucosal wounds more slowly than men, which is the opposite of the sex effect reported in dermal wound healing. Often, dermal and mucosal wounds are equated and direct comparisons are commonly made between their underlying healing processes. However, our findings suggest that heal-

Figure 2. Sex-related changes in wound healing parameters. Statistical significance was determined with 2-tailed t tests and χ² tests. A, Wound sizes for 7 days after wounding. Men had smaller wounds than women on days 1 through 5. Error bars indicate SEM. B, Percentage of subjects considered healed (wounds <10% of original size) on days 4 through 7. Compared with men, a lower proportion of women were considered healed on days 5 and 6. *P<.05 (men vs women).

†P<.01 (men vs women).
ing in these tissues is differentially modulated and that direct comparisons between dermal and mucosal tissues may be inappropriate. These findings also indicate, to our knowl-
dge for the first time, that older adults heal standardized wounds more slowly than younger adults, even when fac-
tors such as medication use and morbidity are removed from the analyses. Thus, both age and sex appear to be influen-
tial factors in mucosal wound healing, and older women may be at the highest risk for delayed healing following oral
or mucosal surgery or injury.

With an increasing number of surgical procedures being performed owing to the rising age of the population
and for aesthetic purposes, a greater emphasis needs to be placed on expediting the healing process. Deter-
mizing the mechanisms that underlie these age and sex differences will help target treatment strategies to re-
duce postsurgical recovery times. This, in turn, will decrease the risk of infection and improve ultimate healing
outcomes.

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REFERENCES

1. US Census Bureau. Interim Projections Consistent With Census 2000. Wash-


7. Ashcroft GS, Horan MA, Ferguson MW. Aging is associated with reduced depo-
sition of specific extracellular matrix components, an upregulation of angiogen-
esis, and an altered inflammatory response in a murine incisional wound heal-


10. van de Kerkhof PC, Van Bergen B, Spruit K, Kuiper JP. Age-related changes in


13. Ashcroft GS, Kiely CM, Horan MA, Ferguson MW. Age-related changes in the
temporal and spatial distributions of fibillin and elastin mRNAs and proteins in


wound healing associated with an increase in TGF-beta levels. Nat Med. 1997;
3:1209-1215.

women deposit more collagen than men during healing of an experimental wound.

mucosal defect in laparoscopic intragastric surgery. Gastric Cancer. 2003;
6:86-98.


19. Heard RH, Mellonig JT, Brunsvoild MA, Lasjo DJ, Meffert RM, Cochran DL.
Clinical evaluation of wound healing following multiple exposures to enamel ma-
trix protein derivative in the treatment of intrabony periodontal defects. J Peri-

1999;57:1288-1294.

longed recovery and delayed healing after third molar surgery. J Oral Maxillofac

22. Benediktsson IS, Wenzel A, Petersen JK, Hintze H. Mandibular third molar re-
move: risk indicators for extended operation time, postoperative pain, and
438-446.


25. Itó I, Hayashi T, Yamada K, Kuraya M, Naito M, Iguchi A. Physiological concen-
tration of estradiol inhibits polymorphonuclear leukocyte chemotaxis via a re-

26. Dovi JV, Szpaderska AM, DiPietro LA. Neutrophil function in the healing wound:

27. Dovi JV, He LK, DiPietro LA. Accelerated wound closure in neutrophil-depleted

28. Lee HG, Eun HC. Differences between fibroblasts cultured from oral mucosa and


30. McCruden AB, Stimson WH. Sex hormones and immune function. In: Ader R,
473-493.

31. Le Goascogne C, Sananes N, Eychene B, Gouezou M, Baulieu EE, Robel P.
Androgen biosynthesis in the stomach: expression of cytochrome P450 17 alpha-
hydroxylase/17,20-lyase messenger ribonucleic acid and protein, and metabo-
lism of pregnenolone and progesterone by parietal cells of the rat gastric mucosa.