

Antler Stem Cells Sustain Regenerative Wound Healing in Deer and in Rats

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Abstract

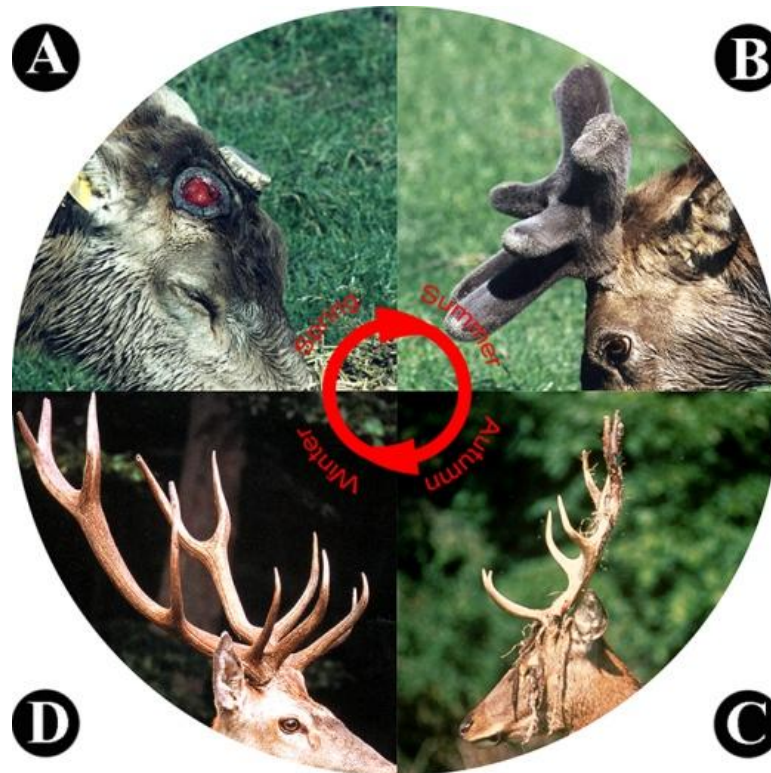
Deer antlers are unique mammalian appendages in that they can fully regenerate following loss from their pedicles (permanent bony protuberances). Antler regeneration starts from regenerative wound healing on top of a pedicle stump. A combination of tissue deletion and transplantation experiments showed that this type of regenerative healing is not skin-specific, but is bestowed by the pedicle periosteum (PP). PP cells express marker genes of both mesenchymal and embryonic stem cells, and can be induced to differentiate into multiple cell lineages in vitro. Therefore, PP cells are termed antler stem cells (AnSCs). Treatment of rats with full-thickness cutaneous wounds (2 × 2 cm) through either direct injection of AnSCs into the rats or topical application of conditioned medium of AnSCs on to the wounds can effectively induce regenerative wound healing. We believe our study has laid the foundations for developing an effective clinical therapy to achieve scar-less wound healing.

Introduction

Wound healing is a stop gap measure that normally results in scar formation even under favorable conditions [1,2]. However, giant wounds on top of pedicles (permanent bony protuberances from which antlers cast and regenerate annually in deer) can rapidly heal and leave almost no visible scar [3,4]. It, therefore, offers us a rare opportunity to learn about how nature has solved the problem of scar-less wound healing in mammals. Deer antlers are unique mammalian organs in that, once lost; they can fully regenerate [5]. Each year in spring, a hard antler is cast from its pedicle, and antler regeneration immediately starts from wound healing (Figure 1A). In summer, antlers are in their fastest growth period (up to 2 cm/day) and are enveloped by velvet-like skin, known as velvet (Figure 1B). In autumn, antler growth slows due to a sharp increase in the level of circulating sex hormones and gradually becomes fully calcified, which causes shedding of the velvet skin (Figure 1C). In winter, exposed hard bony antlers are firmly attached to their living pedicles (Figure 1D)

until spring the next year when they drop off again to trigger another round of antler regeneration (Figure 1).

Figure 1: Antler regeneration cycle. **A:** Spring, bony antler drops off from its pedicle (permanent bony protuberance), and velvet antler regenerates immediately. **B:** Late spring and early summer, rapid antler growth occurs and antlers are covered with velvet skin at their growing phase. **C:** Autumn, antlers become totally calcified and the covering skin starts to shed. **D:** Winter, dead bony antlers are attached to their living pedicles and are eventually cast in spring next year, triggering a new round of antler regeneration.



Wound healing over a pedicle stump in deer - Not a conventional scar, but are generative one in nature: Each year, antler casting creates a big wound (up to 10 cm in diameter) on top of a pedicle (Figure 2A). Interestingly, this wound can heal at an unprecedented speed (within a week; Figure 2B-2C), and also achieve near-perfect regenerative wound healing (Figure 2C-2D). Compared to typical deer scalp/pedicle skin, the velvet skin is shiny and more sparsely populated with hairs (Figure 3A). Histologically [6], this type of skin has a thicker epidermis and is adorned with hair follicles at different developmental stages. These follicles have large and multi-lobed sebaceous glands but lack arrector pili muscles and sweat glands (Figure 3B-3C). Underneath the velvet skin, there is a thin layer of squeezed subcutaneous connective tissue that contains nerves (Figure 3D) and blood vessels. Compared to their conventional counterparts, blood vessels in velvet skin are peculiar in that they have a much thicker wall and narrower lumen (Figure 3E). Overall, velvet skin is a well-structured tissue rather than a disorganized scar.

Figure 2: Wound healing over the top of a pedicle stump following casting of a bony antler. **A:** Pedicle with a fresh casting surface. Notice that a rim (arrow) formed by hairless skin at the distal end of a pedicle surrounds a depressed central bony portion, some blood was retained in the depressed central portion (asterisk). **B:** Apical surface of a pedicle a few days after hard antler casting, the blood in the central portion had formed a scab (S). This scab was surrounded by a ring of shiny skin, which resembles velvet skin. **C:** Apical view of a late wound healing-stage pedicle. Notice that the size of the scab (arrow; lost during sampling) becomes negligible. **D:** A histological section of sagittally cut healing skin. Notice that the skin contains numerous hair follicles (arrow) at different development stages.

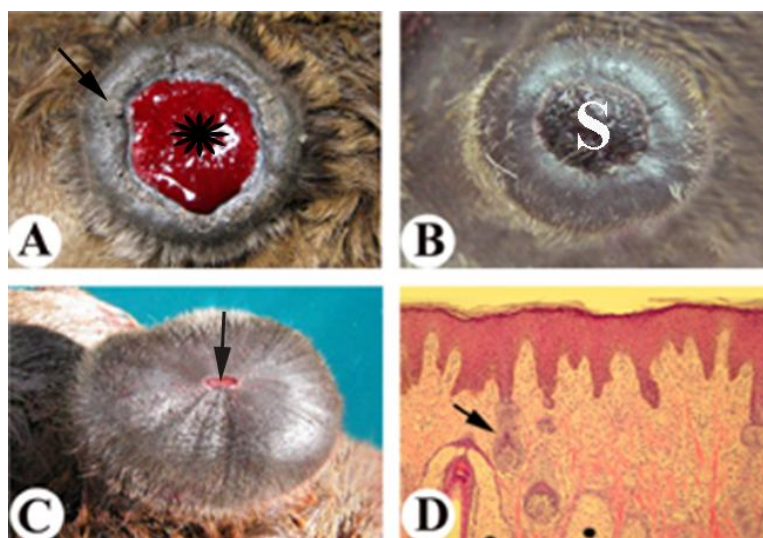
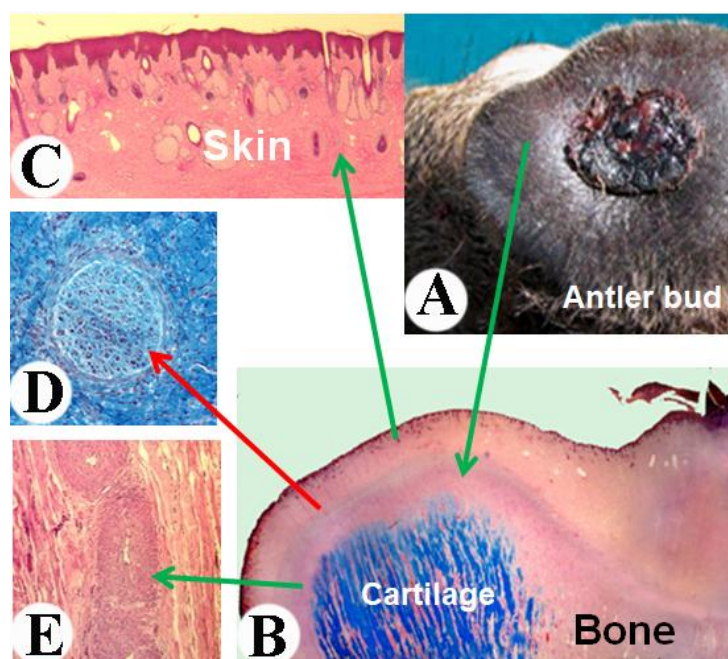
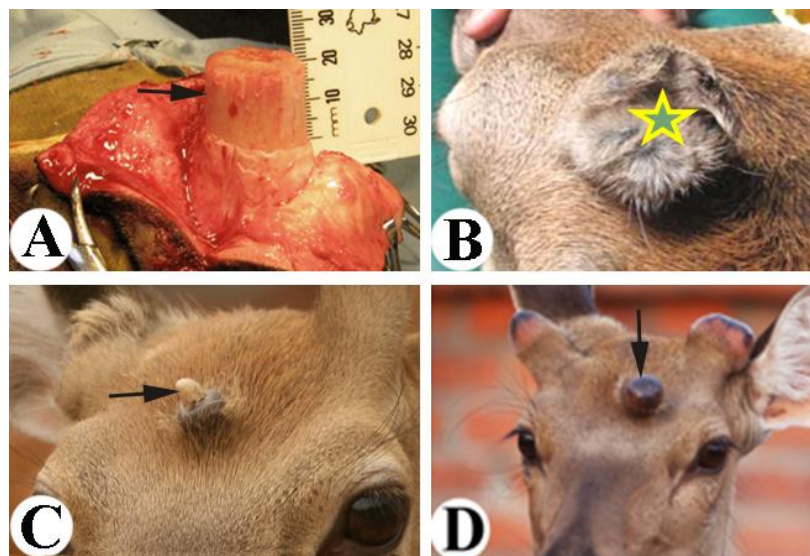


Figure 3: Histological sections of a sagittally cut early regenerating antler bud. **A:** Early regenerating antler bud. **B:** Growth centre for antler first branch, the brow tine. **C:** Velvet skin. Note that this type of skin has a much thicker epidermis, much bigger sebaceous glands, and lacks arrector pili muscle and sweat glands. **D:** Nerve fiber located in the interface between skin and underlying tissue. **E:** Blood vessels. Note that these vessels have a much thicker wall and narrower lumen.



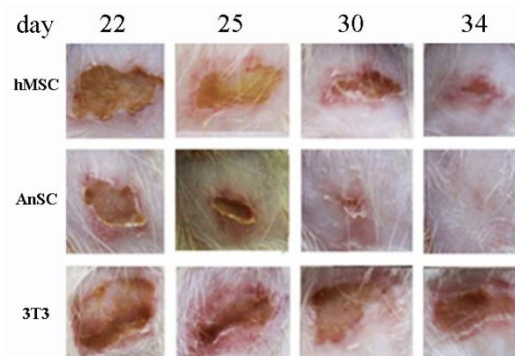
Regenerative wound healing - Not skin type-specific, but due to the presence of pedicle periosteum: A combination of tissue deletion and transplantation demonstrated that it is the pedicle periosteum (PP) that bestows the power of scar-less wound healing on the distal pedicle skin. If PP is totally or partially deleted prior to antler regeneration (Figure 4A), the wound healing over the top of the pedicle can only result in a scar (Figure 4B) [7]. Meanwhile, autologous subcutaneous transplantation of antlerogenic periosteum (antecedent tissue of PP) causes an ectopic pedicle and antler to form [8]. The ectopic antler synchronously becomes calcified (Figure 4C) along with its orthotopic counterparts in autumn and drops off from its pedicle in spring the next year to create a wound on top of the ectopic pedicle. Interestingly, before regeneration of an ectopic antler, the wound heals rapidly and scarlessly likes its orthopedic counterpart due to the presence of PP (Figure 4D) [9]. Therefore, regenerative wound healing occurring on top of a pedicle stump is due to the presence of PP, not because the pedicle skin is capable of doing so itself.

Figure 4: Pedicle periosteum (PP) deletion and transplantation. **A:** One third of PP (arrow) was deleted prior to the initiation of antler regeneration. **B:** Scar healing (star) resulted from the PP-less pedicle. **C:** Ectopic hard antler (arrow) induced from the site of transplanted periosteum. **D:** Scar-less wound healing (arrow) occurred on top of the ectopic pedicle.



Regenerative wound healing -Not animal species-specific, but a stem cell-based process: With the finding that PP can induce regenerative wound healing, we wondered whether PP could do likewise on mammals other than deer. To answer this question, we first tested on rats that had 2×2 cm full thickness punch-cut wounds by direct injection of PP cells through tail veins and using human mesenchymal stem cells (hMSC) and 3T3 fibroblasts as controls (1×10^6 /ml/cell type). The results showed that wound healing was completed on day 34 in the PP group; whereas, the healing rates lagged far behind in both the hMSC and 3T3 groups. At the same time, no visible scar can be detected in the PP cell-treated group (Figure 5; in submission). Therefore, induction of regenerative wound healing by PP cells is not animal species-specific.

Figure 5: Healing over rat wounds (2×2 cm) created by full-thickness skin punch cuts. Three treatments were carried out immediately after creation of these wounds: human mesenchymal stem cells (hMSCs), antler stem cells (AnSCs) and 3T3 fibroblasts (3T3). On day 34, wound healing reached completion in the AnSC group, whereas in both hMSCs and 3T3 groups lagged far behind.



Characterization of PP cells has found that these cells express markers of both mesenchymal stem cells (such as CD73, CD90 and CD105 etc.; Figure 6) and embryonic stem cells (such as Oct4, Nanog and SOX2 etc.; Figure 7), have a self-renewal ability and can be induced to differentiate into multiple cell types (such as chondrocytes, adipocytes and myotubes etc.; Figure 8). Therefore, PP cells are termed antler stem cells (AnSCs) and regenerative wound healing on top of a pedicle is a stem cell-based process [10,11].

Figure 6: Flow cytometry analysis of AnSCs (including PPCs). These cells highly expressed marker genes of mesenchymal stem cells including CD90, CD105 and CD73. APC, antlerogenic periosteal cells (the cells from which PPCs are differentiated); RMC, reserve mesenchymal cells (the cells that are responsible rapid growth of antlers).

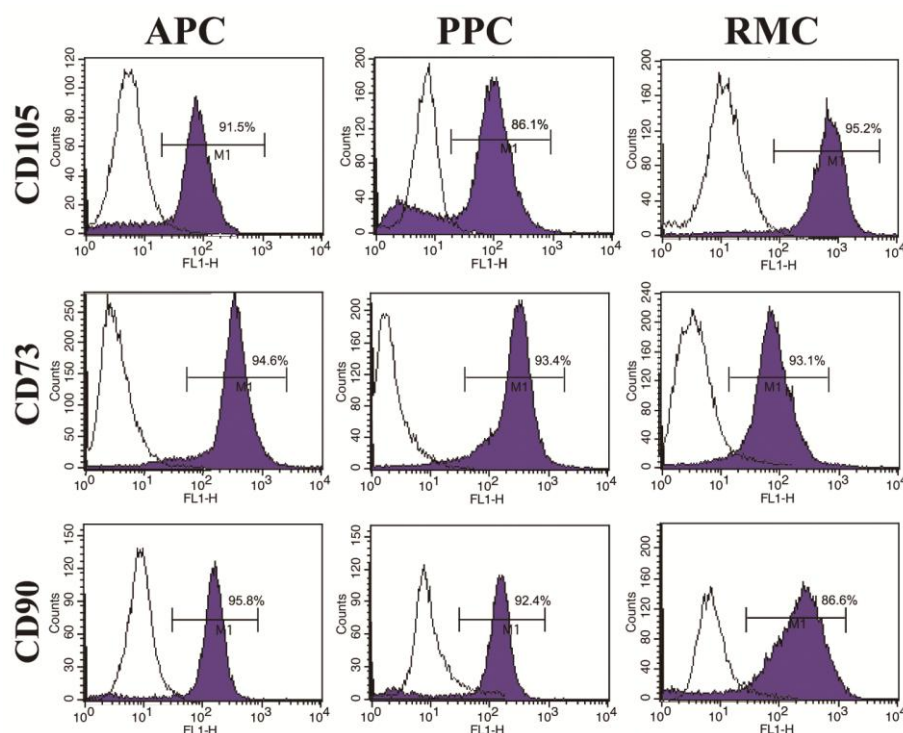


Figure 7: Expression of cell markers and genes associated with embryonic stem cell self-renewal and pluripotency in antler stem cell populations (PPCs and APCs). **A.** CD9 by flow cytometry. Note that the peaks for each cell type were offset for clarity. **B.** Oct4, SOX2 and Nanog by Western blot. **C.** Telomerase by immunocytochemistry (ICC). **D.** Nucleostemin by ICC.

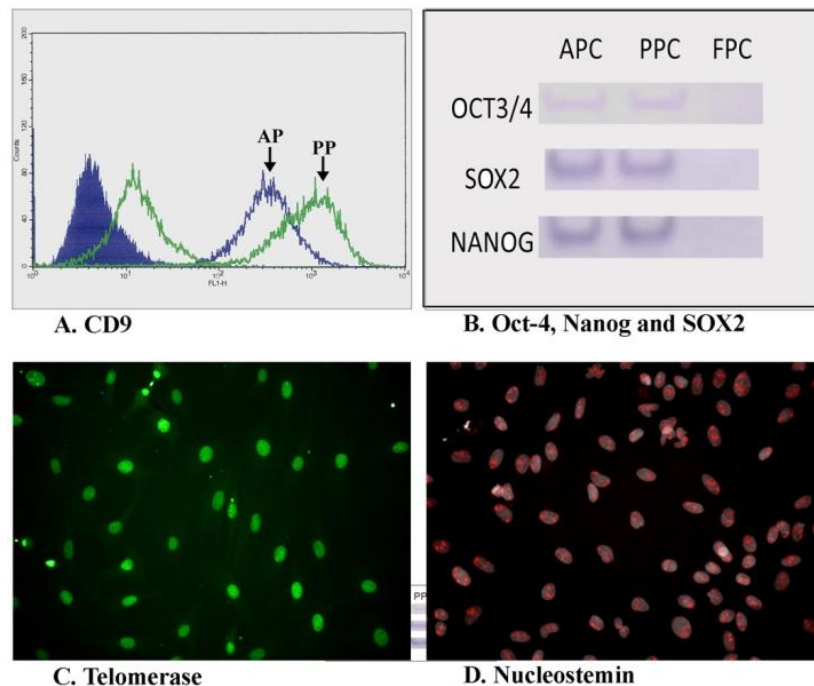
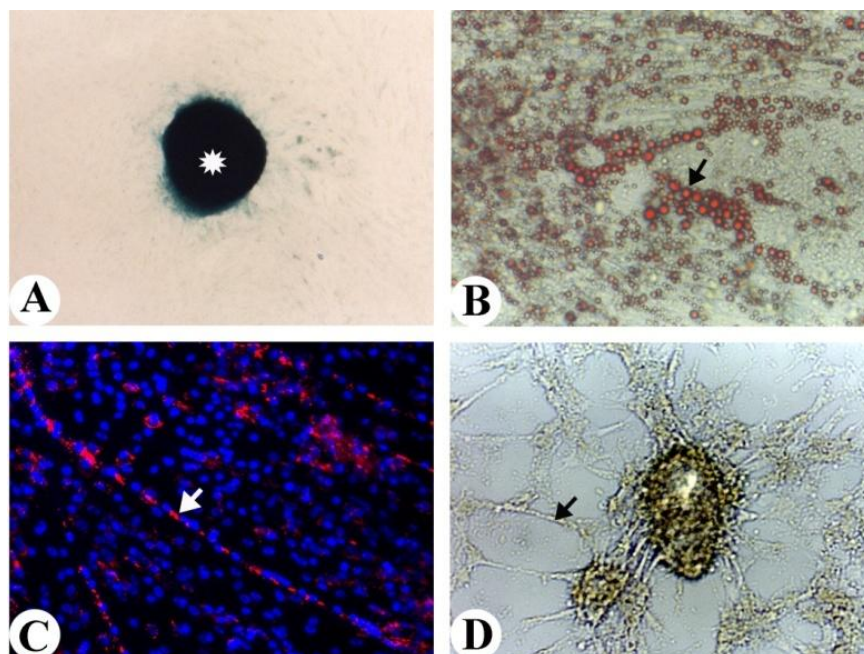
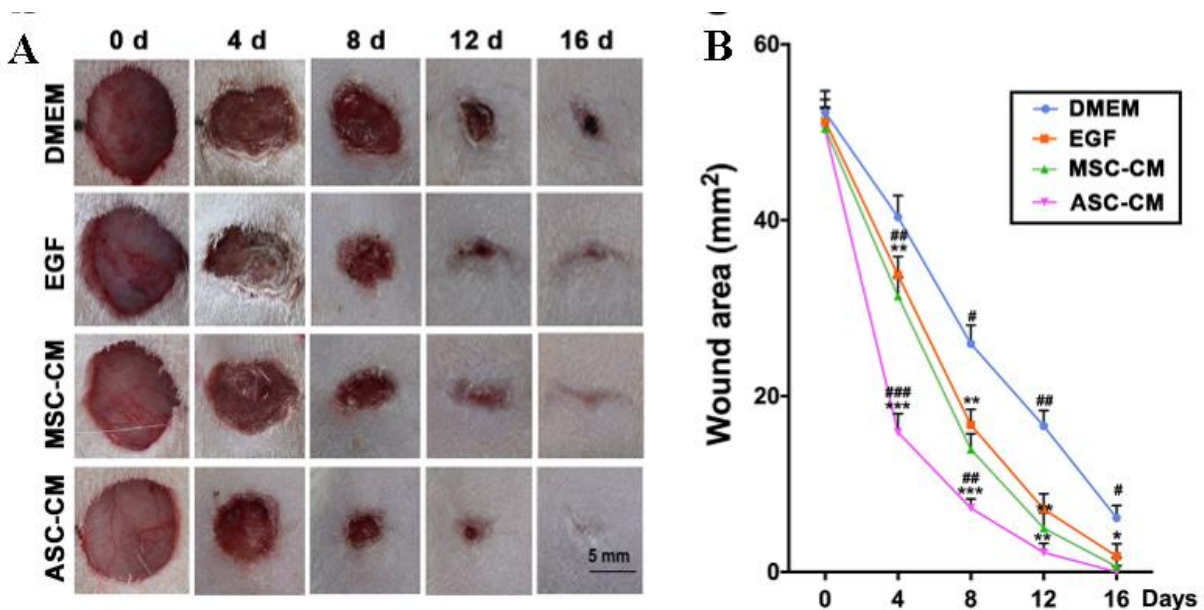


Figure 8: Multipotency of PPCs. **A.** Cartilage nodule (blue) formed while in a micro mass culture. **B.** Adipocytes differentiated while in the culture medium containing linoleic acid. **C.** Myotube (arrows) formed when co-cultured with C2C12 cells (no labelling). **D.** Neuronal-like cells differentiated when cultured in N2 medium.



Regenerative wound healing – not only a natural wonder, but also potentially translatable into clinical use: The fact that AnSCs can induce regenerative wound healing in mammals other than deer themselves has opened up a new avenue for the development of therapies for clinical use. Nonetheless, AnSCs are heterologous to humans and cannot be used via direct injection. Currently, MSCs from different sources have been widely used for the treatment of wound healing, and increasing evidence has demonstrated that MSCs most likely induce wound healing through the paracrine pathway rather than direct participation [12]. MSCs are reported to secrete active molecules to stimulate resident cell proliferation, migration and differentiation; promote angiogenesis [13]; and eventually achieve an optimal wound healing outcome. Following this line, we have recently produced a type of conditional medium by culturing AnSCs in vitro, topically applied it on top of rat wounds, and satisfactorily achieved similar outcomes to directly using AnSCs (Figure 9), i.e. scar-less wound healing [14]. This finding undoubtedly offers a potential opportunity to develop a cell-free therapy from AnSCs for cutaneous regenerative wound healing in the clinic.

Figure 9: Effects of conditioned medium from AnSCs (including PPCs) on rat wound healing rate. A. Overall morphological changes observed during wound healing. B. Changes in wound area during healing. Note that the fastest wound healing rate and smallest wound area occurred in the AnSCs group compared to the other three control groups.



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References

1. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999;341(10):738-46.
2. Hocking AM. Mesenchymal Stem Cell Therapy for Cutaneous Wounds. *Adv Wound Care (New Rochelle)*. 2012;1(4):166-171.

3. Li C, Suttie J, Clark D. Morphological observation of antler regeneration in red deer (*Cervus elaphus*). *J Morphol*. 2004;262(3):731-40.
4. Li C, Suttie J, Clark D. Histological examination of antler regeneration in red deer (*Cervus elaphus*). *AnatRec*. 2005;282A(2):163-174.
5. Goss RJ. *Deer Antlers. Regeneration, Function and Evolution*. New York, NY: Academic Press.1983.
6. Li C, Suttie J. Histological studies of pedicle skin formation and its transformation to antler velvet in red deer (*Cervus elaphus*). *Anat Rec*. 2000;260:62-71.
7. Li C, Mackintosh C, Martin S, Clark D. Identification of key tissue type for antler regeneration through pedicle periosteum deletion. *Cell and Tissue Res*.2007;328:65–75.
8. Goss RJ, Powel RS. Induction of deer antlers by transplanted periosteum. I. Graft size and shape. *J Exp Zool*. 1985;235(3):359-73.
9. Gao Z, Yang F, McMahon C, Li C. Mapping the morphogenetic potential of antler fields through deleting and transplanting subregions of antlerogenic periosteum in sika deer (*Cervus nippon*). *J Anat*. 2012;(2):131-143.
10. Wang D, Berg D, Ba H, Sun H, Wang Z, Li C. Deer antler stem cells are a novel type of cells that sustain full regeneration of a mammalian organ—deer antler. *Cell Death and Dis*.2019;10(6):443.
11. Li C, Yang F, Sheppard A. Adult stem cells and mammalian epimorphic regeneration---Insights from studying annual renewal of deer antlers. *Curr Stem Cell Rest*. 2009;4:237-251.
12. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem cells (Dayton, Ohio)*.2007;25(10):2648-2659.
13. Kusindarta DL, Wihadmadyatami H, Fibrianto YH, Nugroho WS, Susetya H, Musana DK, et al. Human umbilical mesenchymal stem cells conditioned medium promote primary wound healing regeneration. *Vet World*.2016;9(6):605-610.
14. Rong X, Chu W, Zhang H, Wang Y, Qi X, Zhang Y, Zhang G, Wang Y, Li C. Antler stem cell-conditioned medium stimulates regenerative wound healing in rats. *Stem Cell Res Ther*. 2019;10:326.