

北京协和医院实验动物福利伦理委员会

实验动物福利伦理审查申请表

Peking Union Medical College Hospital

Application Form For Welfare & Ethical Inspection in Animal

Experimentation

编号(No. for the application): XHDW-2020-01

批准日期(Approved Date): 2020-2-18

项目名称(Name of the project): SIRT1 基因修饰的脂肪干细胞外泌体在促进皮肤创伤修复中的作用机制研究

申请人(Name of applicant): 王智 项目负责人(Name of supervisor): 王智

申请类别 (Type of the application)	☒ 初次申请 (First application) ☐ 延期 (Application for extention) ☐ 修改原申请 (Applicate for modification) 原申请编号(No. of previous applification):				
从事动物实验 的人员情况 (General information of the people for animal experiment)	姓名 (Name)	职称 (Professional title)	学历 (Degree)	专业 (Major)	上岗证书编号(No. of animal experiment certificate)
	王智	副教授	研究生	整形外科	1121060300059
项目概述 及意义(The content and importance of the research)	糖尿病发病率高，糖尿病足是糖尿病的严重慢性并发症之一，其以长病程、难治愈、高财务支出、致残致死率高为特点，目前治疗手段为手术治疗和药物治疗.现在有研究指示干细胞可以治疗创面愈合，本研究的主要目的是探究干细胞治疗糖尿病足溃烂的效果和机制。 The incidence of diabetes is high. Diabetic foot is one of the serious chronic complications from diabetes. It is characterized by a long course of disease, incurable, high financial expenditure, and high disability mortality rate. The current treatment methods are surgical treatment and drug treatment. There is researching now. It indicates that stem cells can treat wound healing. The main purpose of this study is to explore the effect and mechanism of stem cells in treating diabetic foot ulcers.				
项目 经费情况 (Fundings of the research)	是否获得资 助 (Funding status)	☒ 是 (Yes) ☐ 否 (N)		动物实验经费来源 (Resource of the funding): 北京市自然科学基金 (7192160)	

项目摘要 (Abstract of the funding):	在此研究中, 我们使用外泌体对糖尿病溃疡小鼠模型进行实验, 研究外泌体对糖尿病小鼠皮肤创面愈合。 In this study, we used exosomes to conduct experiments on diabetic ulcer mouse models to study the effects of exosomes on skin wound healing in diabetic mice.	
所用实验动物的概况 (General information for the animal used)	品 种 (Animal species)	☐ 大鼠 (rat) ☒ 小鼠 (mice) ☐ 兔 (rat) ☐ 小型猪 (Pig) ☐ 犬 (Dog)
	大 鼠 品 系 (Strain of rat)	☐ SD ☐ Wistar ☐ BN ☐ Lewis ☐ 其它大鼠品系 (other rat strain)
	小鼠品系 (Strain of Mice)	☒ BALB/c ☐ C57BL/6 ☐ CD-1 ☐ KM ☐ B/c-nu ☐ SCID ☐ NOD SCID ☐ 其它小鼠品系 (other mice strain)
	使用动物数量 (Amount of the animal used)	大鼠(Rat) 小鼠(Mice) 24 兔(Rabbit) 犬(Dog) 猪(Pig)
	实验周期 (Period for experiment)	3 月 (Month)
所需动物品种的理由(The reason for the use of the animal species)	做糖尿病伤口愈合实验, 需要构建糖尿病溃疡小鼠模型, 小鼠容易成模, 且和我们分离的小鼠来源脂肪干细胞外泌体匹配 To do diabetic wound healing experiments, we need to build a diabetic ulcer mouse model, which is easy to model and matches the adipose stem cell exosomes derived from mice we isolated.	
所需动物量计算理由(The calculation of the amount of the animal will be used)	每组 6 只, 分为 4 组, 共 24 只 A total of 4 groups, Sample size was 6 for each group	
环境设施许可证编号 (The Certificate No. of the animal facility)	SYXK(京)2020-0026	
动物实验内容 (干预方法) (Description of the experiment)	1、小鼠购入后需适应性饲养 5-7 日, 减少应激反应给动物带来的不适, 然后开始造模, 用 Balb/c 小鼠并通过单次腹腔内注射溶解在 0.1 M 柠檬酸盐缓冲液 (pH 4.5) 中的 60 mg/kg 链脲佐菌素 (STZ) 诱导糖尿病。在 STZ 给药后 3 天, 我们通过测量从尾静脉获得的血液样本中的空腹血糖水平证实了糖尿病的发展, 造模成功后, 对多饮多尿糖尿病小鼠进行及时换垫料。 These include the adaptive feeding of purchased animals for 5-7 days to reduce the discomfort caused by stress response to animals. We utilized mice and induced diabetes through single	

	intraperitoneal injection of 60 mg/kg streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH 4.5). At 3 d following STZ administration, we confirmed diabetes development through measuring fasting blood glucose levels in blood samples obtained from tail vein, and timely change of pad materials for polydipsia and polyuria diabetic mice after the success of the model. 2. 麻醉后用剃须刀剔除脚面毛发, 用无菌活检穿孔机用于产生全厚度 4mm 的切除创面 After anesthesia, use a razor to remove the instep hair, and use a sterile biopsy puncher to produce a total thickness of 4mm resection wounds 3. 术后需给予曲马朵(20mg/kg, p.o, s.c)镇痛。 Tramadol (20mg/kg, p.o, s.c) was given for postoperative analgesia 4、戊巴比妥钠过量麻醉安乐死, 并联合使用局部麻醉药利多卡因, 以帮助动物缓解疼痛。 In the case of pentobarbital overdose euthanasia, a combination of a local anesthetic may be used to help the animal relieve pain
饲养管理 (Management and breeding of the animal)	☒ 屏障环境(Specific pathogen free environment) ☐ 普通环境(Clear environment)
给药方式 (Method for the delivery of drugs)	☐ 腹腔注射(i. p.) ☐ 静脉注射(i. v.) ☐ 皮下注射(i. h.) ☒ 皮内注射(i. d.) ☐ 肌肉注射(i. m.) ☐ 灌胃(i. g.) ☐ 直肠给药(Rectally) ☐ 滴鼻(Intranasally) ☐ 点眼药(Eye dropper) ☐ 气管给药(Intratracheal) ☐ 其它方式(other way) +单次鞘内给药
麻醉方式 (Method for anaesthesia)	☐ 吸入麻醉(Inhalation anesthesia) ☒ 注射麻醉(Injection anesthesia)
手术操作描述 (Description of the operation)	(持续时间长, 麻醉过程平稳)。 持续时间长: 10 min ; 麻醉后用剃须刀剔除脚面毛发, 用无菌活检穿孔机用于产生全厚度 4mm 的切除创面。 After anesthesia, use a razor to remove the instep hair, and use a sterile biopsy puncher to produce a total thickness of 4mm resection wounds
预期对实验动物的伤害 (Anticipated injury to the animal)	保定动物方式是否伤害到动物(Whether animal is hurt when fastening): ☐ 是(Yes) ☒ 否(No) 造模后是否有肢体受限(Whether limbs is limited after experiment): ☐ 是(Yes) ☒ 否(No) 术后是否有感染(Whether animals is infected after surgery): ☐ 是(Yes) ☒ 否(No)

	是否有可预期的其他伤害情况 (Any other anticipated injury): ☐ 是(Yes) ☒ 否(No)	
疼痛情况及麻醉镇痛方法 (The pain for the animal and method to calm)	A. 轻微、一过性 or 无疼痛 ☐ (Mild, transient or without pain) B. 有疼痛但可以解除 ☒ (Have pain but can be get rid of) C. 不能缓解的疼痛 ☐ (Have pain but can not be get rid of) 出现 B、C 类情况说明麻醉和止痛方法及药物选择(The method to anesthesia and analgesia; medicine will be used when B and C can not be avoided): 麻醉: 戊巴比妥钠, 50 mg/kg 50 mg/kg pentobarbital 止痛药物: 曲马朵 (20mg/kg, p.o, s.c) Tramadol 20mg/kg, p.o, s.c)	
使用有毒(害)物质情况(The usage of toxical material)	☐ 同位素 (Isotope) ☐ 有毒化学品 (Toxic chemical) ☒ 生物制品 (Biological material) ☐ 重组DNA (Recombinant DNA)	
使用特殊仪器设备或特殊环境(Special instrument or environment will be used)	小动物呼吸机 (自备)。	
仁慈终点安乐死方式 (Way of euthanasia)	仁慈终点选择依据 (Evidence of euthanasia)	☒ 疼痛 (Pain) ☐ 应激反应 (Stress response) ☐ 濒死表现 (Moribund appearance)
	安乐死方式 (Way of euthanasia)	☐ 颈椎脱臼 (Cervical dislocation) ☒ 过量麻醉 (Anesthesia overdose) ☐ 空气栓塞 (Air embolism) ☐ CO2 过量麻醉使用的是 100mg/kg 戊巴比妥钠腹腔注射; 小鼠术后慢性疼痛的国际定义是术后 21d, 所以, 所有模型小鼠均观察到术后 21d 后, 即刻行过量麻醉使其安乐死, 以尽量减少术后疼痛带来的痛苦。
负责人声明 (The claim for the applicant)	我本人及参与实验的所有人员, 经过相应培训, 经按照实验动物福利伦理的要求开展动物实验, 并对本实验设计的科学性、合理性和可行性负责。 I declare that all the people involved in the experiment conducted in accordance with the requirements of animal welfare and ethics. Also, all the people are responsible for the design of the experiment.	
备注(Remarks)		
审查意见 (Result of the inspection)	同意 时间(Date): 2020年(Year) 2月(Month) / 8日(Day)	
委员签字 (Signature of the committee member)	康爱君 张栋 马东来 吕威	