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一种双层骨修复膜材料及其制备方法

(57)摘要

一种双层骨修复膜材料及其制备方法，具体为一种具有抗菌消炎及促成骨功效的骨修复材料及其制备方法，属于生物材料领域。该材料以具有生物相容性的可降解脂肪族聚酯及天然高分子为主要原材料，使用静电纺丝方法制备。具有双层膜结构，包括添加抗菌药物的外层和添加促成骨物质的内层。本发明材料具有优异的生物相容性以及可控且长期的药物释放性能，同时双层膜结构的外层膜能够抑制骨缺损发生后易出现的细菌性感染及炎症，防止细菌进入缺损部位，内层膜能够促进骨缺损的修复。该材料可以按需要实现可控的体内降解，不必进行二次手术取出材料。

1. 双层骨修复膜材料，其特征是：以具有生物相容性的可降解脂肪族聚酯及天然可降解聚合物作为基体材料，具有多孔结构；材料中含有两层紧密结合的膜，并在内层膜中添加促成骨成分，外层膜添加抗菌药物，其中：

外层膜以可降解脂肪族聚酯及可降解天然高分子的混合物作为基体材料，其中可降解脂肪族聚酯与可降解天然高分子的质量比为10/90-50/50，抗菌药物质量与基体材料即可降解脂肪族聚酯和可降解天然高分子的总质量之比为1/100-30/100，内层膜以可降解脂肪族聚酯及可降解天然高分子的混合物作为基体材料，其中可降解脂肪族聚酯与可降解天然高分子的质量比为50/50-90/10，促成骨物质的质量与基体材料即可降解脂肪族聚酯和可降解天然高分子的总质量之比为1/100-40/100，含有多载药缓释系统骨修复材料的结构特征为具有多孔结构，平均搭桥孔径为2-6μm。

2. 根据权利要求1所述的双层骨修复膜材料，其特征在于，该材料由两层紧密结合的膜组成；内层膜贴近骨组织，添加有促成骨物质；外层膜贴近组织，含有抑菌药物。

3. 根据权利要求1所述的双层骨修复膜材料，其特征在于，可降解合成高分子包括：聚乳酸、聚己内酯、聚乳酸-羟基乙酸共聚物、聚乳酸-己内酯共聚物、聚乳酸-羟基乙酸-己内酯共聚物其中的一种或两种以上的混合物；可降解天然高分子材料包括：I型胶原、明胶、壳聚糖、淀粉、纤维素、弹性蛋白中的一种或两种以上的混合物。

4. 根据权利要求1所述的双层骨修复膜材料，其特征在于，载入外层膜基体的药物A包含青霉素类、头孢霉素类、四环素类、氯霉素类、大环内脂类、林可霉素、氟喹诺酮类、硝基咪唑类、多肽类及季铵盐类抗菌药；载入内层膜基体的促成骨物质B羟基磷灰石、氧化石墨烯、碳纳米管、磷酸三钙、淫羊藿苷；生物玻璃包括有45S5、磷灰石-硅灰石活性玻璃、可切削生物活性玻璃；生长因子包括骨形态发生蛋白BMP、成纤维细胞生长因子FGF、转化生长因子TGF-B、血小板衍生生长因子PDGF、血管内皮细胞生长因子VEGF和胰岛素样生长因子IGF。

5. 根据权利要求1所述的双层骨修复膜材料，其特征在于，通过调节膜材料双层膜结构的内外层基体材料组成来控制膜材料的降解速度；外层膜在抑制骨缺损发生后初期易出现的细菌性感染及炎症之后，降解完全；内层膜则将为骨修复全程提供促成骨物质，直至骨修复完成后再行完全降解。

6. 制备如权利要求1-5任意一项所述的双层骨修复膜材料的方法，其特征在于，有如下步骤：

(1) 将可降解脂肪族聚酯溶于有机溶剂中，烘箱中静置0.5-1h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液A；

(2) 向A溶液中加入可降解天然高分子，室温磁力搅拌6-12h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液B，溶液B中可降解脂肪族聚酯与可降解天然高分子的质量比为10/90-50/50；

(3) 向溶液B中加入抗菌药物，室温磁力搅拌6-12h，得到可降解天然高分子浓度为0.04-0.4g/mL的溶液C，溶液C中抗菌药物与可降解脂肪族聚酯和可降解天然高分子总质量的比为1/100-30/100；

(4) 将可降解脂肪族聚酯溶于有机溶剂中，烘箱中静置0.5-1h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液D；

(5) D溶液中加入可降解天然高分子，室温磁力搅拌6-12h，得到可降解脂肪族聚酯质量

浓度为0.04-0.4g/mL的溶液E,溶液E中可降解脂肪族聚酯与可降解天然高分子的质量比为50/50-90/10;

(6) 向溶液E中加入促成骨物质中的一种或多种,室温磁力搅拌6-12h,得到可降解天然高分子浓度为0.04-0.4g/mL的溶液F,溶液F中促成骨物质的总质量与可降解天然高分子的质量比为1/100-40/100;

(7) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-3mL/h,电压7-30kV,接收距离8-30cm,纺丝0.5-30h,得到厚度50-200μm的电纺丝纤维膜;

(8) 用溶液F进行静电纺丝,以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-3mL/h,电压7-30kV,接收距离8-30cm,纺丝0.5-30h,得到厚度250-500μm的静电纺丝纤维膜;

(9) 静电纺丝结束后,将纺丝膜在通风橱中室温放置2-7天,包装消毒。

一种双层骨修复膜材料及其制备方法

技术领域

[0001] 本发明涉及一种双层骨修复膜材料的制备方法，属于生物材料领域，具体涉及一种以可降解脂肪族聚酯所形成的纤维作为主要基体材料并加载抗菌药物的外层膜和加载促成骨物质的内层膜复合而成的双层骨修复膜材料及其制备方法。

背景技术

[0002] 骨缺损是临幊上非常常见的创伤，骨的修复过程是非常漫长的，它主要包括血肿和炎症期、初始骨痴反应期、软骨形成期以及骨形成及改建期。在骨修复过程中细胞分泌多种生长因子以不同时序发挥作用，保证骨缺损的修复。

[0003] 在临幊上，当骨缺损发生时，最好的办法是进行骨移植，主要包括自体骨移植，同种异体骨移植以及非组织修补术等，其中对骨缺损修复效果最好的是自体骨移植。但是，自体骨取用过多会给患者带来新的创伤及并发症；同种异体骨移植可以克服部分自体骨移植带来的问题，但是会受到供体来源、移植时组织状况以及免疫原性等的限制；非组织修补术通常用于关节替代手术，主要问题就是不能跟周围的组织整合进而形成感染的病灶。对于骨缺损或者组织缺损，理想的解决办法就是要克服以上三个方面的问题。生物材料领域为解决这一问题提供了潜在的选择。

[0004] 作为一个理想的骨修复支架材料，需要满足以下四个方面的要求：

[0005] (1) 具有生物相容性、骨传导性、骨诱导性，能够为正常的细胞活动提供支持；

[0006] (2) 可生物降解性，能够为新组织的长入提供空间并逐渐被新组织取代；

[0007] (3) 一定的力学性能，能承受手术操作过程以及骨生长过程中的应力；

[0008] (4) 连贯多孔结构，为新组织的生成运输营养物质和废物。

[0009] 静电纺丝技术是一种制备纳米纤维的简单通用方法，由于其药物加载方式简单易行，在静电纺丝过程中可以将不同的药物容易的加载到纤维中，另外，药物在载入纤维中后不会发生性能变化，仍能保持其性能，可以用来预防术后感染或促进骨的生成。因此，静电纺丝制备的纳米纤维载药膜具有良好的临床应用前景。同时骨缺损处多种生长因子缺乏或是活性不足是影响骨再生的重要原因，因此，研究一种既可以抗菌消炎又可以促进骨修复的生物材料是十分重要的。同时，由于抗感染药物主要作用于发炎的周围组织，而促成骨物质则需直接作用于骨骼。为了提高药物的利用率，使用双层膜结构来分别加载这两种药物是一种很有效的方法。

[0010] 本专利中涉及的促成骨物质主要指羟基磷灰石、氧化石墨烯、碳纳米管、磷酸三钙、淫羊藿昔；生物玻璃包括有45S5、磷灰石-硅灰石活性玻璃、可切削生物活性玻璃；生长因子如骨形态发生蛋白(BMP)、成纤维细胞生长因子(FGF)、转化生长因子(TGF-B)、血小板衍生生长因子(PDGF)、血管内皮细胞生长因子(VEGF)和胰岛素样生长因子(IGF)。其中，生物玻璃是指由SiO₂, Na₂O, CaO和P₂O₅等基本成分组成的硅酸盐玻璃。它具有良好的生物相容性，可以与骨组织紧密结合。由于其良好的促成骨性能和优良的加工性能，生物玻璃在修复骨缺损方面有着广阔的前景。然而，生物玻璃材料本身较硬脆，因此不适合独立作为支架诱

导骨再生。通过静电纺丝技术将生物玻璃作为促成骨因子混入纤维中,可以有效的规避这个问题。同时,生物玻璃的加入也可以有效的改善纤维膜的机械性能,大大扩展了材料的应用范围。生长因子是指一类通过与特异的、高亲和的细胞膜受体结合,调节细胞生长与其他细胞功能等多效应的多肽类物质。生长因子对不同种类细胞具有一定的专一性,具有促成骨作用的生长因子可以促进生成大量的成骨细胞、抑制破骨细胞。在治疗骨质疏松、股骨头坏死、关节炎、风湿病和因钙缺乏导致的疾病方面具有很好的效果。

[0011] 由于材料植入过程中的感染而引起的发炎主要发生在材料周围的组织中,而促成骨物质则要作用骨组织,二者的作用位置并不相同。为了提高药物的利用效率,提高治疗效果,可以将两种药物添加到不同的两层膜中。内层膜贴近骨骼,添加促进骨生成物质;外层膜贴近组织,添加抑菌药物。由于两层膜成分相近,因此可以较为紧密的结合在一起,不易脱落或分离。

发明内容

[0012] 本发明的目的是提供一种双层骨修复膜材料的制备方法,实现抗菌药物和促成骨药物的定向释放,可以促进骨缺损修复,不必二次手术,还可抑制缺损发生后易发生的细菌性感染及炎症。

[0013] 双层骨修复膜材料,其特征是:以具有生物相容性的可降解脂肪族聚酯及天然可降解聚合物作为基体材料,具有多孔结构;材料中含有两层紧密结合的膜,并在内层膜中添加促成骨成分,外层膜添加抗菌药物,其中:

[0014] 外层膜以可降解脂肪族聚酯及可降解天然高分子的混合物作为基体材料,其中可降解脂肪族聚酯与可降解天然高分子的质量比为10/90-50/50,抗菌药物质量与基体材料即可降解脂肪族聚酯和可降解天然高分子的总质量之比为1/100-30/100,内层膜以可降解脂肪族聚酯及可降解天然高分子的混合物作为基体材料,其中可降解脂肪族聚酯与可降解天然高分子的质量比为50/50-90/10,促成骨物质的质量与基体材料即可降解脂肪族聚酯和可降解天然高分子的总质量之比为1/100-40/100,含有多载药缓释系统骨修复材料的结构特征为具有多孔结构,平均搭桥孔径为2-6μm。

[0015] 进一步,该材料由两层紧密结合的膜组成;内层膜贴近骨组织,添加有促成骨物质;外层膜贴近组织,含有抑菌药物。

[0016] 进一步,可降解合成高分子包括:聚乳酸、聚己内酯、聚乳酸-羟基乙酸共聚物、聚乳酸-己内酯共聚物、聚乳酸-羟基乙酸-己内酯共聚物其中的一种或两种以上的混合物;可降解天然高分子材料包括:I型胶原、明胶、壳聚糖、淀粉、纤维素、弹性蛋白中的一种或两种以上的混合物。

[0017] 进一步,载入外层膜基体的药物A包含青霉素类、头孢霉素类、四环素类、氯霉素类、大环内脂类、林可霉素、氟喹诺酮类、硝基咪唑类、多肽类及季铵盐类抗菌药;载入内层膜基体的促成骨物质B羟基磷灰石、氧化石墨烯、碳纳米管、磷酸三钙、淫羊藿苷;生物玻璃包括有45S5、磷灰石-硅灰石活性玻璃、可切削生物活性玻璃;生长因子包括骨形态发生蛋白BMP、成纤维细胞生长因子FGF、转化生长因子TGF-B、血小板衍生生长因子PDGF、血管内皮细胞生长因子VEGF和胰岛素样生长因子IGF。

[0018] 进一步,通过调节膜材料双层膜结构的内外层基体材料组成来控制膜材料的降解

速度；外层膜在抑制骨缺损发生后初期易出现的细菌性感染及炎症之后，降解完全；内层膜则将为骨修复全程提供促成骨物质，直至骨修复完成后再行完全降解。

[0019] 所述的双层骨修复膜材料制备方法有如下步骤：

[0020] (1) 将可降解脂肪族聚酯溶于有机溶剂中，烘箱中静置0.5-1h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液A；

[0021] (2) 向A溶液中加入可降解天然高分子，室温磁力搅拌6-12h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液B，溶液B中可降解脂肪族聚酯与可降解天然高分子的质量比为10/90-50/50；

[0022] (3) 向溶液B中加入抗菌药物1，室温磁力搅拌6-12h，得到可降解天然高分子浓度为0.04-0.4g/mL的溶液C，溶液C中抗菌药物1与可降解脂肪族聚酯和可降解天然高分子总质量的比为1/100-30/100。

[0023] (4) 将可降解脂肪族聚酯溶于有机溶剂中，烘箱中静置0.5-1h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液D；

[0024] (5) D溶液中加入可降解天然高分子，室温磁力搅拌6-12h，得到可降解脂肪族聚酯质量浓度为0.04-0.4g/mL的溶液E，溶液E中可降解脂肪族聚酯与可降解天然高分子的质量比为50/50-90/10；

[0025] (6) 向溶液E中加入促成骨物质2中的一种或多种，室温磁力搅拌6-12h，得到可降解天然高分子浓度为0.04-0.4g/mL的溶液F，溶液F中促成骨物质2的总质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为1/100-40/100。

[0026] (7) 用溶液C进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-3mL/h，电压7-30kV，接收距离8-30cm，纺丝0.5-30h，得到厚度50-200μm的电纺丝纤维膜；

[0027] (8) 用溶液F进行静电纺丝，以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-3mL/h，电压7-30kV，接收距离8-30cm，纺丝0.5-30h，得到厚度250-500μm的静电纺丝纤维膜；

[0028] (9) 静电纺丝结束后，将纺丝膜在通风橱中室温放置2-7天，包装消毒。

[0029] 本发明采用静电纺丝的方法制备纳米纤维，但是本发明不限于静电纺丝，溶液浇注、3D打印等制备的材料以及水凝胶等都适用于本发明。

附图说明

[0030] 图1为双层骨修复膜材料示意图；

[0031] 图2为实施例1的双层骨修复膜材料的电镜图片；

[0032] 图3为实施例1-6的双层骨修复膜材料的抑菌圈照片，模型菌为金黄色葡萄球菌。

具体实施方式

[0033] 下面结合实施例对本发明做进一步说明，但本发明并不限于以下实施例。

[0034] 实施例1

[0035] (1) 取4g的聚己内酯，加入20mL三氟乙醇溶剂中，在烘箱中静置1h，得到聚己内酯质量浓度为0.2g/mL的溶液A；

[0036] (2) 向A溶液中加入4g的明胶,室温磁力搅拌12h,得到溶液B,溶液B中聚己内酯与明胶的质量比为50/50;

[0037] (3) 向溶液B中加入0.08g抗菌药物环丙沙星,室温磁力搅拌6h,得到溶液C,溶液C中环丙沙星与聚己内酯和明胶总质量的比为1/100;

[0038] (4) 取7.2g的聚己内酯,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚己内酯质量浓度为0.36/mL的溶液D;

[0039] (5) 向D溶液中加入0.8g的明胶,室温磁力搅拌12h,得到溶液E,溶液E中聚己内酯与明胶的质量比为90/10;

[0040] (6) 向溶液E中加入0.08g促成骨物质45S5生物玻璃,室温磁力搅拌6h,得到溶液F,溶液F中生物玻璃与聚己内酯和明胶总质量的比为1/100;

[0041] (7) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h;

[0042] (8) 用溶液F进行静电纺丝,以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h,得到电纺丝纤维膜;

[0043] (9) 静电纺丝结束后,将纺丝膜在通风橱中室温放置2天,包装消毒。

[0044] 实施例2

[0045] (1) 取0.8g的聚己内酯,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚己内酯质量浓度为0.04g/mL的溶液A;

[0046] (2) 向A溶液中加入7.2g的明胶,室温磁力搅拌12h,得到溶液B,溶液B中聚己内酯与明胶的质量比为10/90;

[0047] (3) 向溶液B中加入2.4g抗菌药物环丙沙星,室温磁力搅拌6h,得到溶液C,溶液C中环丙沙星与聚己内酯和明胶总质量的比为30/100;

[0048] (4) 取4g的聚己内酯,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚己内酯质量浓度为0.2/mL的溶液D;

[0049] (5) 向D溶液中加入4g的明胶,室温磁力搅拌12h,得到溶液E,溶液E中聚己内酯与明胶的质量比为50/50;

[0050] (6) 向溶液E中加入3.2g促成骨物质45S5生物玻璃,室温磁力搅拌6h,得到溶液F,溶液F中生物玻璃与聚己内酯和明胶总质量的比为40/100;

[0051] (7) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h;

[0052] (8) 用溶液F进行静电纺丝,以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h,得到电纺丝纤维膜;

[0053] (9) 静电纺丝结束后,将纺丝膜在通风橱中室温放置2天,包装消毒。

[0054] 实施例3

[0055] (1) 取0.8g的聚己内酯,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚己内酯质量浓度为0.04g/mL的溶液A;

[0056] (2) 向A溶液中加入7.2g的明胶,室温磁力搅拌12h,得到溶液B,溶液B中聚己内酯

与明胶的质量比为10/90；

[0057] (3) 向溶液B中加入0.08g抗菌药物环丙沙星，室温磁力搅拌6h，得到溶液C，溶液C中环丙沙星与聚己内酯和明胶总质量的比为1/100；

[0058] (4) 取7.2g的聚己内酯，加入20mL三氟乙醇溶剂中，在烘箱中静置1h，得到聚己内酯质量浓度为0.36/mL的溶液D；

[0059] (5) 向D溶液中加入0.8g的明胶，室温磁力搅拌12h，得到溶液E，溶液E中聚己内酯与明胶的质量比为90/10；

[0060] (6) 向溶液E中加入3.2g促成骨物质生物玻璃，室温磁力搅拌6h，得到溶液F，溶液F中生物玻璃与聚己内酯和明胶总质量的比为40/100；

[0061] (7) 用溶液C进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为300rpm，纺丝液流动速率为2.5mL/h，电压28kV，接收距离10cm，纺丝8h；

[0062] (8) 用溶液F进行静电纺丝，以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置，滚筒转动速率为300rpm，纺丝液流动速率为2.5mL/h，电压28kV，接收距离10cm，纺丝8h，得到电纺丝纤维膜；

[0063] (9) 静电纺丝结束后，将纺丝膜在通风橱中室温放置2天，包装消毒。

[0064] 实施例4

[0065] (1) 取4g的聚乳酸，加入20mL三氟乙醇溶剂中，在烘箱中静置1h，得到聚乳酸质量浓度为0.2g/mL的溶液A；

[0066] (2) 向A溶液中加入4g的壳聚糖，室温磁力搅拌12h，得到溶液B，溶液B中聚乳酸与壳聚糖的质量比为50/50；

[0067] (3) 向溶液B中加入0.08g抗菌药物甲硝唑，室温磁力搅拌6h，得到溶液C，溶液C中甲硝唑与聚乳酸和壳聚糖总质量的比为1/100；

[0068] (4) 取7.2g的聚乳酸，加入20mL三氟乙醇溶剂中，在烘箱中静置1h，得到聚乳酸质量浓度为0.36/mL的溶液D；

[0069] (5) 向D溶液中加入0.8g的壳聚糖，室温磁力搅拌12h，得到溶液E，溶液E中聚乳酸与壳聚糖的质量比为90/10；

[0070] (6) 向溶液E中加入0.08g促成骨物质磷酸三钙，室温磁力搅拌6h，得到溶液F，溶液F中磷酸三钙与聚乳酸和壳聚糖总质量的比为1/100；

[0071] (7) 用溶液C进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为300rpm，纺丝液流动速率为2.5mL/h，电压28kV，接收距离10cm，纺丝8h；

[0072] (8) 用溶液F进行静电纺丝，以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置，滚筒转动速率为300rpm，纺丝液流动速率为2.5mL/h，电压28kV，接收距离10cm，纺丝8h，得到电纺丝纤维膜；

[0073] (9) 静电纺丝结束后，将纺丝膜在通风橱中室温放置3天，包装消毒。

[0074] 实施例5

[0075] (1) 取0.8g的聚乳酸，加入20mL三氟乙醇溶剂中，在烘箱中静置1h，得到聚乳酸质量浓度为0.04g/mL的溶液A；

[0076] (2) 向A溶液中加入7.2g的壳聚糖，室温磁力搅拌12h，得到溶液B，溶液B中聚乳酸与壳聚糖的质量比为10/90；

[0077] (3) 向溶液B中加入2.4g抗菌药物甲硝唑,室温磁力搅拌6h,得到溶液C,溶液C中甲硝唑与聚乳酸和壳聚糖总质量的比为30/100;

[0078] (4) 取4g的聚乳酸,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚乳酸质量浓度为0.2/mL的溶液D;

[0079] (5) 向D溶液中加入4g的壳聚糖,室温磁力搅拌12h,得到溶液E,溶液E中聚乳酸与壳聚糖的质量比为50/50;

[0080] (6) 向溶液E中加入3.2g促成骨物质磷酸三钙,室温磁力搅拌6h,得到溶液F,溶液F中磷酸三钙与聚乳酸和壳聚糖总质量的比为40/100;

[0081] (7) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h;

[0082] (8) 用溶液F进行静电纺丝,以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h,得到电纺丝纤维膜;

[0083] (9) 静电纺丝结束后,将纺丝膜在通风橱中室温放置3天,包装消毒。

[0084] 实施例6

[0085] (1) 取0.8g的聚乳酸,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚乳酸质量浓度为0.04g/mL的溶液A;

[0086] (2) 向A溶液中加入7.2g的壳聚糖,室温磁力搅拌12h,得到溶液B,溶液B中聚乳酸与壳聚糖的质量比为10/90;

[0087] (3) 向溶液B中加入0.08g抗菌药物甲硝唑,室温磁力搅拌6h,得到溶液C,溶液C中甲硝唑与聚乳酸和壳聚糖总质量的比为1/100;

[0088] (4) 取7.2g的聚乳酸,加入20mL三氟乙醇溶剂中,在烘箱中静置1h,得到聚乳酸质量浓度为0.36/mL的溶液D;

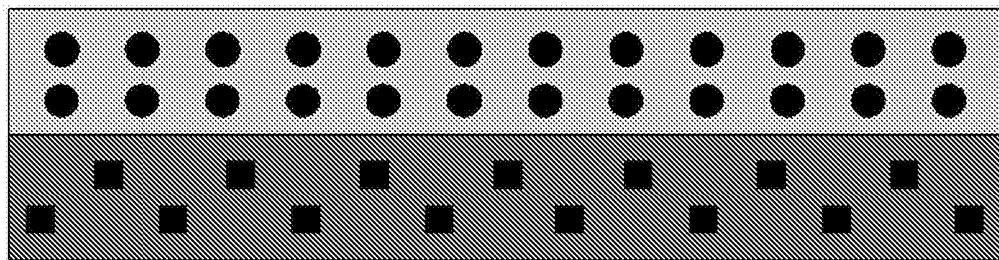
[0089] (5) 向D溶液中加入0.8g的壳聚糖,室温磁力搅拌12h,得到溶液E,溶液E中聚乳酸与壳聚糖的质量比为90/10;

[0090] (6) 向溶液E中加入3.2g促成骨物质磷酸三钙,室温磁力搅拌6h,得到溶液F,溶液F中磷酸三钙与聚乳酸和壳聚糖总质量的比为40/100;

[0091] (7) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h;

[0092] (8) 用溶液F进行静电纺丝,以步骤(7)中带有纤维膜的以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2.5mL/h,电压28kV,接收距离10cm,纺丝8h,得到电纺丝纤维膜;

[0093] 静电纺丝结束后,将纺丝膜在通风橱中室温放置3天,包装消毒。



外层膜 内层膜
● 抗菌药物 ■ 促成骨物质

图1

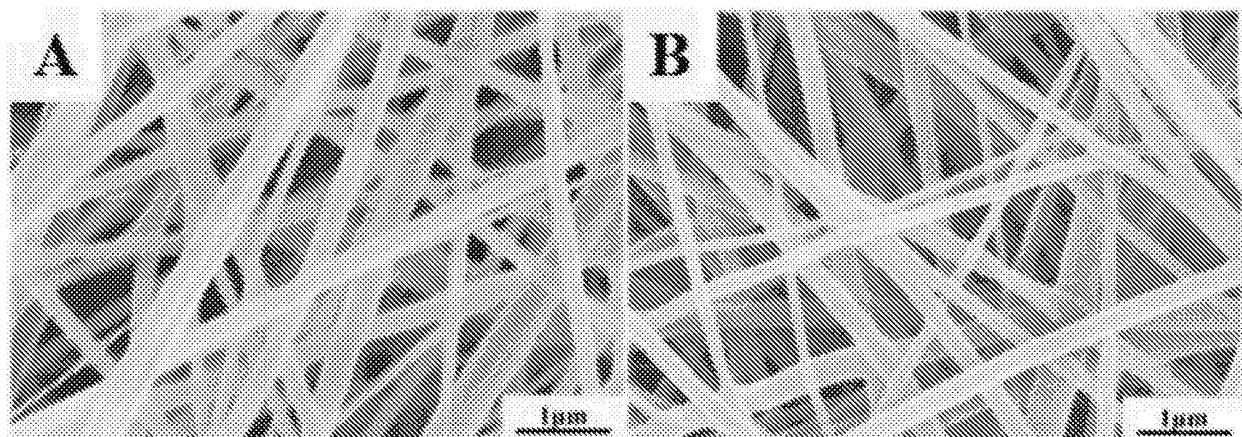


图2

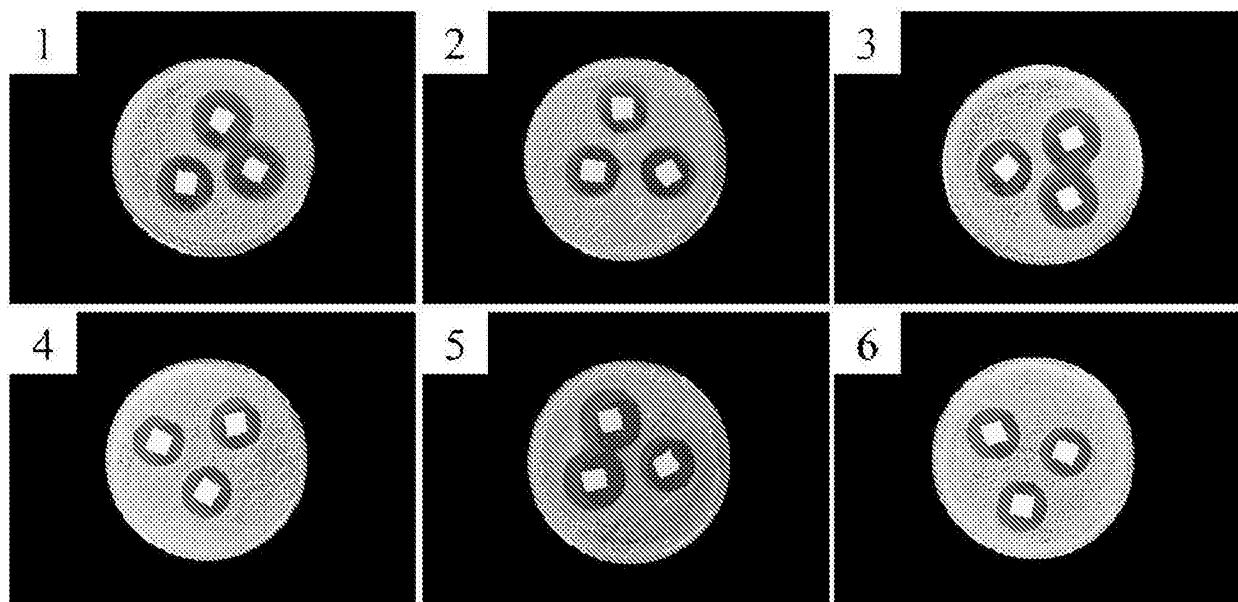


图3

Patents

CN106975106A



> CN106975106A—A kind of double-deck Bone Defect Repari membrane material and preparation method thereof

A kind of double-deck Bone Defect Repari membrane material and preparation method thereof

Abstract

A kind of double-deck Bone Defect Repari membrane material and preparation method thereof, it is specially a kind of that there is anti-inflammation and facilitate bone renovating material of bone effect and preparation method thereof, belong to technical field of biological material. The material is prepared using degradable aliphatic polyester and natural polymer with biocompatibility as main raw material(s) using electrospinning process. Facilitate the internal layer of bone material with double membrane structure, including the outer layer of addition antibacterials and addition. Material of the present invention has excellent biocompatibility and controllable and long-term medicine-releasing performance, the outer membrane of double membrane structure can suppress bacterial infection and the inflammation easily occurred after Cranial defect occurs simultaneously, prevent bacterium from entering defect, inner layer film can promote the reparation of Cranial defect. The material can realize controllable internal degraded on demand, it is not necessary to carry out second operation and take out material.

Classifications

■ A61L27/18 Macromolecular materials obtained otherwise than by reactions only involving carbon-to-carbon unsaturated bonds

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Current Assignee: Beijing University of Chemical Technology

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External links: [Espacenet](#), [Global Dossier](#), [Discuss](#)

Claims (6)

[Hide Dependent](#) ^

1. double-deck Bone Defect Repari membrane material, it is characterized in that: It can with the degradable aliphatic polyester with biocompatibility and naturally drop Depolymerization compound is as matrix material, with loose structure; Containing the film combined closely for two layers in material, and added in inner layer film Facilitate bone component, outer membrane adds antibacterials, wherein:

Outer membrane is using degradable aliphatic polyester and the high molecular mixture of degradable natural as matrix material, wherein degradable Aliphatic polyester is 10/90-50/50, antibacterials quality and matrix material with the high molecular mass ratio of degradable natural The ratio between degrading aliphatic polyester and the high molecular gross mass of degradable natural are 1/100-30/100, and inner layer film is with degradable aliphatic Adoption ester and the high molecular mixture of degradable natural are used as matrix material, wherein degradable aliphatic polyester and degradable natural High molecular mass ratio is 50/50-90/10, facilitates the quality of bone material and matrix material i.e. degradable aliphatic polyester and can The ratio between gross mass of degraded natural polymer is 1/100-40/100, and the structure containing overloading medicine slow-released system bone renovating material is special Levy as with loose structure, averagely bridging aperture is 2-6 μm.

2. double-deck Bone Defect Repari membrane material according to claim 1, it is characterised in that the film that the material is combined closely by two layers Composition; Inner layer film presses close to bone tissue, added with facilitating bone material; Outer membrane presses close to tissue, contains antibacterial medicines.

3. double-deck Bone Defect Repari membrane material according to claim 1, it is characterised in that degradable synthesized polymer includes: It is poly-Lactic acid, polycaprolactone, Poly(D,L-lactide-co-glycolide, PLA-caprolactone copolymer, poly lactic-co-glycolic acid-onself in Ester copolymer one or more kinds of mixture therein; Degradable natural high polymer material includes: Type i collagen, gelatin, shell Mixture more than one or both of glycan, starch, cellulose, elastin laminin.

4. double-deck Bone Defect Repari membrane material according to claim 1, it is characterised in that be loaded into the medicine A bags of outer membrane matrix Containing penicillins, cephalosporin class, Tetracyclines, chloromycetin, macrolide, lincomycin, fluoroquinolones, nitro miao Azole, polypeptide and quaternary ammonium salt antimicrobial; Be loaded into inner layer film matrix facilitate bone material B hydroxyapatites, graphene oxide, CNT, tricalcium phosphate, icarin; Bio-vitric includes 45S5, apatite-wollastonite activity glass, machinable life Thing activity glass; Growth factor includes bone morphogenic protein BMP-2, fibroblast growth factor FGF, TGF TGF- B, platelet derived growth factor PDGF, blood vessel endothelial cell growth factor VEGF and insulin-like growth factor I GF.

5. double-deck Bone Defect Repari membrane material according to claim 1, it is characterised in that pass through adjusting film materials double-layer membrane structure Ectonexine matrix material constitute to control the degradation speed of membrane material; Outer membrane easily occurs at the initial stage after Cranial defect occurs that suppresses Bacterial infection and inflammation after, degraded is complete; Inner layer film will then provide for Bone Defect Repari whole process facilitates bone material, until bone is repaiied Gone again after the completion of multiple degradable.

6. prepare the method for the double-deck Bone Defect Repari membrane material as described in claim 1-5 any one, it is characterised in that have as follows Step:

(1) degradable aliphatic polyester is dissolved in organic solvent, 0.5-1h is stood in baking oven, obtain degradable aliphatic polyester Mass concentration is 0.04-0.4g/mL solution A;

(2) degradable natural macromolecule is added into solution A, room temperature magnetic agitation 6-12h obtains degradable aliphatic polyester matter Measure degradable aliphatic polyester and the high molecular mass ratio of degradable natural in the solution B that concentration is 0.04-0.4g/mL, solution B For 10/90-50/50;

(3) antibacterials are added into solution B, room temperature magnetic agitation 6-12h, obtaining degradable natural polymer concentration is Antibacterials and degradable aliphatic polyester and degradable natural macromolecule gross mass in 0.04-0.4g/mL solution C, solution C Ratio be 1/100-30/100;

(4) degradable aliphatic polyester is dissolved in organic solvent, 0.5-1h is stood in baking oven, obtain degradable aliphatic polyester Mass concentration is 0.04-0.4g/mL solution D;

(5) degradable natural macromolecule is added in solution D, room temperature magnetic agitation 6-12h obtains degradable aliphatic polyester quality Concentration is 0.04-0.4g/mL solution E, and degradable aliphatic polyester is with the high molecular mass ratio of degradable natural in solution E 50/50-90/10;

(6) one or more facilitated in bone material are added into solution E, room temperature magnetic agitation 6-12h obtains degradable natural Polymer concentration is 0.04-0.4g/mL solution F, facilitates the gross mass of bone material and degradable natural high molecular in solution F Mass ratio is 1/100-40/100;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 100-600rpm, is spun Silk liquid flow rate is 0.5-3mL/h, voltage 7-30kV, receives apart from 8-30cm, spinning 0.5-30h, obtains thickness 50-200 μM electricity spinning fibre film;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), roller Slewing rate is 100-600rpm, and spinning solution flow rate is 0.5-3mL/h, voltage 7-30kV, is received apart from 8-30cm, spinning 0.5-30h, obtains the electrospun fibers film of 250-500 μm of thickness;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 2-7 days in fume hood, package sterilization.

Description

A kind of double-deck Bone Defect Repari membrane material and preparation method thereof

Technical field

The present invention relates to a kind of preparation method of double-deck Bone Defect Repari membrane material, belong to technical field of biological material, and in particular to one Kind of the fiber formed using degradable aliphatic polyester is as major matrix material and loads outer membrane and the loading of antibacterials Facilitate double-deck Bone Defect Repari membrane material that the inner layer film of bone material is composited and preparation method thereof.

Background technology

Cranial defect is clinically very common wound, and the repair process of bone is very very long, and it mainly includes hemotoncus With inflammatory phase, initial poroma reaction phase, Subchondral drilling phase and bone formation and reconstruction phase. Cell secretion is more during Bone Defect Repari Plant growth factor to play a role with different sequential, it is ensured that the reparation of Cranial defect.

Clinically, when Cranial defect occurs, best bet is to carry out bone collection, mainly including autologous bone transplanting, together Kind of allogenic bone transplantation and non-tissue repairing's art etc., wherein to bone defect healing effect most preferably autologous bone transplanting. But, from Body bone is taken can excessively bring new wound and complication to patient; Homogeneous allogenic bone transplantation can overcome part autologous bone transplanting The problem of bringing, but can be limited by organization factors and immunogenicity etc. when donor source, transplanting; Non- tissue repairing's art It is generally used for joint replacement surgery, subject matter is exactly the focus that can not be infected with the organizational integration of surrounding and then formation. For Cranial defect or tissue defect, the problem of preferable solution seeks to overcome three above aspect. Technical field of biological material is Solve this problem and provide potential selection.

As a preferable bone renovating bracket material, it is necessary to the requirement in terms of meeting following four:

(1) there is biocompatibility, osteoconductive, osteoinductive, support can be provided for normal cellular activity;

(2) biodegradable, can be that neoblastic grow into provides space and gradually replaced by new organization;

(3) certain mechanical property, can bear the stress during operative process and bone uptake;

(4) link up loose structure, is neoblastic generation transport nutrient and waste.

Electrostatic spinning technique is a kind of simple general-purpose method for preparing nanofiber, because its medicine load mode is simply easy OK, different medicines can be readily loaded into during electrostatic spinning in fiber, in addition, medicine is after being loaded into fiber Will not occur performance change, remain to keep its performance, can infect or promote the generation of bone for prevention of postoperative. Therefore, electrostatic Nanofiber medicine carrying membrane prepared by spinning has good potential applicability in clinical practice. Simultaneously at Cranial defect a variety of growth factors lack or It is that active deficiency is to influence the major reason of osteanagenesis, therefore, research is a kind of not only with anti-inflammation but also can to promote Bone Defect Repari Biomaterial be highly important. Simultaneously as anti-infectives mainly act on the surrounding tissue of inflammation, and facilitate bone thing Matter then needs to directly act on bone. In order to improve the utilization rate of medicine, both medicines are loaded respectively using double membrane structure It is a kind of effectively method.

What is be related in this patent facilitates bone material to refer mainly to hydroxyapatite, graphene oxide, CNT, tricresyl phosphate Calcium, icariin; Bio-vitric includes 45S5, apatite-wollastonite activity glass, machinable bioactivity glass; Growth The factor such as bone morphogenetic protein (BMP), fibroblast growth factor (FGF), TGF (TGF-B), blood platelet Derivative growth factor (PDGF), vascular endothelial growth factor (VEGF) and IGF (IGF). Wherein, it is raw Thing glass refers to by SiO₂, Na₂O, CaO and P₂O₅ The silicate glass constituted etc. basis. It has good bio-compatible Property, it can be combined closely with bone tissue. Due to its good rush bone formation performance and excellent processing characteristics, bio-vitric is being repaired There is wide prospect in terms of Cranial defect. However, bioglass material is harder crisp in itself, therefore be not suitable for independently luring as support Lead osteanagenesis. Bio-vitric can effectively be evaded by this as facilitating bone factor to be mixed into fiber by electrostatic spinning technique Individual problem. Meanwhile, the addition of bio-vitric can also effectively improve the mechanical performance of tunica fibrosa, greatly expand answering for material Use scope. Growth factor refers to that a class, by being combined with special, high affine cell-membrane receptor, adjusts cell growth and other The polypeptides matter of the manifold effects such as cell function. Growth factor has certain selectivity to variety classes cell, with facilitating The growth factor of bone effect can promote to generate substantial amounts of Gegenbaur's cell, suppress osteoclast. In treatment osteoporosis, femoral head Necrosis, arthritis, rheumatism and there is good effect caused by calcium deficiency in terms of disease.

Inflammation is occurred mainly in the tissue of surrounding materials caused by the infection in material implantation process, and is facilitated Bone material will then act on bone tissue, and the active position of the two is simultaneously differed. In order to improve the utilization ratio of medicine, curative effect is controlled in raising Really, two kinds of medicines can be added in different two membranes. Inner layer film presses close to bone, and addition promotes osmosis material; Outer layer Film presses close to tissue, adds antibacterial medicines. It because two layers of film component is close, therefore more can closely be combined together, be difficult Come off or separate.

The content of the invention

It is an object of the invention to provide a kind of preparation method of double-deck Bone Defect Repari membrane material, realize antibacterials and facilitate bone The targeted release of medicine, can promote bone defect healing, it is not necessary to second operation, may also suppress incident bacterium after defect occurs Sexuality dye and inflammation.

Double-deck Bone Defect Repari membrane material, it is characterized in that: With the degradable aliphatic polyester with biocompatibility and naturally may be used Degradation polymer is as matrix material, with loose structure; Containing the film combined closely for two layers in material, and add in inner layer film Plus facilitate bone component, outer membrane adds antibacterials, wherein:

Outer membrane is using degradable aliphatic polyester and the high molecular mixture of degradable natural as matrix material, wherein can Degrading aliphatic polyester is 10/90-50/50, antibacterials quality and matrix material with the high molecular mass ratio of degradable natural i.e. the ratio between high molecular gross mass of degradable aliphatic polyester and degradable natural is 1/100-30/100, and inner layer film is with degradable Aliphatic polyester and the high molecular mixture of degradable natural as matrix material, wherein degradable aliphatic polyester with it is degradable The mass ratio of natural polymer is 50/50-90/10, facilitates the quality and matrix material i.e. degradable aliphatic polyester of bone material It is 1/100-40/100, the knot containing overloading medicine slow-released system bone renovating material with the ratio between the high molecular gross mass of degradable natural Structure is characterized by loose structure, and averagely bridging aperture is 2-6 μm .

Further, the film that the material is combined closely by two layers is constituted; Inner layer film presses close to bone tissue, added with facilitating bone thing Matter; Outer membrane presses close to tissue, contains antibacterial medicines.

Further, degradable synthesized polymer includes: It is PLA, polycaprolactone, Poly(D,L-lactide-co-glycolide, poly-Lactic acid-caprolactone copolymer, poly lactic-co-glycolic acid-caprolactone copolymer one or more kinds of mixture therein; Can Degraded natural macromolecular material includes: One or both of type I collagen, gelatin, chitosan, starch, cellulose, elastin laminin Mixture above.

Further, the medicine A for being loaded into outer membrane matrix includes penicillins, cephalosporin class, Tetracyclines, chloramphenicol Class, macrolide, lincomycin, fluoroquinolones, nitro glyoxaline, polypeptide and quaternary ammonium salt antimicrobial; It is loaded into internal layer Film matrix facilitates bone material B hydroxyapatites, graphene oxide, CNT, tricalcium phosphate, icariin; Bio-vitric Include 4SS5, apatite-wollastonite activity glass, machinable bioactivity glass; Growth factor includes Bones morphology and occurs egg It is white BMP, fibroblast growth factor FGF, TGF-TGF-B, platelet derived growth factor PDGF, intravascular Skin cell growth factor VEGF and insulin-like growth factor IGF.

Further, it is made up of to control the degraded of membrane material the ectonexine matrix material of adjusting film materials double-layer membrane structure Speed; Outer membrane is after the bacterial infection and inflammation that initial stage easily occurs after suppressing Cranial defect generation, and degraded is complete; Inner layer film It will then be provided for Bone Defect Repari whole process and facilitate bone material, until being gone again after the completion of Bone Defect Repari degradable.

Described double-deck Bone Defect Repari membrane material preparation method for material has the following steps:

(1) degradable aliphatic polyester is dissolved in organic solvent, 0.5-1h is stood in baking oven, obtain degradable aliphatic Polyester mass concentration is 0.04-0.4g/mL solution A;

(2) degradable natural macromolecule is added into solution A, room temperature magnetic agitation 6-12h obtains degradable aliphatic adoption Ester mass concentration is degradable aliphatic polyester and the high molecular matter of degradable natural in 0.04-0.4g/mL solution B, solution B Amount is than being 10/90-50/50;

(3) antibacterials 1 are added into solution B, room temperature magnetic agitation 6-12h obtains degradable natural polymer concentration For 0.04-0.4g/mL solution C, antibacterials 1 and degradable aliphatic polyester and degradable natural macromolecule are total in solution C The ratio of quality is 1/100-30/100.

(4) degradable aliphatic polyester is dissolved in organic solvent, 0.5-1h is stood in baking oven, obtain degradable aliphatic Polyester mass concentration is 0.04-0.4g/mL solution D;

(5) degradable natural macromolecule is added in solution D, room temperature magnetic agitation 6-12h obtains degradable aliphatic polyester Mass concentration is degradable aliphatic polyester and the high molecular quality of degradable natural in 0.04-0.4g/mL solution E, solution E Than for 50/50-90/10;

(6) one or more facilitated in bone material 2 are added into solution E, room temperature magnetic agitation 6-12h obtains dropping Solve the gross mass and degradable aliphatic for facilitating bone material 2 in the solution F that natural polymer concentration is 0.04-0.4g/mL, solution F Adoption ester and the ratio of degradable natural macromolecule gross mass are 1/100-40/100.

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 100- 600rpm, spinning solution flow rate is 0.5-3mL/h, voltage 7-30kV, receives apart from 8-30cm, spinning 0.5-30h, obtains thickness The electricity spinning fibre film of 50-200 μm of degree;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 100-600rpm, and spinning solution flow rate is 0.5-3mL/h, voltage 7-30kV, is received apart from 8-30cm, Spinning 0.5-30h, obtains the electrospun fibers film of 250-500 μm of thickness;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 2-7 days in fume hood, package sterilization.

The present invention prepares nanofiber using the method for electrostatic spinning, but the invention is not restricted to electrostatic spinning, solution is poured Material and hydrogel of the preparations such as note, 3D printing etc. are suitable for the present invention.

Brief description of the drawings

Fig. 1 is double-deck Bone Defect Repari membrane material schematic diagram;

Fig. 2 is the electron microscopic picture of the double-deck Bone Defect Repari membrane material of embodiment 1;

Fig. 3 is the inhibition zone photo of embodiment 1-6 double-deck Bone Defect Repari membrane material, and model bacterium is staphylococcus aureus.

Embodiment

With reference to embodiment, the present invention will be further described, but the present invention is not limited to following examples.

Embodiment 1

(1) 4g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, polycaprolactone is obtained Mass concentration is 0.2g/mL solution A;

(2) add 4g gelatin into solution A, room temperature magnetic agitation 12h, obtain in solution B, solution B polycaprolactone with The mass ratio of gelatin is 50/50;

(3) 0.08g antibacterials Ciprofloxacin is added into solution B, room temperature magnetic agitation 6h obtains solution C, solution C Middle Ciprofloxacin and polycaprolactone and the ratio of gelatin gross mass are 1/100;

(4) 7.2g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, obtains gathering in oneself Ester mass concentration is 0.36/mL solution D;

(5) 0.8g gelatin is added into solution D, room temperature magnetic agitation 12h obtains polycaprolactone in solution E, solution E Mass ratio with gelatin is 90/10;

(6) 0.08g is added into solution E and facilitates bone material 45S5 bio-vitrics, room temperature magnetic agitation 6h obtains solution F, Bio-vitric and polycaprolactone and the ratio of gelatin gross mass are 1/100 in solution F;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 2 days in fume hood, package sterilization.

Embodiment 2

(1) 0.8g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, obtains gathering in oneself Ester mass concentration is 0.04g/mL solution A;

(2) 7.2g gelatin is added into solution A, room temperature magnetic agitation 12h obtains polycaprolactone in solution B, solution B Mass ratio with gelatin is 10/90;

(3) 2.4g antibacterials Ciprofloxacins are added into solution B, room temperature magnetic agitation 6h is obtained in solution C, solution C Ciprofloxacin and polycaprolactone and the ratio of gelatin gross mass are 30/100;

(4) 4g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, polycaprolactone is obtained Mass concentration is 0.2/mL solution D;

(5) add 4g gelatin into solution D, room temperature magnetic agitation 12h, obtain in solution E, solution E polycaprolactone with The mass ratio of gelatin is 50/50;

(6) 3.2g is added into solution E and facilitates bone material 45S5 bio-vitrics, room temperature magnetic agitation 6h obtains solution F, Bio-vitric and polycaprolactone and the ratio of gelatin gross mass are 40/100 in solution F;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 2 days in fume hood, package sterilization.

Embodiment 3

(1) 0.8g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, obtains gathering in oneself Ester mass concentration is 0.04g/mL solution A;

(2) 7.2g gelatin is added into solution A, room temperature magnetic agitation 12h obtains polycaprolactone in solution B, solution B Mass ratio with gelatin is 10/90;

(3) 0.08g antibacterials Ciprofloxacins are added into solution B, room temperature magnetic agitation 6h obtains solution C, solution C Middle Ciprofloxacin and polycaprolactone and the ratio of gelatin gross mass are 1/100;

(4) 7.2g polycaprolactone is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, obtains gathering in oneself Ester mass concentration is 0.36/mL solution D;

(5) 0.8g gelatin is added into solution D, room temperature magnetic agitation 12h obtains polycaprolactone in solution E, solution E Mass ratio with gelatin is 90/10;

(6) 3.2g is added into solution E and facilitates bone material bio-vitric, room temperature magnetic agitation 6h obtains solution F, solution F Middle bio-vitric and polycaprolactone and the ratio of gelatin gross mass are 40/100;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 2 days in fume hood, package sterilization.

Embodiment 4

(1) 4g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA quality is obtained Concentration is 0.2g/mL solution A;

(2) add 4g chitosan into solution A, room temperature magnetic agitation 12h, obtain in solution B, solution B PLA with The mass ratio of chitosan is 50/50;

(3) 0.08g antibacterials metronidazoles are added into solution B, room temperature magnetic agitation 6h is obtained in solution C, solution C Metronidazole and PLA and the ratio of chitosan gross mass are 1/100;

(4) 7.2g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA matter is obtained Measure the solution D that concentration is 0.36/mL;

(5) 0.8g chitosan is added into solution D, room temperature magnetic agitation 12h obtains PLA in solution E, solution E Mass ratio with chitosan is 90/10;

(6) 0.08g is added into solution E and facilitates bone material tricalcium phosphate, room temperature magnetic agitation 6h obtains solution F, solution Tricalcium phosphate and PLA and the ratio of chitosan gross mass are 1/100 in F;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 3 days in fume hood, package sterilization.

Embodiment 5

(1) 0.8g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA matter is obtained Measure the solution A that concentration is 0.04g/mL;

(2) 7.2g chitosan is added into solution A, room temperature magnetic agitation 12h obtains PLA in solution B, solution B Mass ratio with chitosan is 10/90;

(3) 2.4g antibacterials metronidazoles are added into solution B, room temperature magnetic agitation 6h obtains first in solution C, solution C Nitre azoles and PLA and the ratio of chitosan gross mass are 30/100;

(4) 4g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA quality is obtained Concentration is 0.2/mL solution D;

(5) add 4g chitosan into solution D, room temperature magnetic agitation 12h, obtain in solution E, solution E PLA with The mass ratio of chitosan is 50/50;

(6) 3.2g is added into solution E and facilitates bone material tricalcium phosphate, room temperature magnetic agitation 6h obtains solution F, solution F Middle tricalcium phosphate and PLA and the ratio of chitosan gross mass are 40/100;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

(9) after electrostatic spinning terminates, by spinning film, room temperature is placed 3 days in fume hood, package sterilization.

Embodiment 6

(1) 0.8g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA matter is obtained Measure the solution A that concentration is 0.04g/mL;

(2) 7.2g chitosan is added into solution A, room temperature magnetic agitation 12h obtains PLA in solution B, solution B Mass ratio with chitosan is 10/90;

(3) 0.08g antibacterials metronidazoles are added into solution B, room temperature magnetic agitation 6h is obtained in solution C, solution C Metronidazole and PLA and the ratio of chitosan gross mass are 1/100;

(4) 7.2g PLA is taken, adds in 20mL trifluoroethanol solvents, 1h is stood in an oven, PLA matter is obtained Measure the solution D that concentration is 0.36/mL;

(5) 0.8g chitosan is added into solution D, room temperature magnetic agitation 12h obtains PLA in solution E, solution E Mass ratio with chitosan is 90/10;

(6) 3.2g is added into solution E and facilitates bone material tricalcium phosphate, room temperature magnetic agitation 6h obtains solution F, solution F Middle tricalcium phosphate and PLA and the ratio of chitosan gross mass are 40/100;

(7) electrostatic spinning is carried out with solution C, using stainless steel drum as reception device, roller slewing rate is 300rpm, is spun Silk liquid flow rate is 2.5mL/h, voltage 28kV, is received apart from 10cm, spinning 8h;

(8) electrostatic spinning is carried out with solution F, with tunica fibrosa using stainless steel drum as reception device in the step (7), Roller slewing rate is 300rpm, and spinning solution flow rate is 2.5mL/h, voltage 28kV, receives apart from 10cm, spinning 8h, obtains To electricity spinning fibre film;

After electrostatic spinning terminates, by spinning film, room temperature is placed 3 days in fume hood, package sterilization.

Patent Citations (6)

Publication number	Priority date	Publication date	Assignee	Title
CN102277737A *	2011-04-27	2011-12-14	南昌大学	Preparation method and application of polycaprolactone/natural high-molecular composite porous scaffold
CN103007364A *	2012-12-20	2013-04-03	北京市意华健科贸有限责任公司	Aliphatic polyester double-layered asymmetric guided tissue regeneration membrane and preparation method thereof
CN103736153A *	2013-12-30	2014-04-23	北京市创伤骨科研究所	Single-layer and double-layer polycaprolactone-based guided tissue regeneration membranes and preparation method thereof
CN104474589A *	2014-12-23	2015-04-01	山东国际生物科技园发展有限公司	Guided tissue regeneration membrane as well as preparation method and application thereof
CN104524643A *	2014-11-26	2015-04-22	北京化工大学	Halloysite-nanotube-containing drug-loaded type guide tissue regeneration membrane and preparation method thereof
CN103948974B *	2013-12-30	2017-06-06	北京化工大学	Carry Types of Medicine guide tissue regeneration film and preparation method thereof

Family To Family Citations

* Cited by examiner, † Cited by third party

Cited By (24)

Publication number	Priority date	Publication date	Assignee	Title
CN107366040A *	2017-08-11	2017-11-21	广东富琳健康产业有限公司	A kind of fiber for graphene far-infrared magnet therapeutic knee-pad and preparation method thereof
CN107625995A *	2017-08-18	2018-01-26	北京市创伤骨科研究所	A kind of coaxial fiber Bone Defect Repari membrane material of multilayer and preparation method thereof
CN107693845A *	2017-08-08	2018-02-16	上海纳米技术及应用国家工程研究中心有限公司	The bilayer that ectonexine loads HA and VEGF respectively imitates periosteum material
CN107982579A *	2017-11-21	2018-05-04	上海纳米技术及应用	3D printing carries preparation method of the nano combined artificial bone of



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(56)对比文件

CN 103007364 A, 2013.04.03,

CN 101172164 A, 2008.05.07,

CN 102166378 A, 2011.08.31,

CN 101584885 A, 2009.11.25,

陈佳佳 等.纳米羟基磷灰石与聚己内酯复合电纺纤维膜.《功能高分子学报》.2012,第25卷(第1期),第40-46页.

审查员 樊培伟

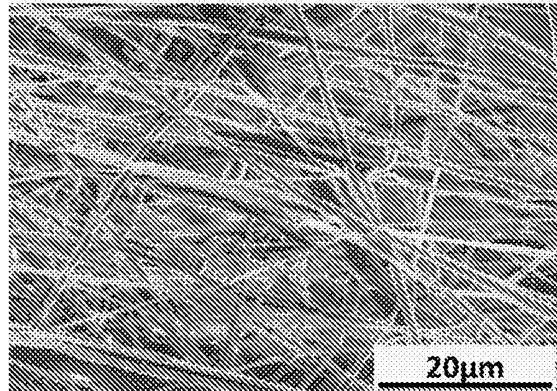
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(54)发明名称

载药型引导组织再生膜及其制备方法

(57)摘要

本发明是载药型引导组织再生膜及其制备方法。它是以聚己内酯和其他可降解脂肪族聚酯为原材料并加入抗菌药物，通过静电纺丝的方法制备单层引导组织再生膜；或以聚己内酯和可降解脂肪族聚酯材料为致密层，可降解天然高分子材料及生物活性粒子为疏松层，并在致密层加入抗菌药物，通过层层静电纺丝的方法制备成具有不用孔结构和生物活性的双层引导组织再生膜。本发明膜材料具有优异的生物相容性、机械性能和与组织修复进程一致的降解性能，能有效的阻止成纤维细胞等向组织缺损处的长入，同时促进组织的再生修复，不必二次手术取出，还可以有效的抑制手术后易发生的细菌性感染及炎症。可广泛用于引导组织再生、术后防粘连、药物缓释膜等医疗领域。



1. 一种载药型引导组织再生膜,其特征是:以聚己内酯作为主要基体材料,并含有抗菌药物,具有单层或双层多孔结构,其中:

(1) 单层膜以纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混作为基体材料,并添加抗菌药物;其中聚己内酯与可降解脂肪族聚酯的质量比为50/50-100/0,抗菌药物与聚合物的质量比为0.5/100-50/100,所述聚合物为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混物;结构特征为具有无规排列的纳米级纤维结构,平均搭桥孔径为2-6μm,纤维直径为200-1000nm,膜厚度为50-500μm;

(2) 双层膜由致密层和疏松层构成:

a) 致密层基体材料为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混材料,并添加抗菌药物,其中聚己内酯与可降解脂肪族聚酯的质量比为50/50-100/0,药物与聚合物的质量比为0.5/100-50/100,所述聚合物为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混物;结构特征为具有无规或网格状排列的纳米至微米级纤维结构,平均孔径为2-6μm,纤维直径为200nm-1200nm,膜厚度为25-250μm;

b) 疏松层基体材料为纯聚己内酯与无机生物活性粒子的共混材料,或聚己内酯与可降解天然高分子材料和无机生物活性粒子制备的共混复合材料,或者纯聚己内酯,或者聚己内酯与可降解天然高分子材料的共混复合材料;采用交联剂对可降解天然高分子材料进行交联;其中聚己内酯与可降解天然高分子材料的质量比为10/90-100/0,无机生物活性粒子与聚合物的质量比为0/100-50/100;疏松层结构特征为平均孔径5-100μm,纤维直径为200nm-7μm,厚度为25-250μm。

2. 根据权利要求1所述的一种载药型引导组织再生膜,其特征在于可降解脂肪族聚酯包括:聚己内酯、聚乳酸-己内酯共聚物、聚乳酸-羟基乙酸-己内酯共聚物其中的一种或一种以上混合物。

3. 根据权利要求1所述的一种载药型引导组织再生膜,其特征在于所述的可降解天然高分子材料包括:I型胶原、明胶、壳聚糖、淀粉、纤维素、弹性蛋白中的一种或一种以上混合物。

4. 根据权利要求1所述的一种载药型引导组织再生膜,其特征在于所述的抗菌药物,包含青霉素类、头孢霉素类、四环素类、氯霉素类、大环内脂类、林可霉素和克林霉素、氟喹诺酮类及硝基咪唑类抗菌药。

5. 权利要求1所述的一种载药型引导组织再生膜,其特征在于无机生物活性粒子包括:颗粒尺寸为1-100nm的羟基磷灰石、β-磷酸三钙及生物玻璃粒子中的一种或一种以上混合物。

6. 根据权利要求1所述的一种载药型引导组织再生膜,其特征在于所述的交联剂包括:甲醛、戊二醛、京尼平、EDC/NHS中的一种。

7. 根据权利要求1所述的一种载药型引导组织再生膜的制备方法,其特征在于所述的单层引导组织再生膜的制备方法含有如下步骤:

(1) 将聚己内酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液A;

(2) 将可降解脂肪族聚酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液B;

(3) 按一定体积比例将溶液A与溶液B混合，并加入一定质量的抗菌药物，室温磁力搅拌12h，得到聚合物质量浓度为0.04-0.2g/mL的溶液C，溶液C中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0，抗菌药物与聚合物的质量比为0.5/100-50/100；

(4) 用溶液C进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-10mL/h，电压7-20kV，接收距离8-30cm，纺丝0.5-30h，得到厚度50-500μm的电纺丝纤维膜；

(5) 静电纺丝结束后，将纺丝膜在通风橱中室温放置2-7天，包装消毒。

8. 根据权利要求1所述的一种载药型引导组织再生膜的制备方法，其特征在于所述的双层引导组织再生膜的制备方法含有如下步骤：

(1) 将聚己内酯溶于有机溶剂中，室温磁力搅拌6-24h，得到质量浓度为0.04-0.2g/mL的溶液A；

(2) 将可降解脂肪族聚酯溶于有机溶剂中，室温磁力搅拌6-24h，得到质量浓度为0.04-0.2g/mL的溶液B；

(3) 将可降解天然高分子材料溶于有机溶剂中，室温磁力搅拌6-24h，得到质量浓度为0.04-0.2g/mL的溶液C；

(4) 按一定体积比例将溶液A与溶液B混合，并加入一定质量的抗菌药物，室温磁力搅拌12h，得到聚合物质量浓度为0.04-0.2g/mL的溶液D，溶液D中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0，抗菌药物与聚合物的质量比为0.5/100-50/100；

(5) 在溶液D中加入一定量的生物活性粒子，超声30min-2h，室温下磁力搅拌12h，得到质量浓度为0.04-0.2g/mL的溶液E，其中无机生物活性粒子与聚合物的质量比为0/100-50/100；

(6) 按一定体积比例将溶液A与溶液C充分混合，室温磁力搅拌12h，得到聚合物质量浓度为0.04-0.2g/mL的溶液F，溶液F中聚己内酯与可降解天然高分子材料的质量比10/90-100/0；

(7) 在溶液F中加入一定量的生物活性粒子，超声30min-2h，室温下磁力搅拌12h，得到质量浓度为0.04-0.2g/mL的溶液G，其中无机生物活性粒子与聚合物的质量比为0/100-50/100；

(8) 用溶液D进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-10mL/h，电压7-20kV，接收距离8-30cm，纺丝0.5-15h，得到厚度25-250μm的致密层膜；

(9) 在致密层膜的基础上，用溶液E或溶液G进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-10mL/h，电压7-20kV，接收距离8-30cm，纺丝0.5-15h，得到厚度25-250μm的疏松层膜；

(10) 静电纺丝结束后，将电纺丝纤维膜浸泡在浓度为0.01%-3%的交联剂乙醇溶液中交联10min-12h，浸泡结束后在去离子水中浸洗5-10次，将纺丝膜在通风橱中室温放置2-7天，包装消毒。

9. 根据权利要求1所述的一种载药型引导组织再生膜的制备方法，其特征在于所述的双层引导组织再生膜的另一种制备方法含有如下步骤：

(1) 将聚己内酯溶于有机溶剂中，室温磁力搅拌6-24h，得到质量浓度为0.04-0.2g/mL

的溶液A；将可降解脂肪族聚酯溶于有机溶剂中，室温磁力搅拌6-24h，得到质量浓度为0.04-0.2g/mL的溶液B；按一定体积比例将溶液A与溶液B混合，并加入一定质量的抗菌药物，室温磁力搅拌12h，得到聚合物质量浓度为0.04-0.2g/mL的溶液C，溶液C中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0，抗菌药物与聚合物的质量比为0.5/100-50/100；用溶液C进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-10mL/h，电压7-20kV，接收距离8-30cm，纺丝0.5-30h，得到厚度25-250μm的致密层膜；

(2) 将致密层膜铺在平底容器底部，将疏松层的组成材料溶解于有机溶剂中，然后浇铸在致密层膜的表面，将其在-60℃--20℃下冷冻6-12h后，放入真空干燥器中真空干燥4-12h，即得双层引导组织再生膜。

10. 根据权利要求6、7或8所述的一种载药型引导组织再生膜的制备方法，其特征在于所述的有机溶剂为六氟异丙醇、三氟乙醇、三氯甲烷、甲醇、二氯甲烷、N,N'-二甲基甲酰胺中的一种或几种混合溶剂。

载药型引导组织再生膜及其制备方法

技术领域

[0001] 本发明属于生物材料领域,具体涉及一种以聚己内酯作为主要基体材料并加载抗菌药物的引导组织再生膜材料及其制备方法。

背景技术

[0002] 由于长期缺牙、牙周病、外伤、腭裂和颌面部肿瘤造成的骨缺损,往往无法进行义齿修复和种植体植入,必须采用骨修复重建。应用生物膜引导牙周组织再生的引导组织再生术(guided tissue regeneration,GTR)被认为是近二十年来最有效的口腔骨缺损修复技术,已被较广泛地用于牙周临床。引导组织再生技术是在牙周结缔组织瓣与牙根间放置一种膜,作为屏障,阻止牙龈在结缔组织内生长,选择性引导再生潜能的细胞向牙根表面增殖,产生新牙骨质及新牙周韧带。引导组织再生膜的类型和性能成为制约引导组织再生技术发展的关键因素。

[0003] 虽然GTR技术可取得长期稳定的疗效,但由于术后膜暴露、残留在牙周病损中的细菌等因素引发的感染将影响其牙周新组织的获得。目前对于术后抗感染还主要采取全身系统用药,但药物利用率较低,且容易引起胃肠道副反应。局部释药可以增强治疗效果,降低不良反应。然而目前还没有抑菌型引导组织再生膜产品问世。在研的新型引导组织再生膜也很少有关于抑菌性能的研究。

[0004] 静电纺丝技术是一种制备纳米纤维的简单通用方法,由于其药物加载方式简单易行,在静电纺丝或纺丝后处理过程中不同的药物及生物大分子很容易加载到纤维内部和表面,另外,抗菌药物在载入纤维中后不会发生性能变化,仍能保持其抗菌性能,可以用来预防术后感染。因此,电纺丝制备的纳米纤维载药膜具有良好的临床应用前景。

发明内容

[0005] 一种载药型引导组织再生膜,其特征是:以聚己内酯作为主要基体材料,并含有抗菌药物,具有单层或双层多孔结构,其中:

[0006] (1) 单层膜以纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混作为基体材料,并添加抗菌药物;其中聚己内酯与可降解脂肪族聚酯的质量比为50/50-100/0,抗菌药物与聚合物的质量比为0.5/100-50/100,所述聚合物为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混物;结构特征为具有无规排列的纳米级纤维结构,平均搭桥孔径为2-6μm,纤维直径为200-1000nm,膜厚度为50-500μm;

[0007] (2) 双层膜由致密层和疏松层构成:

[0008] a) 致密层基体材料为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混材料,并添加抗菌药物,其中聚己内酯与可降解脂肪族聚酯的质量比为50/50-100/0,药物与聚合物的质量比为0.5/100-50/100,所述聚合物为纯聚己内酯或聚己内酯与可降解脂肪族聚酯共混物;结构特征为具有无规或网格状排列的纳米至微米级纤维结构,平均孔径为2-6μm,纤维直径为200nm-1200nm,膜厚度为25-250μm;

[0009] b) 疏松层基体材料为纯聚己内酯与无机生物活性粒子的共混材料,或聚己内酯与可降解天然高分子材料和无机生物活性粒子制备的共混复合材料,采用交联剂对可降解天然高分子材料进行交联;其中聚己内酯与可降解天然高分子材料的质量比为10/90-100/0,无机生物活性粒子与聚合物的质量比为0/100-50/100;疏松层结构特征为平均孔径5-100μm,纤维直径为200nm-7μm,厚度为25-250μm。

[0010] 可降解脂肪族聚酯包括:聚乳酸、聚己内酯、聚乳酸-羟基乙酸共聚物、聚乳酸-己内酯共聚物、聚乳酸-羟基乙酸-己内酯共聚物其中的一种或一种以上混合物。

[0011] 所述的可降解天然高分子材料包括:I型胶原、明胶、壳聚糖、淀粉、纤维素、弹性蛋白中的一种或一种以上混合物;

[0012] 所述的抗菌药物,包含青霉素类、头孢霉素类、四环素类、氯霉素类、大环内脂类、林可霉素和克林霉素、氟喹诺酮类及硝基咪唑类抗菌药。

[0013] 所述的无机生物活性粒子包括:颗粒尺寸为1-100nm的羟基磷灰石、β-磷酸三钙及生物玻璃粒子中的一种或一种以上混合物。

[0014] 述的交联剂包括:甲醛、戊二醛、京尼平、EDC/NHS中的一种;

[0015] 单层引导组织再生膜的制备方法含有如下步骤:

[0016] (1) 将聚己内酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液A;

[0017] (2) 将可降解脂肪族聚酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液B;

[0018] (3) 按一定体积比例将溶液A与溶液B混合,并加入一定质量的抗菌药物,室温磁力搅拌12h,得到聚合物质量浓度为0.04-0.2g/mL的溶液C,溶液C中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0,抗菌药物与聚合物的质量比为0.5/100-50/100;

[0019] (1) 用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-10mL/h,电压7-20kV,接收距离8-30cm,纺丝0.5-30h,得到厚度50-500μm的电纺丝纤维膜;

[0020] (5) 静电纺丝结束后,将纺丝膜在通风橱中室温放置2-7天,包装消毒。

[0021] 双层引导组织再生膜的制备方法含有如下步骤:

[0022] (1) 将聚己内酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液A;

[0023] (2) 将可降解脂肪族聚酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液B;

[0024] (3) 将可降解天然高分子材料溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液C;

[0025] (4) 按一定体积比例将溶液A与溶液B混合,并加入一定质量的抗菌药物,室温磁力搅拌12h,得到聚合物质量浓度为0.04-0.2g/mL的溶液D,溶液D中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0,抗菌药物与聚合物的质量比为0.5/100-50/100;

[0026] (5) 在溶液D中加入一定量的生物活性粒子,超声30min-2h,室温下磁力搅拌12h,得到质量浓度为0.04-0.2g/mL的溶液E,其中无机生物活性粒子与聚合物的质量比为0/100-50/100;

[0027] (6) 按一定体积比例将溶液A与溶液C充分混合,室温磁力搅拌12h,得到聚合物质量浓度为0.04-0.2g/mL的溶液F,溶液F中聚己内酯与可降解天然高分子材料的质量比10/90-100/0;

[0028] (7) 在溶液F中加入一定量的生物活性粒子,超声30min-2h,室温下磁力搅拌12h,得到质量浓度为0.04-0.2g/mL的溶液G,其中无机生物活性粒子与聚合物的质量比为0/100-50/100;

[0029] (8) 用溶液D进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-10mL/h,电压7-20kV,接收距离8-30cm,纺丝0.5-15h,得到厚度25-250μm的致密层膜;

[0030] (9) 在致密层膜的基础上,用溶液E或溶液G进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-10mL/h,电压7-20kV,接收距离8-30cm,纺丝0.5-15h,得到厚度25-250μm的疏松层膜;

[0031] (10) 静电纺丝结束后,将电纺丝纤维膜浸泡在浓度为0.01%-3%的交联剂乙醇溶液中交联10min-12h,浸泡结束后在去离子水中浸洗5-10次,将纺丝膜在通风橱中室温放置2-7天,包装消毒。

[0032] 双层引导组织再生膜的另一种制备方法含有如下步骤:

[0033] (1) 将聚己内酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液A;将可降解脂肪族聚酯溶于有机溶剂中,室温磁力搅拌6-24h,得到质量浓度为0.04-0.2g/mL的溶液B;按一定体积比例将溶液A与溶液B混合,并加入一定质量的抗菌药物,室温磁力搅拌12h,得到聚合物质量浓度为0.04-0.2g/mL的溶液C,溶液C中聚己内酯与可降解脂肪族聚酯的质量比50/50-100/0,抗菌药物与聚合物的质量比为0.5/100-50/100;用溶液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为100-600rpm,纺丝液流动速率为0.5-10mL/h,电压7-20kV,接收距离8-30cm,纺丝0.5-30h,得到厚度25-250μm的致密层膜;

[0034] (2) 将致密层膜铺在平底容器底部,将疏松层的组成材料溶解于有机溶剂中,然后浇铸在致密层膜的表面,将其在-60℃--20℃下冷冻6-12h后,放入真空干燥器中真空干燥4-12h,即得双层引导组织再生膜。

[0035] 所述的有机溶剂为六氟异丙醇、三氟乙醇、三氯甲烷、甲醇、二氯甲烷、N,N'-二甲基甲酰胺中的一种或几种混合溶剂;

[0036] 它是以聚己内酯和其他可降解脂肪族聚酯为主要原材料并加入抗菌药物,通过静电纺丝的方法制备单层引导组织再生膜;或以聚己内酯和可降解脂肪族聚酯材料为致密层,可降解天然高分子材料及生物活性粒子为疏松层,并在致密层加入抗菌药物,通过层层静电纺丝的方法制备成具有不用孔结构和生物活性的双层引导组织再生膜。本发明膜材料具有优异的生物相容性、机械性能和与组织修复进程一致的降解性能,能有效的阻止成纤维细胞等向组织缺损处的长入,同时促进组织的再生修复,不必二次手术取出,还可以有效的抑制手术后易发生的细菌性感染及炎症。可广泛用于引导组织再生、术后防粘连、药物缓释膜等医疗卫生领域。

附图说明

[0037] 图1是本发明方法所制备的聚己内酯载不同量甲硝唑(1%-40%质量比)的单层引导组织再生膜的相关实施效果图,图片中样品的命名方式为:P1表示纺丝PCL纳米纤维膜中MNA与PCL的质量比为1%,P5表示纺丝PCL纳米纤维膜中MNA与PCL的质量比为5%,其他样品类推。

- [0038] (a) 是上述单层纺丝纤维膜SEM照片。
- [0039] (b) 是上述单层纺丝纤维膜湿态下的应力-应变曲线。
- [0040] (c) 是上述单层纺丝纤维膜水接触角示意图。
- [0041] (d) 是上述单层纺丝纤维膜的载药率表格。
- [0042] (e) 是上述单层纺丝纤维膜的药物释放曲线。
- [0043] (f) 是上述单层纺丝纤维膜体外降解30天的质量损失图。
- [0044] (g) 是上述单层纺丝纤维膜降解30天后的SEM图。
- [0045] (h) 是上述单层纺丝纤维膜细胞毒性结果柱状图(细胞毒性检测采用L929细胞,RGR表示细胞的相对增殖率)。
- [0046] (i) 是上述单层纺丝纤维膜细胞毒性实验中,细胞在材料的浸提液中生长24h时的状态,表明材料的浸提液对细胞的生长和形貌没有影响。
- [0047] (j) 是L929细胞在上述单层纺丝纤维膜上生长不同时间时的O.D值,表明随着时间的延长,细胞可以在材料上粘附增殖。
- [0048] (k) 是上述单层纺丝纤维膜的生物相容性能——L929小鼠成纤维细胞在纤维膜上增殖7天的SEM细胞形貌图。
- [0049] (1) 是上述单层纺丝纤维膜中,载药率为30%时,纤维膜在兔子体内埋植不同时间后的组织切片图,表明材料在体内具有很好的生物相容性,不会引起明显的免疫反应,无炎症反应和感染的现象发生。
- [0050] 图2是本发明所制备的以聚己内酯与聚乳酸共混载甲硝唑10%的单层纤维膜照片。
- [0051] 图3是本发明以聚己内酯与明胶共混载甲硝唑30%的单层纤维膜照片。
- [0052] 图4是本发明以聚己内酯载氯霉素20%为致密层,聚己内酯和明胶5:5共混为疏松层,制备的双层纤维膜照片(a为致密层一侧,b为疏松层一侧)。
- [0053] 图5是本发明以聚己内酯载盐酸四环素40%为致密层,聚己内酯和明胶5:5共混,纳米羟基磷灰石与聚合物质量比为20%为疏松层制备的双层纤维膜照片(a为致密层一侧,b为疏松层一侧)。

具体实施方式

- [0054] 下面通过实施例进一步说明本发明,但本发明并不限于这些实例。
- [0055] 实施例1
- [0056] 1. 将2g聚己内酯与0.2g甲硝唑溶于7.2g DMF与10.8g DCM的混合溶剂中,室温磁力搅拌12h,得到聚己内酯浓度为10%(w/w)、甲硝唑与聚己内酯质量比为10%的纺丝液。
- [0057] 2. 取溶液进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转速为200rpm,电压10KV,接收距离为16cm,纺丝液进液速率4mL/h,纺丝5h。得到厚度为250μm左右的单层引导组织再生膜。
- [0058] 3. 将得到的纤维膜置于室温下通风橱中干燥72h,保证残余溶剂充分挥发。

[0059] 实施例2

[0060] 1、将聚己内酯与甲硝唑溶于DCM/DMF=7:3的混合有机溶剂中,室温磁力搅拌12h,得到聚己内酯浓度为20%,甲硝唑与聚己内酯的质量比为50%的纺丝液;

[0061] 2、室温进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为600rpm,纺丝流动速率为3mL/h,电压12kV,接收距离15cm,纺丝5h,得到厚度200μm左右的单层引导组织再生膜。

[0062] 3、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0063] 实施例3

[0064] 1、将1.5g聚己内酯与0.3g盐酸四环素溶于13.5g三氟乙醇中,室温磁力搅拌12h,得到溶液A;

[0065] 2、将1.5g聚乳酸与0.3g盐酸四环素溶于13.5g三氟乙醇中,室温磁力搅拌12h,得到溶液B;

[0066] 3、将溶液A与溶液B混合,磁力搅拌12h,得到聚己内酯与聚乳酸的质量比为1:1,聚合物浓度为10%,药物与聚合物质量比为20%的纺丝液C;

[0067] 4、室温用纺丝液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为200rpm,纺丝流动速率为2mL/h,电压13kV,接收距离20cm,纺丝10h,得到厚度250μm左右的单层电纺丝纤维膜。

[0068] 5、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0069] 实施例4

[0070] 1、将聚己内酯与克林霉素溶于六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯浓度为6%,克林霉素与聚己内酯质量比为30%的溶液A;

[0071] 2、将胶原溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为6%的溶液B;

[0072] 3、将溶液A与溶液B混合,磁力搅拌6h,得到胶原与聚己内酯的质量比为1:1,聚合物浓度为6%,药物与聚合物的质量比为30%的纺丝液C;

[0073] 4、室温用纺丝液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝流动速率1mL/h,电压10kV,接收距离15cm,纺丝20h,得到厚度250μm左右的电纺丝膜;

[0074] 5、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0075] 实施例5

[0076] 1、将聚己内酯溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为8%的溶液A;

[0077] 2、将明胶溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为8%的溶液B;

[0078] 3、将溶液A与溶液B混合,磁力搅拌6h,得到明胶与聚己内酯的质量比3:7,聚合物浓度为8%的纺丝液C;

[0079] 4、将聚己内酯与甲硝唑溶于六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯浓度为10%,甲硝唑与聚己内酯质量比为40%的纺丝液D;

[0080] 5、室温用纺丝液C进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝流动速率2mL/h,电压10kV,接收距离15cm,纺丝10h,得到厚度为250μm左右的电纺丝疏松层膜;

[0081] 6、更换纺丝液D继续进行静电纺丝于步骤5所纺的纤维膜上,纺丝条件为滚筒转动

速率为200rpm,纺丝液流动速率为4mL/h,电压12kV,接收距离15cm,纺丝5h,得到总厚度500 μm 左右的双层引导组织再生膜。

[0082] 7、将纺丝纤维膜置于浓度为0.5%的京尼平的乙醇溶液中,使明胶发生交联,交联反应30min后,将膜在去离子水中浸洗10次,洗去未反应的京尼平及乙醇溶剂。

[0083] 8、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0084] 实施例6

[0085] 1、将聚己内酯溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为6%的溶液A;

[0086] 2、将聚乳酸溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为6%的溶液B;

[0087] 3、将壳聚糖溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为6%的溶液C;

[0088] 4、将溶液A与溶液B混合,并加入甲硝唑,磁力搅拌12h,得到聚乳酸与聚己内酯的质量比4:6,聚合物浓度为6%,药物与聚合物的质量比为40%的纺丝液D;

[0089] 5、将溶液A与溶液C混合,磁力搅拌12h,得到壳聚糖与聚己内酯的质量比5:5,聚合物浓度为6%的纺丝液E;

[0090] 6、室温用纺丝液D进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为500rpm,纺丝液流动速率3mL/h,电压12kV,接收距离18cm,纺丝5h,得到厚度为200 μm 左右的致密层膜;

[0091] 7、更换纺丝液E继续进行静电纺丝于步骤6所纺的纤维膜上,纺丝条件为滚筒转动速率为200rpm,纺丝液流动速率为1mL/h,电压12kV,接收距离18cm,纺丝15h,得到总厚度400 μm 左右的双层引导组织再生膜。

[0092] 8、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0093] 实施例7

[0094] 1、将聚己内酯溶于六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯浓度为6%的溶液A;

[0095] 2、将聚己内酯与头孢霉素溶于六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯浓度为10%,头孢霉素与聚己内酯的质量比为50%的溶液B;

[0096] 3、将明胶溶于六氟异丙醇中,室温磁力搅拌12h,得到浓度为6%的溶液C;

[0097] 4、将溶液A与溶液C混合,并加入一定质量的纳米羟基磷灰石,超声1h,磁力搅拌12h,得到明胶与聚己内酯的质量比5:5,聚合物浓度为6%,纳米羟基磷灰石与聚合物的质量比为20%的纺丝液D;

[0098] 5、室温用纺丝液B进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率1.5mL/h,电压10kV,接收距离18cm,纺丝10h,得到厚度200 μm 左右的电纺丝致密层膜;

[0099] 6、更换纺丝液D继续进行静电纺丝于步骤5所纺的纤维膜上,纺丝条件为滚筒转动速率为300rpm,纺丝液流动速率为1mL/h,电压13kV,接收距离18cm,纺丝15h,得到总厚度400 μm 左右的双层引导组织再生膜。

[0100] 7、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0101] 实施例8

[0102] 1、将聚己内酯溶于六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯浓度为8%的溶液A;

[0103] 2、将聚羟基乙酸溶于六氟异丙醇中,室温磁力搅拌12h,得到聚羟基乙酸浓度为8%的溶液B;

[0104] 3、将壳聚糖溶于六氟异丙醇中,室温磁力搅拌12h,得到壳聚糖浓度为8%的溶液C;

[0105] 4、将溶液A与B混合,并加入一定量的氯霉素,磁力搅拌12h,得到聚羟基乙酸与聚己内酯的质量比3:7,聚合物浓度为8%,氯霉素与聚合物的质量比为30%的纺丝液D;

[0106] 5、将溶液A与溶液C混合,并加入一定质量的生物活性玻璃,磁力搅拌12h,得到壳聚糖与聚己内酯的质量比4:6,聚合物浓度为8%,生物活性玻璃与聚合物的质量比为30%的纺丝液E;

[0107] 6、室温用纺丝液D进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为300rpm,纺丝液流动速率为2mL/h,电压12kV,接收距离18cm,纺丝5h,得到厚度150μm左右的电纺丝致密层膜;

[0108] 7、更换纺丝液E继续进行静电纺丝于步骤6所纺的纤维膜上,纺丝条件为滚筒转动速率为300rpm,纺丝液流动速率为1mL/h,电压12kV,接收距离18cm,纺丝10h,得到总厚度为300μm左右的双层引导组织再生膜。

[0109] 8、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

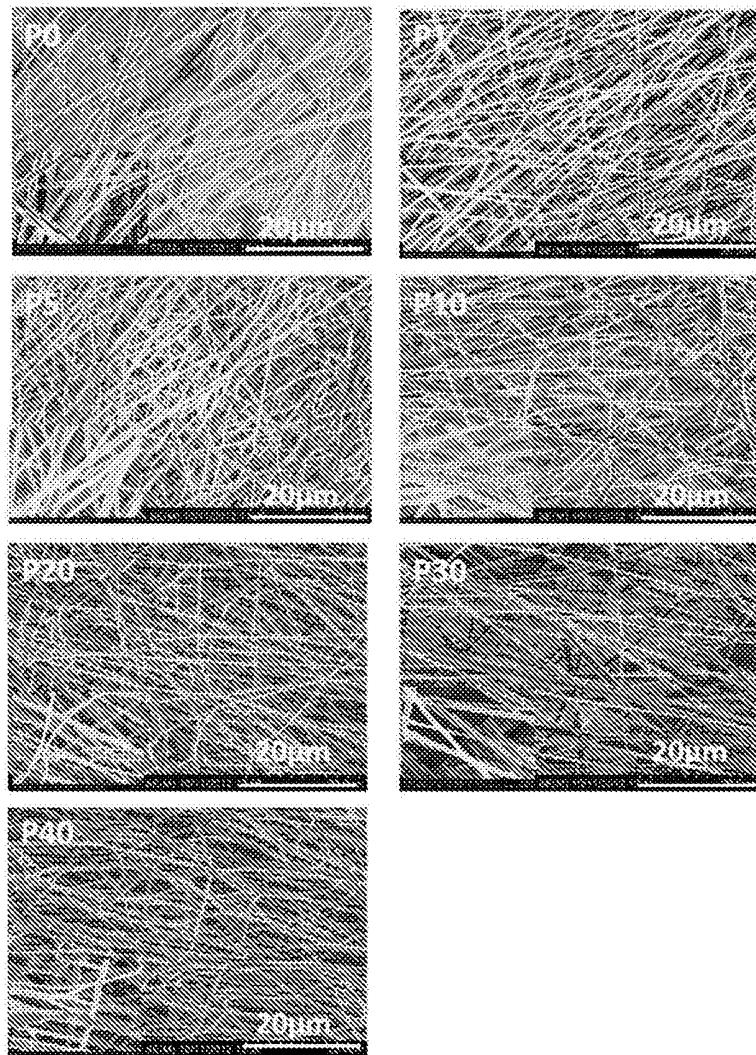
[0110] 实施例9

[0111] 1、将聚己内酯和头孢霉素溶于DCM/DMF=6:4的混合有机溶剂中,室温磁力搅拌12h,得到聚己内酯浓度为10%,头孢霉素与聚己内酯的质量比为40%的纺丝液;

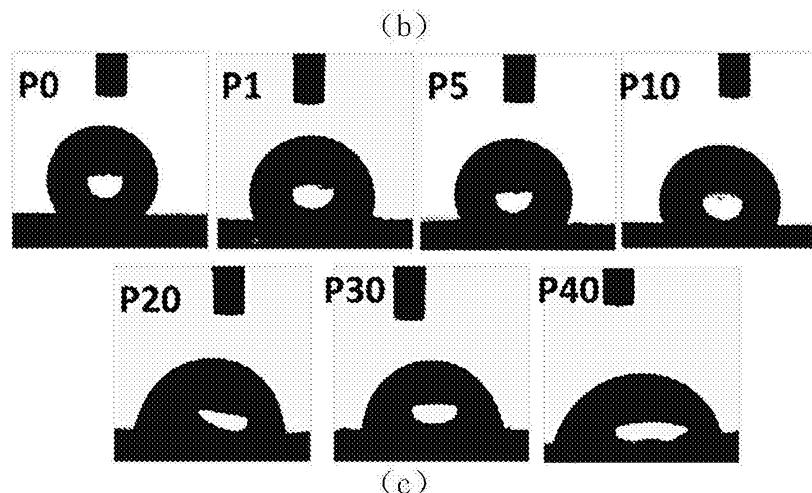
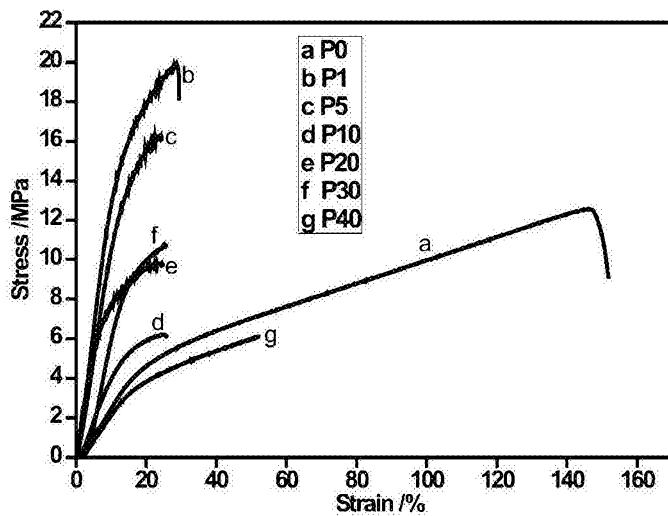
[0112] 2、室温用纺丝液进行静电纺丝,以不锈钢滚筒为接收装置,滚筒 转动速率为500rpm,纺丝液流动速率为3mL/h,电压12kV,接收距离15cm,纺丝5h,得到厚度200μm左右的电纺丝纤维膜。

[0113] 3、将得到的纤维膜置于室温下通风橱中干燥72h,使残余溶剂充分挥发。

[0114] 4、将上述纤维膜平铺于培养皿底部,在其上浇铸浓度为20%(w/w)的明胶水溶液,-40℃冷冻干燥12h,得到双层膜。



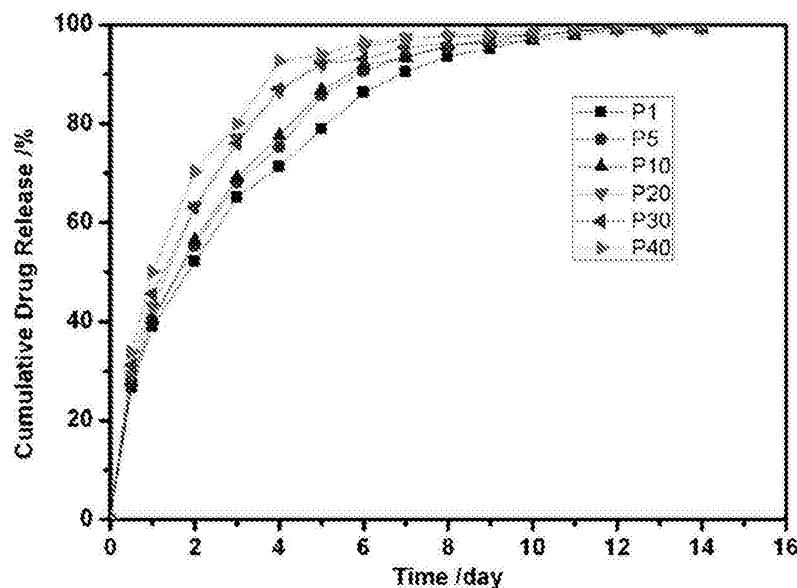
(a)



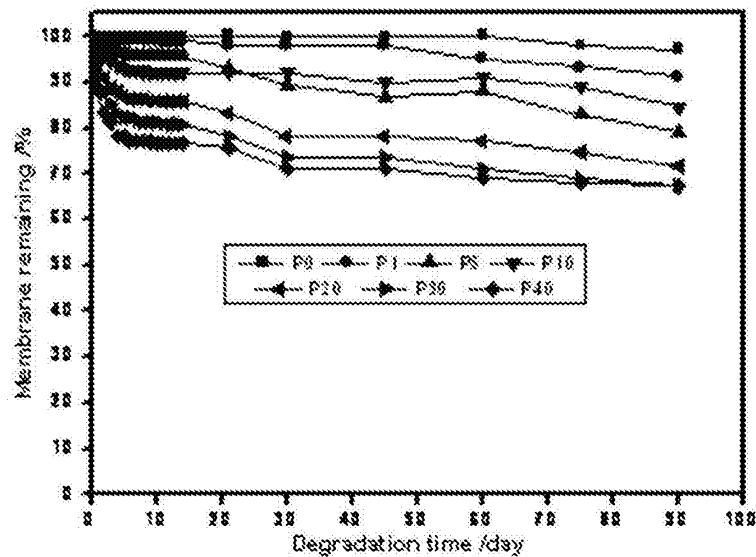
Sample	Drug encapsulation efficiency (%)
P1	92.3
P5	89.2

P10	86.5
P20	84.7
P30	83.2
P40	81.7

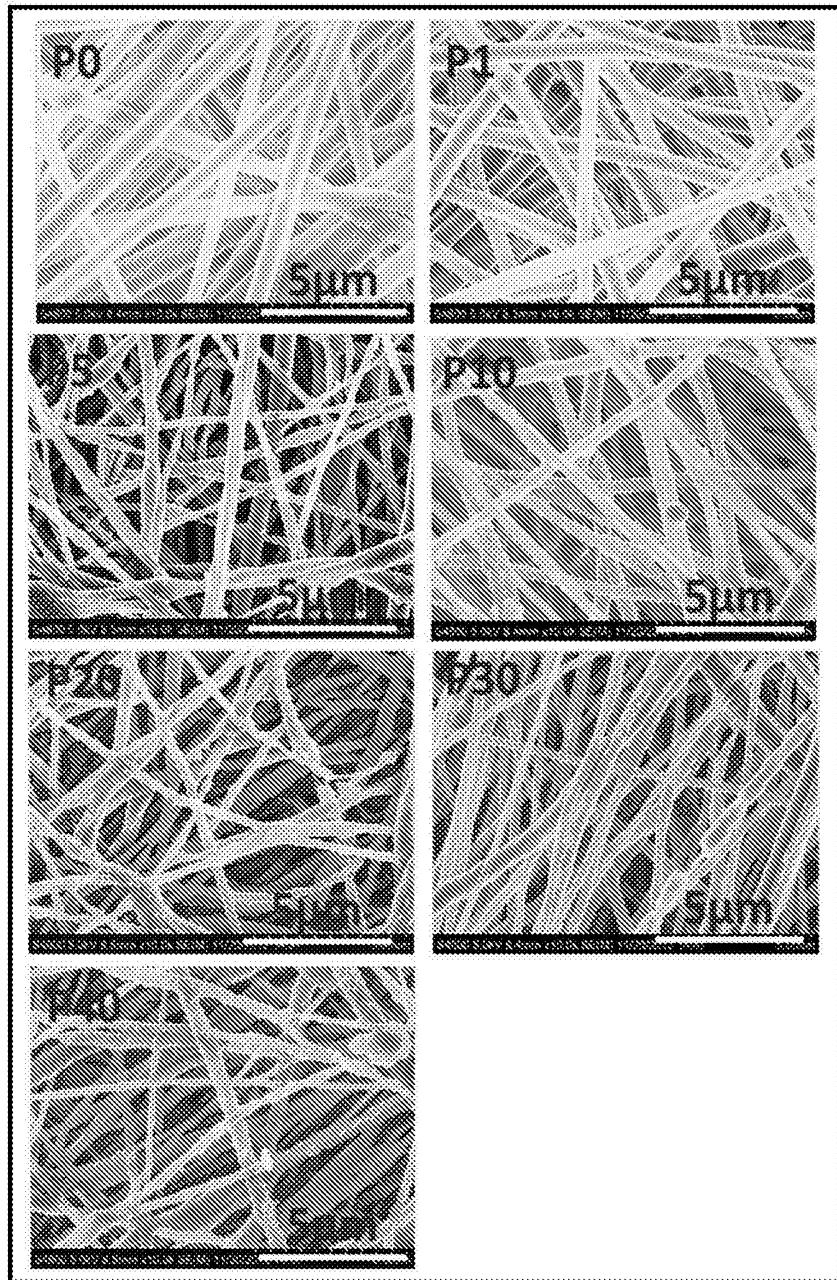
(d)



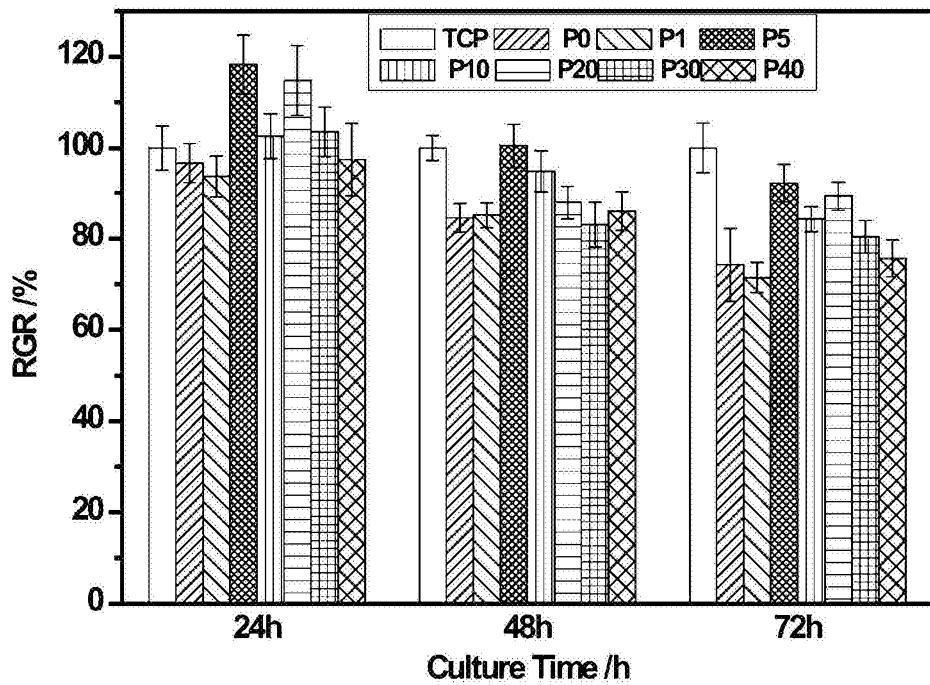
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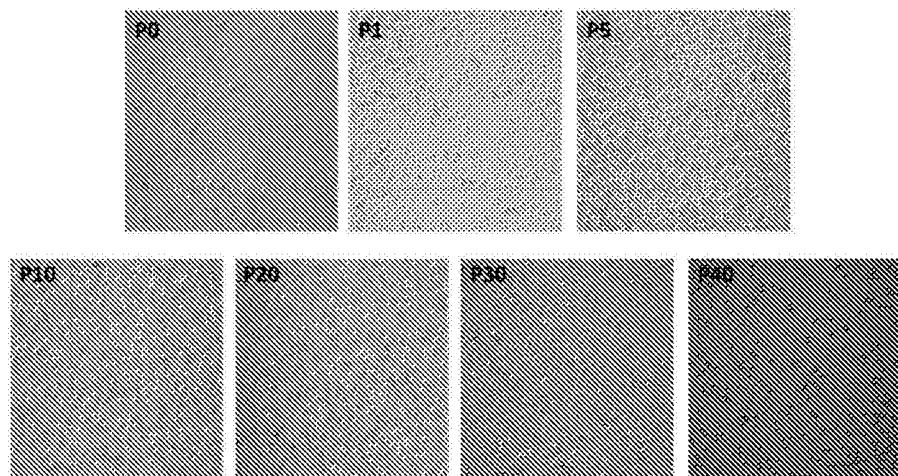
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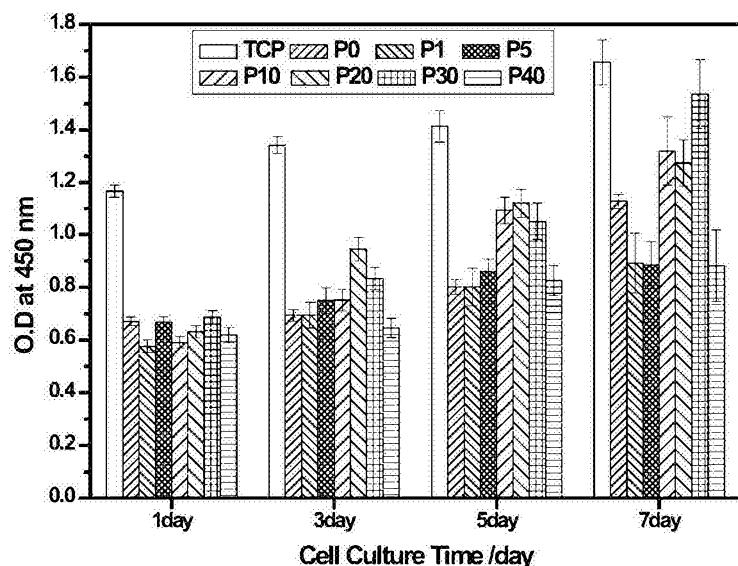
(g)



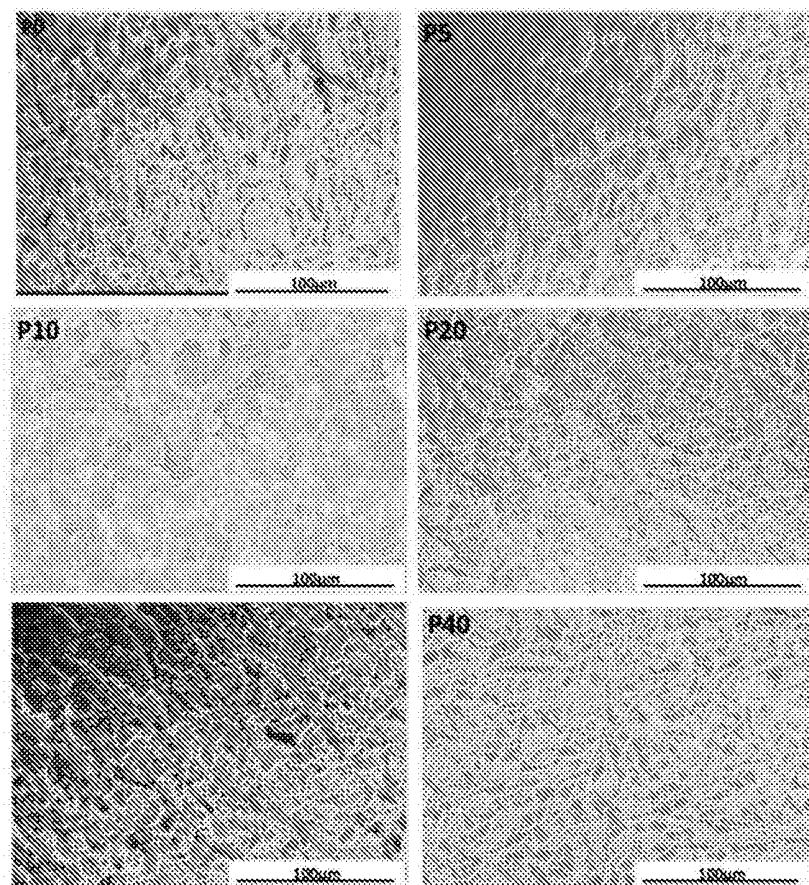
(h)



(i)



(j)



(k)

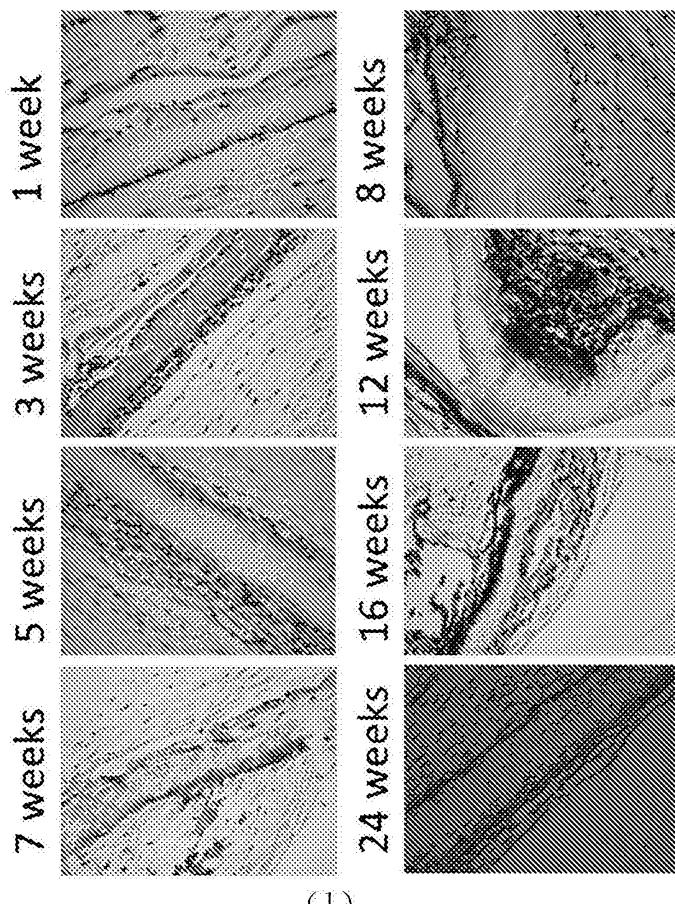


图1

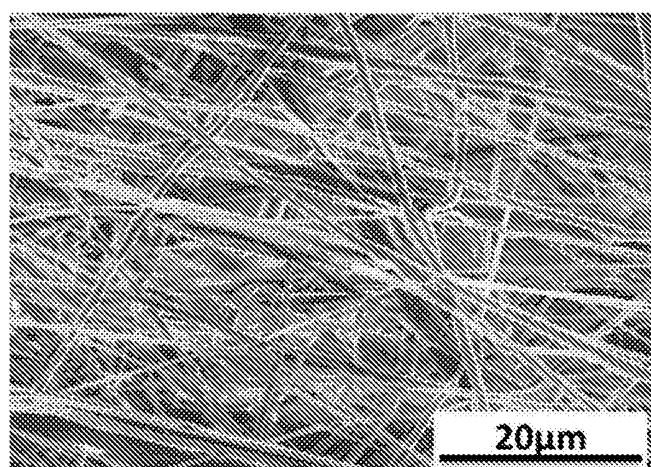


图2

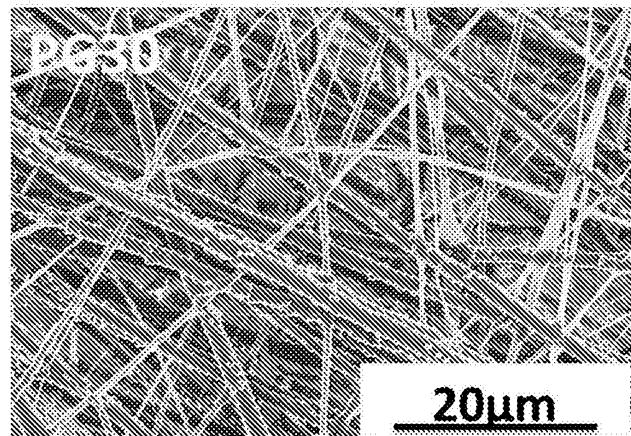


图3

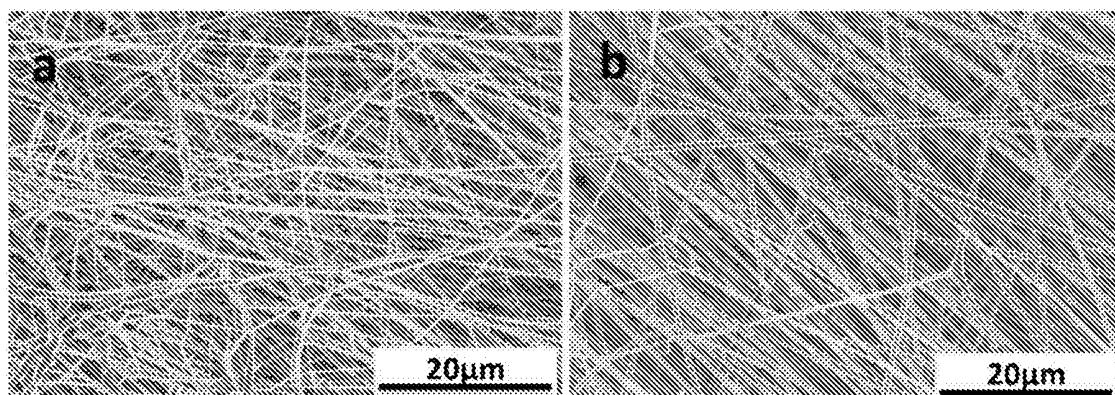


图4

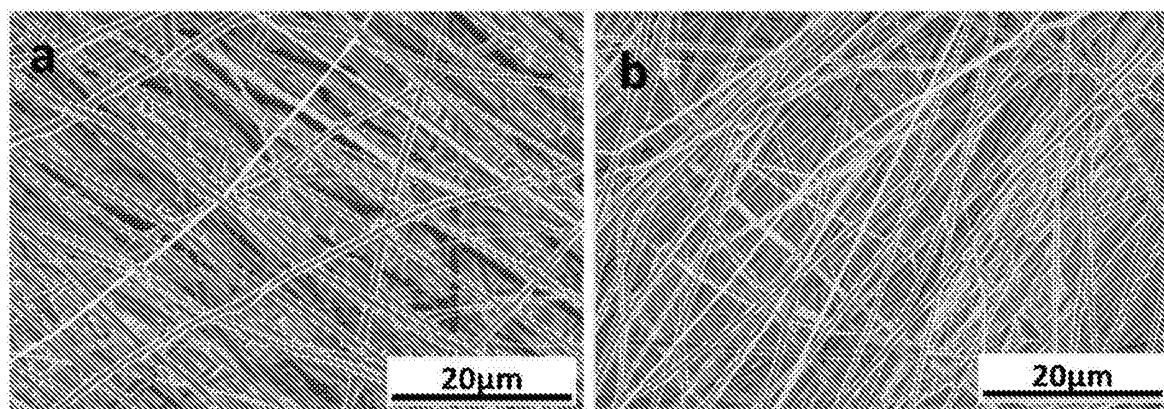


图5

Carry Types of Medicine guide tissue regeneration film and preparation method thereof

Abstract

The present invention is to carry Types of Medicine guide tissue regeneration film and preparation method thereof. It is with polycaprolactone and other degradable aliphatic polyesters as raw material and adds antibacterials, and individual layer guide tissue regeneration film is prepared by the method for electrostatic spinning; Or with polycaprolactone and degradable aliphatic polyester material as compacted zone, degradable natural macromolecular material and bioactive particles are weaker zone, and antibacterials are added in compacted zone, it is prepared into the double-deck guide tissue regeneration film without pore structure and bioactivity by the method for layer upon layer electrostatic spinning. Inventive film material has excellent biocompatibility, mechanical performance and the degradation property consistent with tissue repair process, fibroblast etc. can effectively be prevented to growing at tissue defect, while the Regeneration and Repair of promotion organization, need not second operation take out, can also effectively suppress the incident bacterial infection of Post operation and inflammation. Can be widely used for the medical fields such as guide tissue regeneration, post-operation adhesion preventing, medical releasing film.

CN103948974B

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External links: [Espacenet](#), [Global Dossier](#), [Discuss](#)

Claims (10)

[Hide Dependent ^](#)

1. it is a kind of to carry Types of Medicine guide tissue regeneration film, it is characterized in that: Using polycaprolactone as major matrix material, and contain antibacterial Medicine, with individual layer or double-layer porous structure, wherein:

(1) monofilms is blended as matrix material using pure polycaprolactone or polycaprolactone with degradable aliphatic polyester, and is added Antibacterials; Wherein polycaprolactone and the mass ratio of degradable aliphatic polyester is 50/50-100/0, antibacterials and polymer Mass ratio be 0.5/100-50/100, the polymer is that pure polycaprolactone or polycaprolactone are total to degradable aliphatic polyester Mixed thing; Architectural feature is the nano-scale fiber structure with random arrangement, and average bridging aperture is 2-6 μm, and fibre diameter is 200-1000nm, film thickness is 50-500 μm;

(2) duplcature is made up of compacted zone and weaker zone:

A) compacted zone matrix material is pure polycaprolactone or polycaprolactone and degradable aliphatic polyester intermingling material, and adds anti-Bacterium medicine, wherein polycaprolactone and the quality that the mass ratio of degradable aliphatic polyester is 50/50-100/0, medicine and polymer Than being 0.5/100-50/100, the polymer is pure polycaprolactone or polycaprolactone and degradable aliphatic polyester blend; Architectural feature is that, with random or latticed array nanometer to micron order fibre structure, average pore size is 2-6 μm, fibre diameter It is 200nm-1200nm, film thickness is 25-250 μm;

B) weaker zone matrix material is the intermingling material of pure polycaprolactone and inorganic bioactivity particle, or polycaprolactone with can drop Blended compound material prepared by solution natural macromolecular material and inorganic bioactivity particle, or pure polycaprolactone, or gather oneself The blended compound material of lactone and degradable natural macromolecular material; Degradable natural macromolecular material is carried out using crosslinking agent Crosslinking; Wherein polycaprolactone and the mass ratio of degradable natural macromolecular material is 10/90-100/0, inorganic bioactivity particle It is 0/100-50/100 with the mass ratio of polymer; Weaker zone architectural feature is 5-100 μm of average pore size, and fibre diameter is 200nm-7 μm, thickness is 25-250 μm.

2. one kind according to claim 1 carries Types of Medicine guide tissue regeneration film, it is characterised in that degradable aliphatic polyester Including: Polycaprolactone, PLA-caprolactone copolymer, poly lactic-co-glycolic acid-caprolactone copolymer one kind therein or one Plant thing mixed above.

3. one kind according to claim 1 carries Types of Medicine guide tissue regeneration film, it is characterised in that described degradable natural Macromolecular material includes: One or more mixing in NTx, gelatin, shitosan, starch, cellulose, elastin laminin Thing.

4. one kind according to claim 1 carries Types of Medicine guide tissue regeneration film, it is characterised in that described antibacterials, bag Containing PCs, cephalosporin class, Tetracyclines, chloromycetin, macrolide, lincomycin and clindamycin, fluorine quinoline promise Ketone and nitro glyoxaline antimicrobial.

5. the one kind described in claim 1 carries Types of Medicine guide tissue regeneration film, it is characterised in that inorganic bioactivity particle includes: Particle size is one or more mixing in hydroxyapatite, bata-tricalcium phosphate and the bio-vitric particle of 1-100nm Thing.

6. one kind according to claim 1 carries Types of Medicine guide tissue regeneration film, it is characterised in that described crosslinking agent includes: One kind in formaldehyde, glutaraldehyde, Geniposide, EDC/NHS.

7. it is according to claim 1 it is a kind of carry Types of Medicine guide tissue regeneration film preparation method, it is characterised in that it is described The preparation method of individual layer guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04-0.2g/mL Solution A;

(2) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04- The solution B of 0.2g/mL;

(3) example mixes solution A with solution B according to a certain volume, and adds the antibacterials of certain mass, room temperature magnetic agitation 12h, obtains the solution C that polymer residual is 0.04-0.2g/mL, polycaprolactone and degradable aliphatic polyester in solution C Mass ratio 50/50-100/0, the mass ratio of antibacterials and polymer is 0.5/100-50/100;

(4) electrostatic spinning is carried out with solution C, with stainless steel drum as reception device, roller slewing rate is 100-600rpm, is spun Silk liquid flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-30h, obtains thickness 50-500 µm electricity spinning fibre film;

(5) after electrostatic spinning terminates, by spinning film, room temperature is placed 2-7 days in fume hood, package sterilization.

8. it is according to claim 1 it is a kind of carry Types of Medicine guide tissue regeneration film preparation method, it is characterised in that it is described The preparation method of double-deck guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04-0.2g/mL Solution A;

(2) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04- The solution B of 0.2g/mL;

(3) degradable natural macromolecular material is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtaining mass concentration is The solution C of 0.04-0.2g/mL;

(4) example mixes solution A with solution B according to a certain volume, and adds the antibacterials of certain mass, room temperature magnetic agitation 12h, obtains the solution D that polymer residual is 0.04-0.2g/mL, polycaprolactone and degradable aliphatic polyester in solution D Mass ratio 50/50-100/0, the mass ratio of antibacterials and polymer is 0.5/100-50/100;

(5) a certain amount of bioactive particles are added in solution D, ultrasonic 30min-2h, magnetic agitation 12h, obtains at room temperature Mass concentration is the solution E of 0.04-0.2g/mL, and wherein inorganic bioactivity particle and the mass ratio of polymer is 0/100-50/ 100;

(6) with solution C be sufficiently mixed solution A by example according to a certain volume, room temperature magnetic agitation 12h, obtains polymer quality dense The solution F for 0.04-0.2g/mL is spent, the mass ratio 10/90- of polycaprolactone and degradable natural macromolecular material in solution F 100/0;

(7) a certain amount of bioactive particles are added in solution F, ultrasonic 30min-2h, magnetic agitation 12h, obtains at room temperature Mass concentration is the solution G of 0.04-0.2g/mL, and wherein inorganic bioactivity particle and the mass ratio of polymer is 0/100-50/ 100;

(8) electrostatic spinning is carried out with solution D, with stainless steel drum as reception device, roller slewing rate is 100-600rpm, is spun Silk liquid flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-15h, obtains thickness 25-250 µm fine and close tunic;

(9) on the basis of fine and close tunic, electrostatic spinning is carried out with solution E or solution G, with stainless steel drum as reception device, Roller slewing rate is 100-600rpm, and spinning solution flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8- 30cm, spinning 0.5-15h, obtain the loose tunic of 25-250 µm of thickness;

(10) after electrostatic spinning terminates, electricity spinning fibre film is immersed in the crosslinking agent ethanol solution that concentration is 0.01%-3% Crosslinking 10min-12h, immersion is embathed 5-10 times in deionized water after terminating, and by spinning film, room temperature places 2-7 in fume hood My god, package sterilization.

9. it is according to claim 1 it is a kind of carry Types of Medicine guide tissue regeneration film preparation method, it is characterised in that it is described Another preparation method of double-deck guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04-0.2g/mL Solution A; Degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtaining mass concentration is The solution B of 0.04-0.2g/mL; Example mixes solution A with solution B according to a certain volume, and adds the antimicrobial of certain mass Thing, room temperature magnetic agitation 12h, obtain polymer residual be 0.04-0.2g/mL solution C, in solution C polycaprolactone with The mass ratio 50/50-100/0 of degradable aliphatic polyester, antibacterials are 0.5/100-50/100 with the mass ratio of polymer; Electrostatic spinning is carried out with solution C, with stainless steel drum as reception device, roller slewing rate is 100-600rpm, spinning solution stream Dynamic speed is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-30h, obtains the cause of 25-250 µm of thickness Close tunic;

(2) fine and close tunic is layered on Flat bottom container bottom, the composition material of weaker zone is dissolved in organic solvent, then cast On the surface of fine and close tunic, by it at -60 DEG C – after freezing 6-12h at 20 DEG C, it is put into vacuum desiccator and is vacuum dried 4- 12h, obtains final product double-deck guide tissue regeneration film.

10. the preparation method of a kind of load Types of Medicine guide tissue regeneration film according to claim 6,7 or 8, it is characterised in that Described organic solvent is hexafluoroisopropanol, trifluoroethanol, chloroform, methyl alcohol, dichloromethane, N, N'-dimethyl formamide In one or more mixed solvents.

Description

Carry Types of Medicine guide tissue regeneration film and preparation method thereof

Technical field

The invention belongs to technical field of biological material, and in particular to a kind of so that polycaprolactone is as major matrix material and loads anti- Guide tissue regeneration film material of bacterium medicine and preparation method thereof.

Background technology

Due to the Cranial defect that long-term agomphosis, periodontosis, wound, cleft palate and Oral and facial tumor are caused, justice cannot be often carried out. Tooth is repaired and planting body implantation, it is necessary to rebuild using Bone Defect Repair. Using biomembrane Guide Periodontal Tissue Regeneration guiding tissue again Raw art (guided tissue regeneration, GTR) is considered as the maximally effective oral cavity bone defect healing of recent two decades Technology, by wide clinical for periodontal. Guide tissue regeneration technology is placed between periodontal connective tissue valve and root of the tooth. A kind of film, as barrier, prevents gum from being grown in connective tissue, and the cell of selectivity guiding regeneration potential increases to root surface Grow, produce new cementum and new periodontal ligament. The type and performance of guide tissue regeneration film turn into restricting guide regeneration skill. The key factor of art development.

Although GTR technologies can obtain curative effect steady in a long-term, due to the exposure of postoperative film, remain in it is thin in periodontal disease damage. The infection that the factors such as bacterium trigger will influence the neoblastic acquisition of its periodontal. At present anti-infective whole body system is also mainly taken for postoperative System medication, but utilization ratio of drug is relatively low, and easily cause gastrointestinal side effect. Local delivery of drug can strengthen therapeutic effect, reduce Adverse reaction. But come out there is presently no antibacterial type guide tissue regeneration film product. In the new guide tissue regeneration film for grinding Also few researchs on bacteriostasis property.

Electrostatic spinning technique is a kind of simple general-purpose method for preparing nanofiber, because its medicine load mode is simply easy OK, medicine different in electrostatic spinning or spinning last handling process and large biological molecule are easy to be loaded into fibrous inside and table Face, in addition, antibacterials will not occur performance change after being loaded into fiber, remains to keep its anti-microbial property, can be used to pre-Anti-POI. Therefore, the nanofiber medicine carrying membrane that prepared by Electrospun has good potential applicability in clinical practice.

The content of the invention

One kind carries Types of Medicine guide tissue regeneration film, it is characterized in that: Using polycaprolactone as major matrix material, and contain Antibacterials, with individual layer or double-layer porous structure, wherein:

(1) Monofilm is blended as matrix material using pure polycaprolactone or polycaprolactone with degradable aliphatic polyester, and Addition antibacterials; The wherein mass ratio of polycaprolactone and degradable aliphatic polyester is 50/50-100/0, antibacterials with it is poly- The mass ratio of compound is 0.5/100-50/100, and the polymer is pure polycaprolactone or polycaprolactone and degradable aliphatic adoption Ester blend; Architectural feature is the nano-scale fiber structure with random arrangement, and average bridging aperture is 2-6 μm , fibre diameter It is 200-1000nm, film thickness is 50-500 μm ;

(2) Duplication is made up of compacted zone and weaker zone:

A) compacted zone matrix material is pure polycaprolactone or polycaprolactone and degradable aliphatic polyester intermingling material, and is added Plus antibacterials, wherein polycaprolactone and the mass ratio of degradable aliphatic polyester is 50/50-100/0, medicine and polymer Mass ratio is 0.5/100-50/100, and the polymer is that pure polycaprolactone or polycaprolactone are blended with degradable aliphatic polyester Thing; Architectural feature is that, with random or latticed array nanometer to micron order fibre structure, average pore size is 2-6 μm , fiber A diameter of 200nm-1200nm, film thickness is 25-250 μm ;

B) weaker zone matrix material is the intermingling material of pure polycaprolactone and inorganic bioactivity particle, or polycaprolactone with Blended compound material prepared by degradable natural macromolecular material and inorganic bioactivity particle, using crosslinking agent to degradable day Right macromolecular material is crosslinked; Wherein polycaprolactone and the mass ratio of degradable natural macromolecular material is 10/90-100/0, Inorganic bioactivity particle is 0/100-50/100 with the mass ratio of polymer; Weaker zone architectural feature is average pore size 5-100 μm , fibre diameter is 200nm-7 μm , and thickness is 25-250 μm .

Degradable aliphatic polyester includes: PLA, polycaprolactone, Poly(D,L-lactide-co-glycolide, PLA-oneself Lactone copolymers, poly lactic-co-glycolic acid-caprolactone copolymer one or more mixtures therein.

Described degradable natural macromolecular material includes: NTx, gelatin, shitosan, starch, cellulose, elastic egg One or more mixtures in white;

Described antibacterials, comprising PCs, cephalosporin class, Tetracyclines, chloromycetin, macrolide, Lincomycin and clindamycin, FQNS and nitro glyoxaline antimicrobial.

Described inorganic bioactivity particle includes: Particle size is the hydroxyapatite of 1-100nm, bata-tricalcium phosphate and One or more mixtures in bio-vitric particle.

The crosslinking agent stated includes: One kind in formaldehyde, glutaraldehyde, Geniposide, EDC/NHS;

The preparation method of individual layer guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04- The solution A of 0.2g/mL;

(2) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtaining mass concentration is The solution B of 0.04-0.2g/mL;

(3) example mixes solution A with solution B according to a certain volume, and adds the antibacterials of certain mass, room temperature magnetic force Stirring 12h, obtains the solution C that polymer residual is 0.04-0.2g/mL, polycaprolactone and degradable aliphatic in solution C The mass ratio 50/50-100/0 of polyester, antibacterials are 0.5/100-50/100 with the mass ratio of polymer;

(4) electrostatic spinning is carried out with solution C, with stainless steel drum as reception device, roller slewing rate is 100- 600rpm, spinning solution flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-30h, is obtained The electricity spinning fibre film of 50-500 μm of thickness;

(5) after electrostatic spinning terminates, by spinning film, room temperature is placed 2-7 days in fume hood, package sterilization.

The preparation method of double-deck guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04- The solution A of 0.2g/mL;

(2) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtaining mass concentration is The solution B of 0.04-0.2g/mL;

(3) degradable natural macromolecular material is dissolved in organic solvent, room temperature magnetic agitation 6-24h obtains quality dense Spend the solution C for 0.04-0.2g/mL;

(4) example mixes solution A with solution B according to a certain volume, and adds the antibacterials of certain mass, room temperature magnetic force Stirring 12h, obtains the solution D that polymer residual is 0.04-0.2g/mL, polycaprolactone and degradable aliphatic in solution D The mass ratio 50/50-100/0 of polyester, antibacterials are 0.5/100-50/100 with the mass ratio of polymer;

(5) a certain amount of bioactive particles are added in solution D, ultrasonic 30min-2h, at room temperature magnetic agitation 12h, The solution E that mass concentration is 0.04-0.2g/mL is obtained, wherein inorganic bioactivity particle and the mass ratio of polymer is 0/100-50/100;

(6) with solution C be sufficiently mixed solution A by example according to a certain volume, room temperature magnetic agitation 12h, obtains polymeric material Amount concentration is the solution F of 0.04-0.2g/mL, the mass ratio 10/ of polycaprolactone and degradable natural macromolecular material in solution F 90-100/0;

(7) a certain amount of bioactive particles are added in solution F, ultrasonic 30min-2h, at room temperature magnetic agitation 12h, The solution G that mass concentration is 0.04-0.2g/mL is obtained, wherein inorganic bioactivity particle and the mass ratio of polymer is 0/ 100-50/100;

(8) electrostatic spinning is carried out with solution D, with stainless steel drum as reception device, roller slewing rate is 100- 600rpm, spinning solution flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-15h, is obtained The fine and close tunic of 25-250 μm of thickness;

(9) on the basis of fine and close tunic, electrostatic spinning is carried out with solution E or solution G, is filled by reception of stainless steel drum Put, roller slewing rate is 100-600rpm, spinning solution flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8- 30cm, spinning 0.5-15h, obtain the loose tunic of 25-250 μm of thickness;

(10) after electrostatic spinning terminates, electricity spinning fibre film is immersed in the crosslinking agent ethanol solution that concentration is 0.01%-3% Middle crosslinking 10min-12h, immersion is embathed 5-10 times in deionized water after terminating, and by spinning film, room temperature places 2- in fume hood 7 days, package sterilization.

Another preparation method of double-deck guide tissue regeneration film is containing having the following steps:

(1) polycaprolactone is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains mass concentration for 0.04- The solution A of 0.2g/mL; Degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h obtains quality dense Spend the solution B for 0.04-0.2g/mL; Example mixes solution A with solution B according to a certain volume, and adds the antibacterial of certain mass Medicine, room temperature magnetic agitation 12h obtains the solution C that polymer residual is 0.04-0.2g/mL, polycaprolactone in solution C With the mass ratio 50/50-100/0 of degradable aliphatic polyester, antibacterials are 0.5/100-50/ with the mass ratio of polymer 100; Electrostatic spinning is carried out with solution C, with stainless steel drum as reception device, roller slewing rate is 100-600rpm, spinning Liquid flow rate is 0.5-10mL/h, voltage 7-20kV, is received apart from 8-30cm, spinning 0.5-30h, obtains 25-250 μm of thickness Fine and close tunic;

(2) fine and close tunic is layered on Flat bottom container bottom, the composition material of weaker zone is dissolved in organic solvent, then The surface of fine and close tunic is cast in, by it at -60 DEG C – after freezing 6-12h at 20 DEG C, it is put into vacuum drying in vacuum desiccator 4-12h, obtains final product double-deck guide tissue regeneration film.

Described organic solvent is hexafluoroisopropanol, trifluoroethanol, chloroform, methyl alcohol, dichloromethane, N, N'-diformazan One or more mixed solvents in base formamide;

It is with polycaprolactone and other degradable aliphatic polyesters as main raw material(s) and adds antibacterials, by quiet The method of Electrospun prepares individual layer guide tissue regeneration film;Or with polycaprolactone and degradable aliphatic polyester material as fine and close Layer, degradable natural macromolecular material and bioactive particles are weaker zone, and add antibacterials in compacted zone, by layer by layer The method of electrostatic spinning is prepared into the double-deck guide tissue regeneration film without pore structure and bioactivity.Inventive film material With excellent biocompatibility, mechanical performance and the degradation property consistent with tissue repair process, fibre can be effectively prevented into Dimension cell etc. is acceptable effective while the Regeneration and Repair of promotion organization, it is not necessary to which second operation takes out to growing at tissue defect The incident bacterial infection of suppression Post operation and inflammation.Guide tissue regeneration, post-operation adhesion preventing, medicine is can be widely used for delay Release the medicine and hygiene fieldses such as film.

Brief description of the drawings

Fig. 1 is that the polycaprolactone prepared by the inventive method carries not same amount metronidazole (1%-40% mass ratios) Individual layer guiding The related implementation result figure of tissue regeneration membrane, the naming method of sample is in picture: P1 is represented in spinning PCL nano fibrous membranes The mass ratio of MNA and PCL represents that the mass ratio of MNA and PCL in spinning PCL nano fibrous membranes is 5%, other sample classes for 1%, P5 Push away.

- (a) It is above-mentioned individual layer spinning fibre film SEM photograph.
- (b) It is the load-deformation curve under above-mentioned individual layer spinning fibre film hygrometric state.
- (c) It is above-mentioned individual layer spinning fibre film water contact angle schematic diagram.
- (d) It is the carrying drug ratio form of above-mentioned individual layer spinning fibre film.
- (e) It is the drug release patterns of above-mentioned individual layer spinning fibre film.
- (f) It is the above-mentioned individual layer spinning fibre film external degradation mass loss figure of 30 days.
- (g) It is SEM figure of the above-mentioned individual layer spinning fibre membrane degradation after 30 days.
- (h) It is above-mentioned individual layer spinning fibre theca cell toxicity data block diagram (Cytotoxicity detection uses L929 cells, RGR represents the relative proliferation rate of cell) .
- (i) In being above-mentioned individual layer spinning fibre theca cell toxicity test, when cell grows 24h in the leaching liquor of material State, show the leaching liquor of material does not influence on the growth of cell and pattern.
- (j) It is O.D values when L929 cells grow different time on above-mentioned individual layer spinning fibre film, shows over time Extension, cell can adhere to propagation on material.
- (k) It is the bio-compatible performance of above-mentioned individual layer spinning fibre film -- L929 I cells are on tunica fibrosa The propagation SEM cell morphology figures of 7 days.
- (l) In being above-mentioned individual layer spinning fibre film, when carrying drug ratio is 30%, tunica fibrosa healing-in different time in rabbit body Histotomy figure afterwards, shows that material has good biocompatibility in vivo, will not cause obvious immune response, without inflammation Disease is reacted and the phenomenon of infection occurs.

Fig. 2 is the single layer fibre film photo that metronidazole 40% is carried with polycaprolactone and polyactic acid blend prepared by the present invention.

Fig. 3 is the single layer fibre film photo that the present invention carries metronidazole 30% with polycaprolactone and gelatin cross-blend.

Fig. 4 is that the present invention carries chloramphenicol 20% as compacted zone with polycaprolactone, polycaprolactone and gelatin 5: 5 blendings are loose Layer, the double layer fibre film photo (a is compacted zone side, and b is weaker zone side) of preparation.

Fig. 5 is that the present invention carries quadracycline 40% as compacted zone with polycaprolactone, polycaprolactone and gelatin 5:5 blendings, receive Rice hydroxyapatite is with polymer quality than for the 20% double layer fibre film photo prepared for weaker zone, (a is compacted zone side, and b is Weaker zone side).

Specific embodiment

The present invention is further illustrated below by embodiment, but the present invention is not limited to these examples.

Embodiment 1

1. 2g polycaprolactones and 0.2g metronidazoles are dissolved in the mixed solvent of 7.2g DMF and 10.8g DCM, room temperature magnetic Power stirs 12h, and it is 10% to obtain polycaprolactone concentration (w/w), metronidazole and spinning solution that polycaprolactone mass ratio is 10%.

2. taking solution carries out electrostatic spinning, and with stainless steel drum as reception device, drum rotation speed is 200rpm, voltage 10KV, it is 16cm, spinning solution feed liquor speed 4mL/h, spinning 5h to receive distance. Obtain the individual layer guiding that thickness is 250 μm or so Tissue regeneration membrane.

3. the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, it is ensured that residual solvent fully volatilizes.

Embodiment 2

1st, polycaprolactone and metronidazole are dissolved in DCM/DMF=7: In 3 mixed organic solvents, room temperature magnetic agitation 12h, It is 20% to obtain polycaprolactone concentration, and the mass ratio of metronidazole and polycaprolactone is 50% spinning solution;

2nd, room temperature carries out electrostatic spinning, and with stainless steel drum as reception device, roller slewing rate is 600rpm, spinning solution Flow rate is 3mL/h, voltage 12kV, is received apart from 15cm, spinning 5h, obtains the individual layer guiding tissue of 200 μm or so of thickness Regeneration membrane.

3rd, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 3

1st, 1.5g polycaprolactones and 0.3g quadracyclines are dissolved in 13.5g trifluoroethanols, room temperature magnetic agitation 12h, Obtain solution A;

2nd, 1.5g PLAs and 0.3g quadracyclines are dissolved in 13.5g trifluoroethanols, room temperature magnetic agitation 12h is obtained To solution B;

3rd, solution A is mixed with solution B, magnetic agitation 12h, it is 1 to obtain polycaprolactone with the mass ratio of PLA:1, gather Compound concentration is 10%, and medicine is with polymer quality than the spinning solution C for 20%;

4th, room temperature carries out electrostatic spinning with spinning solution C, and with stainless steel drum as reception device, roller slewing rate is 200rpm, spinning solution flow rate is 2mL/h, voltage 13kV, is received apart from 20cm, spinning 10h, obtains 250 μm or so of thickness Individual layer electricity spinning fibre film.

5th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 4

1st, polycaprolactone and clindamycin are dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains polycaprolactone dense It is 6% to spend, and clindamycin and polycaprolactone mass ratio are 30% solution A;

2nd, collagen is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution B that concentration is 6%;

3rd, solution A is mixed with solution B, magnetic agitation 6h, it is 1 to obtain collagen with the mass ratio of polycaprolactone:1, polymerization Thing concentration is 6%, and the mass ratio of medicine and polymer is 30% spinning solution C;

4th, room temperature carries out electrostatic spinning with spinning solution C, and with stainless steel drum as reception device, roller slewing rate is 300rpm, spinning solution flow rate 1mL/h, voltage 10kV, receive apart from 15cm, spinning 20h, obtain 250 μm or so of thickness Electrosyn membrane;

5th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 5

1st, polycaprolactone is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution A that concentration is 8%;

2nd, gelatin is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution B that concentration is 8%;

3rd, solution A is mixed with solution B, magnetic agitation 6h, obtains the mass ratio 3 of gelatin and polycaprolactone:7, polymer Concentration is 8% spinning solution C;

4th, polycaprolactone and metronidazole are dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains polycaprolactone dense It is 10% to spend, and metronidazole and polycaprolactone mass ratio are 40% spinning solution D;

5th, room temperature carries out electrostatic spinning with spinning solution C, and with stainless steel drum as reception device, roller slewing rate is 300rpm, spinning solution flow rate 2mL/h, voltage 10kV, receive apart from 15cm, spinning 10h, obtain thickness for 250 μm or so The loose tunic of Electrosyn;

6th, change spinning solution D and proceed electrostatic spinning on the tunica fibrosa that step 5 is spun, spinning condition is rotated for roller Speed is 200rpm, and spinning solution flow rate is 4mL/h, voltage 12KV, is received apart from 15cm, spinning 5h, obtains gross thickness 500 μm or so double-deck guide tissue regeneration film.

7th, spinning fibre film is placed in the ethanol solution of the Geniposide that concentration is 0.5%, makes cross-link gelatin, be crosslinked After reaction 30min, film is embathed in deionized water 10 times, wash away unreacted Geniposide and alcohol solvent.

8th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 6

1st, polycaprolactone is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution A that concentration is 6%;

2nd, PLA is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution B that concentration is 6%;

3rd, shitosan is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution C that concentration is 6%;

4th, solution A is mixed with solution B, and adds metronidazole, magnetic agitation 12h obtains PLA with polycaprolactone Mass ratio 4:6, polymer concentration is 6%, and the mass ratio of medicine and polymer is 40% spinning solution D;

5th, solution A is mixed with solution C, magnetic agitation 12h, obtains the mass ratio 5 of shitosan and polycaprolactone:5, polymerization Thing concentration is 6% spinning solution E;

6th, room temperature carries out electrostatic spinning with spinning solution D, and with stainless steel drum as reception device, roller slewing rate is 500rpm, spinning solution flow rate 3mL/h, voltage 12kV, receive apart from 18cm, spinning 5h, obtain thickness for 200 μm or so Fine and close tunic;

7th, change spinning solution E and proceed electrostatic spinning on the tunica fibrosa that step 6 is spun, spinning condition is rotated for roller Speed is 200rpm, and spinning solution flow rate is 1mL/h, voltage 12kV, is received apart from 18cm, spinning 15h, obtains gross thickness 400 μm or so of double-deck guide tissue regeneration film.

8th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 7

1st, polycaprolactone is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h, obtain polycaprolactone concentration be 6% it is molten Liquid A;

2nd, polycaprolactone and cephalosporin are dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains polycaprolactone dense It is 10% to spend, and the mass ratio of cephalosporin and polycaprolactone is 50% solution B;

3rd, gelatin is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution C that concentration is 6%;

4th, solution A is mixed with solution C, and adds the nanometer hydroxyapatite of certain mass, ultrasonic 1h, magnetic agitation 12h, obtains the mass ratio 5 of gelatin and polycaprolactone:5, polymer concentration is 6%, the quality of nanometer hydroxyapatite and polymer Than the spinning solution D for 20%;

5th, room temperature carries out electrostatic spinning with spinning solution B, and with stainless steel drum as reception device, roller slewing rate is 300rpm, spinning solution flow rate 1.5mL/h, voltage 10kV, receive apart from 18cm, spinning 10h, obtain 200 μm or so of thickness Electrospun densification tunic;

6th, change spinning solution D and proceed electrostatic spinning on the tunica fibrosa that step 5 is spun, spinning condition is rotated for roller Speed is 300rpm, and spinning solution flow rate is 1mL/h, voltage 13kV, is received apart from 18cm, spinning 15h, obtains gross thickness 400 μm or so of double-deck guide tissue regeneration film.

7th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 8

1st, polycaprolactone is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h, obtain polycaprolactone concentration be 8% it is molten Liquid A;

2nd, polyglycolic acid is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h, it is 8% to obtain polyglycolic acid concentration Solution B;

3rd, shitosan is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 12h obtains the solution C that chitosan concentration is 8%;

4th, solution A is mixed with B, and adds a certain amount of chloramphenicol, magnetic agitation 12h obtains polyglycolic acid and gathers The mass ratio 3 of caprolactone:7, polymer concentration is 8%, and the mass ratio of chloramphenicol and polymer is 30% spinning solution D;

5th, solution A is mixed with solution C, and adds the bioactivity glass of certain mass, magnetic agitation 12h obtains shell The mass ratio 4 of glycan and polycaprolactone:6, polymer concentration is 8%, and bioactivity glass is 30% with the mass ratio of polymer Spinning solution E;

6th, room temperature carries out electrostatic spinning with spinning solution D, and with stainless steel drum as reception device, roller slewing rate is 300rpm, spinning solution flow rate 2mL/h, voltage 12kV, receive apart from 18cm, spinning 5h, obtain the electricity of 150 μm or so of thickness Spinning densification tunic;

7th, change spinning solution E and proceed electrostatic spinning on the tunica fibrosa that step 6 is spun, spinning condition is rotated for roller Speed is 300rpm, and spinning solution flow rate is 1mL/h, voltage 12kV, is received apart from 18cm, spinning 10h, and obtaining gross thickness is 300 μm or so of double-deck guide tissue regeneration film.

8th, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

Embodiment 9

1st, polycaprolactone and cephalosporin are dissolved in DCM/DMF=6: In 4 mixed organic solvents, room temperature magnetic agitation 12h, it is 10% to obtain polycaprolactone concentration, and the mass ratio of cephalosporin and polycaprolactone is 40% spinning solution;

2nd, room temperature carries out electrostatic spinning with spinning solution, and with stainless steel drum as reception device, roller slewing rate is 500rpm, spinning solution flow rate is 3mL/h, voltage 12kV, is received apart from 15cm, spinning 5h, obtains 200 μm or so of thickness Electricity spinning fibre film.

3rd, the tunica fibrosa that will be obtained is placed in fume hood at room temperature and dries 72h, residual solvent is fully volatilized.

4th, above-mentioned tunica fibrosa is laid in culture dish bottom, concentration of casting thereon is 20% (w/w) Aqueous gelatin solution , - 40 DEG C of freeze-drying 12h, obtain duplicate.

Patent Citations (4)

Publication number	Priority date	Publication date	Assignee	Title
CN101172164A *	2006-11-03	2008-05-07	中国科学院化学研究所	Biopolymer nano tunica fibrosa material capable of being biological degraded and absorbed, preparing method and uses of the same
CN101584885A *	2009-06-25	2009-11-25	同济大学	Preparation method of three-layer lead tissue regenerating velum with gradient
CN102166378A *	2011-01-13	2011-08-31	北京化工大学	Tissue regeneration guiding membrane and preparation method thereof



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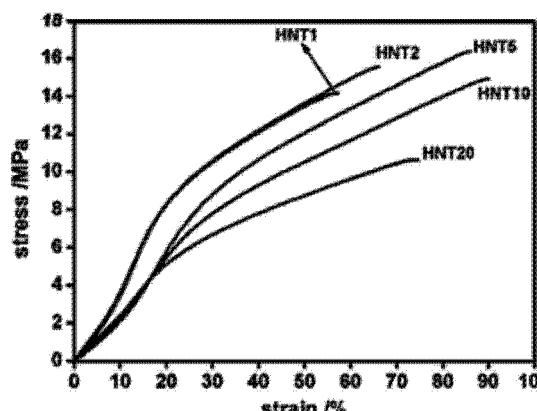
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(54) 发明名称

含有埃洛石纳米管载药型引导组织再生膜及
其制备方法

(57) 摘要

含有埃洛石纳米管载药型引导组织再生膜及
其制备方法属于生物材料领域。它以可降解脂肪
族聚酯为主要原材料并加入抗菌药物和载有抗菌
药物的埃洛石纳米管，通过静电纺丝制备单层引
导组织再生膜；或以可降解脂肪族聚酯与可降解
天然高分子的共混材料为致密层，可降解脂肪族
聚酯与可降解天然高分子材料及生物活性粒子为
疏松层，并在致密层加入抗菌药物和载有抗菌药
物的埃洛石纳米管，通过静电纺丝的方法制备成
具有不同孔结构和生物活性的双层引导组织再生
膜。本发明材料具有优异生物相容性、能有效的阻
止成纤维细胞等向组织缺损处的长入，同时促进
组织的再生修复，不必二次手术，可控及长期的药
物释放还可抑制手术后易发生的细菌性感染及炎
症。



1. 含有埃洛石纳米管载药型引导组织再生膜，其特征是：以可降解脂肪族聚酯形成的纤维作为主要基体材料，并含有载有抗菌药物的埃洛石纳米管，具有单层或双层多孔结构，其中：

(1) 单层膜以可降解脂肪族聚酯或可降解脂肪族聚酯和可降解天然高分子的混合物在静电纺丝中形成的纤维作为基体材料，并添加抗菌药物和载有抗菌药物的埃洛石纳米管；可降解脂肪族聚酯与可降解天然高分子的质量比为 10/90-90/10，载有抗菌药物的埃洛石纳米管质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 1/100-40/100，其中载有抗菌药物的埃洛石纳米管的质量是纯埃洛石纳米管与载入药物的总质量，以下所指载有抗菌药物的埃洛石纳米管的质量均为纯埃洛石纳米管与载入抗菌药物的总质量；直接载入纤维中的抗菌药物质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100；结构特征为具有无规排列的纤维结构，平均搭桥孔径为 2-6 μm，纤维直径为 200-1000nm，膜厚度为 50-500 μm；

(2) 双层膜由致密层和疏松层构成：

a) 致密层以可降解脂肪族聚酯与可降解天然高分子的混合物在静电纺丝中所形成的纤维为基体材料，并添加抗菌药物以及载有抗菌药物的埃洛石纳米管，其中可降解脂肪族聚酯与可降解天然高分子的质量比为 10/90-90/10，载有抗菌药物的埃洛石纳米管质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 1/100-40/100，直接载入纤维中的抗菌药物的质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100；结构特征为具有无规或网格状排列的纤维结构，平均孔径为 2-6 μm，纤维直径为 200nm-1200nm，膜厚度为 25-500 μm；

b) 疏松层以可降解脂肪族聚酯与无机生物活性粒子共混纺丝所形成的纤维，或可降解脂肪族聚酯与可降解天然高分子材料和无机生物活性粒子制备的共混纺丝纤维为基体材料，选择性采用交联剂对可降解天然高分子材料进行交联；其中可降解脂肪族聚酯与可降解天然高分子的质量比为 10/90-90/10，无机生物活性粒子质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100；疏松层结构特征为平均孔径 5-100 μm，纤维直径为 200nm-7 μm，厚度为 25-500 μm。

2. 根据权利要求 1 所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，可降解脂肪族聚酯包括：聚乳酸、聚己内酯、聚乳酸-羟基乙酸共聚物、聚乳酸-己内酯共聚物、聚乳酸-羟基乙酸-己内酯共聚物其中的一种或两种以上的混合物；可降解天然高分子材料包括：I 型胶原、明胶、壳聚糖、淀粉、纤维素、弹性蛋白中的一种或两种以上的混合物。

3. 根据权利要求 1 所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，抗菌药物，包含青霉素类、头孢霉素类、四环素类、氯霉素类、大环内脂类、林可霉素、氟喹诺酮类、硝基咪唑类、多肽类抗菌药；抗菌药物载入埃洛石纳米管，再将载药的埃洛石纳米管通过共混方式加入到纤维中，为了提高载药量，同时在纤维中直接加入另一部分药物，直接加入纤维中的该部分药物与载入埃洛石管内的药物相同或者不同。

4. 根据权利要求 1 所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，无机生物活性粒子包括：颗粒尺寸为 1-100nm 的羟基磷灰石、β-磷酸三钙及生物活性玻璃粒子中的一种或两种以上的混合物。

5. 根据权利要求 1 所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，交联

剂包括：甲醛、戊二醛、京尼平、1-乙基-3-(3-二甲基氨基丙基)-碳化二亚胺(EDC)、N-羟基琥珀酰亚胺(NHS)中的一种。

6. 根据权利要求1所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，单层引导组织再生膜的制备方法有如下步骤：

(1) 取抗菌药物1加入到其良溶剂中，充分搅拌超声使其溶解以得到其饱和溶液A；

(2) 取埃洛石纳米管与溶液A混合，超声分散得到埃洛石纳米管的悬浮液B；将悬浮液B溶液抽真空直至液体表面不再有气泡出现，离心后取出上清液，用抗菌药物1的良溶剂清洗沉淀；重复抽真空步骤至少三次，即可得到载有抗菌药物1的埃洛石纳米管；

(3) 将上述载有抗菌药物1的埃洛石纳米管干燥，研磨；

(4) 将可降解脂肪族聚酯溶于有机溶剂中，室温磁力搅拌6-24h，得到可降解脂肪族聚酯质量浓度为0.04-0.2g/mL的溶液C；

(5) 向C溶液中加入抗菌药物2，室温磁力搅拌6-12h后加入载有抗菌药物1的埃洛石纳米管，磁力搅拌6-12h，得到可降解脂肪族聚酯质量浓度为0.04-0.2g/mL的溶液D，溶液D中载有抗菌药物1的埃洛石纳米管与可降解脂肪族聚酯的质量比1/100-40/100，抗菌药物2与可降解脂肪族聚酯的质量比为5/100-50/100；

(6) 用溶液D进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为100-600rpm，纺丝液流动速率为0.5-10mL/h，电压7-20kV，接收距离8-30cm，纺丝0.5-30h，得到厚度50-500μm的电纺丝纤维膜；

(7) 静电纺丝结束后，将纺丝膜在通风橱中室温放置2-7天，包装消毒。

7. 根据权利要求1所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于，双层引导组织再生膜的制备方法有如下步骤：

(1) 取抗菌药物1加入到其良溶剂中，搅拌使其溶解以得到其饱和溶液A；

(2) 取埃洛石纳米管与溶液A混合，超声分散得到埃洛石纳米管的悬浮液B；将B溶液抽真空直至液体表面不再有气泡出现，离心后取出上清液，用抗菌药物1的良溶剂清洗沉淀，重复上述抽真空步骤至少3次，即可得到载有抗菌药物的埃洛石纳米管；

(3) 将上述载有抗菌药物1的埃洛石纳米管干燥，研磨；

(4) 将可降解脂肪族聚酯溶于有机溶剂中，室温磁力搅拌6-24h，得到可降解脂肪族聚酯质量浓度为0.04-0.2g/mL的溶液C；

(5) 将可降解天然高分子材料溶于有机溶剂中，室温磁力搅拌6-24h，得到可降解天然高分子质量浓度为0.04-0.2g/mL的溶液D；

(6) 按一定体积比例将溶液C与溶液D充分混合，室温磁力搅拌6-12h，得到可降解脂肪族聚酯和可降解天然高分子总质量浓度为0.04-0.2g/mL的溶液E，溶液E中可降解脂肪族聚酯与可降解天然高分子材料的质量比为10/90-90/10；

(7) 在溶液E中加入抗菌药物2，室温下磁力搅拌6-12h后加入载有抗菌药物1的埃洛石纳米管，室温磁力搅拌6-12h，得到可降解脂肪族聚酯与可降解天然高分子总质量浓度为0.04-0.2g/mL的溶液F，溶液F中载有抗菌药物1的埃洛石纳米管的质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为1/100-40/100，抗菌药物2的质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为5/100-50/100；

(8) 在溶液F中加入生物活性粒子，超声30min-2h，室温下磁力搅拌6-12h，得到可降解

脂肪族聚酯和可降解天然高分子总质量浓度为 0.04-0.2g/mL 的溶液 G, 其中无机生物活性粒子质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100;

(9) 在溶液 E 中加入生物活性粒子, 超声 30min-2h, 室温下磁力搅拌 6-12h, 得到可降解脂肪族聚酯和可降解天然高分子总质量浓度为 0.04-0.2g/mL 的溶液 H, 其中无机生物活性粒子质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100;

(10) 用溶液 F 进行静电纺丝, 以不锈钢滚筒为接收装置, 滚筒转动速率为 100-600rpm, 纺丝液流动速率为 0.5-10mL/h, 电压 7-20kV, 接收距离 8-30cm, 纺丝 0.5-15h, 得到厚度 25-250 μm 的致密层膜;

(11) 在致密层膜的基础上, 用溶液 G 或溶液 H 进行静电纺丝, 以不锈钢滚筒为接收装置, 滚筒转动速率为 100-600rpm, 纺丝液流动速率为 0.5-10mL/h, 电压 7-20kV, 接收距离 8-30cm, 纺丝 0.5-20h, 得到厚度 25-500 μm 的疏松层膜;

(12) 静电纺丝结束后, 将电纺丝纤维膜浸泡在质量浓度为 0.01% -3% 的交联剂的乙醇溶液中交联 10min-12h, 浸泡结束后在去离子水中浸洗 5-10 次, 将纺丝膜在通风橱中室温放置 2-7 天, 包装消毒。

8. 根据权利要求 1 所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于, 双层引导组织再生膜的另一种制备方法有如下步骤:

(1) 将可降解脂肪族聚酯溶于有机溶剂中, 室温磁力搅拌 6-24h, 得到可降解脂肪族聚酯质量浓度为 0.04-0.2g/mL 的溶液 A; 将可降解天然高分子溶于有机溶剂中, 室温磁力搅拌 6-24h, 得到可降解天然高分子质量浓度为 0.04-0.2g/mL 的溶液 B; 按一定体积比例将溶液 A 与溶液 B 混合, 加入抗菌药物 2, 室温磁力搅拌 6-12h 后加入载有抗菌药物 1 的埃洛石纳米管, 室温磁力搅拌 6-12h, 得到可降解脂肪族聚酯和可降解天然高分子总质量浓度为 0.04-0.2g/mL 的溶液 C, 溶液 C 中可降解脂肪族聚酯与可降解天然高分子的质量比 10/90-90/10, 载有抗菌药物 1 的埃洛石纳米管质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 1/100-40/100, 抗菌药物 2 的质量与可降解脂肪族聚酯和可降解天然高分子总质量的比为 5/100-50/100; 用溶液 C 进行静电纺丝, 以不锈钢滚筒为接收装置, 滚筒转动速率为 100-600rpm, 纺丝液流动速率为 0.5-10mL/h, 电压 7-20kV, 接收距离 8-30cm, 纺丝 0.5-30h, 得到厚度 25-500 μm 的致密层膜;

(2) 将致密层膜铺在平底容器底部, 将疏松层的组成材料可降解脂肪族聚酯与可降解天然高分子以及的生物活性粒子溶解于有机溶剂中, 然后浇铸在致密层膜的表面, 将其在 -60°C --20°C 下冷冻 6-12h 后, 放入真空干燥器中真空干燥 4-12h, 即得双层引导组织再生膜。

9. 根据权利要求 6-8 任意一项所述的含有埃洛石纳米管载药型引导组织再生膜其特征在于, 所述的抗菌药物的良溶剂为水、乙醇、丙酮、甲苯、六氟异丙醇、三氟乙醇、三氯甲烷、甲醇、二氯甲烷、N,N'-二甲基甲酰胺中的一种或几种混合溶剂, 可降解脂肪族聚酯和可降解天然高分子材料的溶剂为六氟异丙醇、三氟乙醇、三氯甲烷、甲醇、二氯甲烷、N,N'-二甲基甲酰胺中的一种或几种混合溶剂。

含有埃洛石纳米管载药型引导组织再生膜及其制备方法

技术领域

[0001] 本发明属于生物材料领域,具体涉及一种以可降解脂肪族聚酯所形成的纤维作为主要基体材料并加载抗菌药物和载有抗菌药物的埃洛石纳米管的引导组织再生膜材料及其制备方法。

背景技术:

[0002] 引导组织再生 (Guide Tissue Regeneration, GTR) 技术是 80 年代末 90 年代初发展起来的一项新技术。其原理是利用膜的物理屏障功能将受损部位与周围组织隔离,创造一个相对封闭的组织环境,从而使成骨细胞优先迁移、生长。GTR 的应用为牙周病的治疗、牙种植区骨量不足及其它骨缺损的修复、骨折的愈合提供了一个新的有效途径。

[0003] 目前 GTR 膜材料可分为不可吸收和可吸收两大类。不可吸收引导组织再生膜主要由聚四氟乙烯材料制备,主要代表产品为 Gore-Texs。聚四氟乙烯膜虽然具有良好的力学强度,但不能自行降解,需二次手术去除,增加病人的痛苦及手术费用,且细胞亲和性差,易导致伤口裂开,膜早期暴露,影响伤口的愈合。因此,对可吸收膜材料的开发和研究便成为 GTR 技术发展的重要方向。

[0004] 目前应用最为普遍的 GTR 膜以天然材料胶原为主,代表产品为瑞士 Geistlich 公司生产的 Bio-Gide 和美国 Zimmer 公司生产的 Biomend,分别以猪皮胶原及牛跟腱胶原制备。其中 Bio-Gide 也是目前临床应用的金标准。天然胶原 GTR 膜虽然具有良好的生物相容性,但其力学强度相对较弱,降解较快,植入后易造成早期降解塌陷。为了增强及延长降解时间,通常需要加厚膜的厚度,这就为种植和修复提供了不利。此外,天然材料制备的 GTR 膜还存在产品质量受原料来源限制,价格昂贵等缺陷。为了克服天然胶原材料制备的 GTR 膜存在的缺陷,一些人工合成可降解材料制备的 GTR 产品也相继问世。

[0005] 虽然 GTR 技术可取得长期稳定的疗效,但由于手术中和术后以及二次取出过程中,引入到损伤部位的厌氧细菌以及需氧细菌等引发的感染将影响其新组织的获得。目前对于术后抗感染还主要采取全身系统用药,但药物利用率较低,且容易引起胃肠道副反应。局部释药可以增强治疗效果,降低不良反应。

[0006] 静电纺丝技术是一种制备纳米纤维的简单通用方法,由于其药物加载方式简单易行,在静电纺丝或纺丝后处理过程中不同的药物及生物大分子很容易加载到纤维内部和表面,另外,抗菌药物在载入纤维中后不会发生性能变化,仍能保持其抗菌性能,可以用来预防术后感染。因此,电纺丝制备的纳米纤维载药膜具有良好的临床应用前景。

[0007] 在通过静电纺丝制备载药型引导组织再生膜时,药物通过共混的方式加入到纤维内部和表面,药物释放速率比较快,存在突释现象,在引导组织再生膜移植到人体的 5-7 天后,药物释放基本上已达 80-90%,虽然术后一周是发生感染的高危期,但是组织修复过程中感染情况的发生要持续至少 2 周。在组织修复的过程中,感染多为混合感染,即厌氧菌和需氧菌掺杂,两种细菌互相促进生长,因此在预防及治疗引导组织再生术后感染时,须采用分别对需氧菌和厌氧菌敏感的药物来提高疗效。因此研究一种既可以减慢药物释放速率从

而实现药物可持续释放又可以同时杀死厌氧菌和需氧菌的引导组织再生膜是十分重要的。

[0008] 埃洛石纳米管是一种管状的铝硅酸盐,它的外径约为50-80nm,内径约为10-15nm,长度大约是1000nm,负载率大约是15% -20%。埃洛石纳米管的长径比为20-50,在聚合物纤维中取向会显著增强纤维的力学性能。埃洛石纳米管具有天然来源,不会对环境造成污染,并且具有良好的生物相容性。作为管状容器,埃洛石纳米管在化妆品、药物释放、医用移植(如牙齿填料)等方面得到广泛应用。

[0009] 在聚合物体系中加入抗菌药物和载有抗菌药物的埃洛石纳米管,通过静电纺丝技术,制备单层及双层抗菌抗炎型载药引导组织再生膜,若对制得的引导组织再生膜的降解速率或者力学性能以及耐水性有更高的要求,还可以对聚合物基体中的天然高分子进行交联。本材料的载药共分为两部分,一部分是直接存在于纤维内部及纤维表面的抗菌药物,另一部分存在于纤维内部的埃洛石纳米管中。直接存在于纤维内部的抗菌药物扩散至纤维表面然后再转移至损伤部位;纤维内部埃洛石纳米管中的抗菌药物首先要从埃洛石纳米管扩散进入纤维内部,然后再从纤维内部扩散至纤维表面,最后再从纤维表面扩散至损伤部位,从而减缓了药物释放的速率,缓解突释现象。本设计可以提高纳米纤维膜的载药量,减缓药物释放速率,缓解突释现象;同时,纤维和埃洛石纳米管负载药物可以不同,以起到同时杀灭厌氧菌及需氧菌的作用。

附图说明

[0010] 图1是本发明方法所制备的单层聚己内酯-明胶(质量比为9:1)载不同量埃洛石纳米管(HNT)(1%-20%质量比)的单层引导组织再生膜的相关实施效果图,图片中样品的命名方式为:HNT0表示纺丝PG/HNT纳米纤维膜中含甲硝唑埃洛石纳米管质量与聚己内酯和明胶总质量的比为0%,HNT20表示纺丝PG/HNT纳米纤维膜中埃洛石纳米管质量与聚己内酯和明胶总质量的比为20%,其他样品类推。

[0011] (1) 是上述PG/HNT单层纺丝纤维膜SEM照片。

[0012] (2) 是上述PG/HNT单层纺丝纤维膜湿态下的应力-应变曲线, a为沿收集器旋转方向,不添加埃洛石纳米管的纳米纤维膜的拉伸强度约为7.3MPa,添加埃洛石纳米管以后材料的拉伸强度得到明显改善,断裂伸长率无明显变化。

[0013] (3) 是上述PG/HNT单层纺丝纤维膜水接触角示意图。

[0014] (4) 是上述PG/HNT单层纺丝纤维膜细胞增殖后相反侧膜SEM照片。

[0015] (5) 是L929细胞在上述PG/HNT单层纺丝纤维膜上生长5天时的SEM和激光共聚焦显微镜照片,(a)-(g) HNT0,(b)-(h) HNT1,(c)-(i) HNT2,(d)-(j) HNT5,(e)-(k) HNT 10, and (f)-(l) HNT20,以及L929细胞在上述PG/HNT-MNA单层纺丝纤维膜上(m)粘附4h和(n)生长不同时间的OD值(OD值测试使用CCK-8法),测试结果表明随着时间的延长,细胞可以在材料上粘附增殖。

[0016] 图2是不同载药方式制备的材料项性能测试对比图,HNT-MNA是指按照实施例1中所述方法制备所得的载有抗菌药物甲硝唑的埃洛石纳米管,PG-MNA是指聚己内酯/明胶纳米纤维(质量比为9:1)中直接载入抗菌药物甲硝唑(20%的质量比),PG/HNT-MNA是指聚己内酯/明胶(质量比9:1)纤维中加入按照实施例1中所述方法制备所得的含甲硝唑的埃洛石纳米管(20%的质量比),PG-MNA/HNT-MNA是指按照实施例4中所述方法制备得到的

纤维膜，其中聚己内酯和明胶质量比为 9:1，纤维中直接载入甲硝唑的质量与聚己内酯和明胶总质量的比为 20%，载有甲硝唑的埃洛石纳米管与聚己内酯和明胶总质量的比为 20%。

[0017] (1) 是载有 MNA 的 HNT 中 MNA 释放的药物释放曲线。

[0018] (2) 是 HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜浸泡在 PBS 缓冲溶液中药物释放情况。

[0019] (3) 是在琼脂板上进行的抑菌试验情况 :a 是 37 °C 缺氧条件下培养一天时, HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜抑菌圈情况 ;b 是 HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜抑菌圈直径随时间变化的情况。

[0020] (4) 是细胞毒性试验情况 :a 是 L929 细胞分别在 PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜浸提液中生长 24h, 48h, 72h 以后的 O.D 值测试结果柱状图 ;b、c、d 分别是 L929 细胞分别在空白对照和 PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜浸提液中生长 72h 后的细胞形态图。

[0021] (5) 是细胞增殖实验情况 :L929 细胞在空白对照和 PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜上生长 1 天、3 天、5 天、7 天后的 O.D 测试结果柱状图。

[0022] (6)a 是 L929 细胞在空白对照和 PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜上生长 5 天后的 SEM 照片,b 是 L929 细胞在空白对照和 PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA 单层纳米纤维膜上生长 5 天后的激光共聚焦显微镜照片。

具体实施方式

[0023] 下面通过实施例进一步说明本发明，但本发明并不限于这些实例。

[0024] 实施例 1

[0025] 1. 取 4g 抗菌药物甲硝唑加入到三氟乙醇中，搅拌使其溶解以得到其饱和溶液 A；取 1g 埃洛石纳米管与溶液 A 混合，超声分散得到埃洛石纳米管的悬浮液 B；将悬浮液 B 抽真空直至液体表面不再有气泡出现，离心后取出上清液，用三氟乙醇清洗沉淀，重复抽真空步骤 3 次，即可得到载有甲硝唑的埃洛石纳米管；将上述载有甲硝唑的埃洛石纳米管干燥，研磨。

[0026] 2. 将 2g 聚己内酯溶于 7.2g N,N 二甲基甲酰胺与 10.8g 二氯甲烷的混合溶剂中，室温磁力搅拌 24h 后加入 0.1g 甲硝唑，室温磁力搅拌 12h 后加入 0.01g 载有甲硝唑的埃洛石纳米管，室温磁力搅拌 6h 后得到溶液 C，溶液 C 中聚己内酯质量浓度为 10%，载有甲硝唑的埃洛石纳米管与聚己内酯的质量比为 5%，取溶液 C 进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转速为 200rpm，电压 10KV，接收距离为 16cm，纺丝液进液速率 4mL/h，纺丝 5h。得到厚度为 400 μm 左右的单层引导组织再生膜。

[0027] 3. 将得到的纤维膜置于室温下通风橱中干燥 72h，保证残余 N,N 二甲基甲酰胺和二氯甲烷充分挥发。

[0028] 实施例 2

[0029] 1、取 4g 抗菌药物盐酸四环素加入到去离子水中，搅拌使其溶解以得到其饱和溶液 A；取 1g 埃洛石纳米管与溶液 A 混合，超声分散得到埃洛石纳米管的悬浮液 B；将悬浮液 B 抽真空直至液体表面不再有气泡出现，离心后取出上清液，用去离子水清洗沉淀，重复抽

真空步骤 3 次,即可得到载有盐酸四环素的埃洛石纳米管;将上述载有盐酸四环素的埃洛石纳米管干燥,研磨。

[0030] 2、将 2g 聚己内酯溶于 5.6g 二氯甲烷和 2.4gN,N 二甲基甲酰胺的混合有机溶剂中,室温磁力搅拌 24h 后加入 1g 抗菌药物甲硝唑,室温磁力搅拌 12h 后再加入 0.8g 载有盐酸四环素的埃洛石纳米管,室温磁力搅拌 6h 后得到溶液 C,溶液 C 中聚己内酯质量浓度为 20%,甲硝唑与聚己内酯的质量比为 50%,载有盐酸四环素的埃洛石纳米管与聚己内酯的质量比为 40%。

[0031] 3、室温取溶液 C 进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为 600rpm,纺丝液流动速率为 3mL/h,电压 12kV,接收距离 15cm,纺丝 5h,得到厚度 300 μm 左右的单层引导组织再生膜。

[0032] 4、将得到的纤维膜置于室温下通风橱中干燥 72h,使残余二氯甲烷和 N,N 二甲基甲酰胺充分挥发。

[0033] 实施例 3

[0034] 1、取 6g 抗菌药物甲硝唑加入到三氟乙醇中,搅拌使其溶解以得到其饱和溶液 A;取 1.5g 埃洛石纳米管与溶液 A 混合,超声分散得到埃洛石纳米管的悬浮液 B;将悬浮液 B 抽真空直至液体表面不再有气泡出现,离心后取出上清液,用三氟乙醇清洗沉淀,重复抽真空步骤 3 次,即可得到载有甲硝唑的埃洛石纳米管;将上述载有甲硝唑的埃洛石纳米管干燥,研磨。

[0035] 2、将 1.5g 聚己内酯溶于 13.5g 三氟乙醇中,室温磁力搅拌 24h 后得到聚己内酯质量浓度为 10% 的溶液 C;

[0036] 3、将 1.5g 聚乳酸溶于 13.5g 三氟乙醇中,室温磁力搅拌 24h,得到聚乳酸质量浓度为 10% 的溶液 D;

[0037] 4、将溶液 C 与溶液 D 充分混合,加入 0.6g 盐酸四环素,室温磁力搅拌 12h 后加入 1.2g 载有甲硝唑的埃洛石纳米管,磁力搅拌 6h,得到溶液 E,溶液 E 中聚己内酯和聚乳酸总质量浓度为 10%,聚己内酯与聚乳酸的质量比为 1:1,盐酸四环素质量与聚己内酯和聚乳酸总质量的比为 20%,载有甲硝唑的埃洛石纳米管质量与聚己内酯和聚乳酸总质量的比为 40%;

[0038] 5、室温用纺丝液 E 进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为 200rpm,纺丝液流动速率为 2mL/h,电压 13kV,接收距离 20cm,纺丝 10h,得到厚度 300 μm 左右的单层电纺纤维膜。

[0039] 6、将得到的纤维膜置于室温下通风橱中干燥 72h,使残余三氟乙醇充分挥发。

[0040] 实施例 4

[0041] 1、取 4g 抗菌药物甲硝唑加入三氟乙醇中,搅拌使其溶解以得到其饱和溶液 A;取 1g 埃洛石纳米管与溶液 A 混合,超声分散得到埃洛石纳米管的悬浮液 B;将悬浮液 B 抽真空直至液体表面不再有气泡出现,离心后取出上清液,用三氟乙醇清洗沉淀。重复抽真空步骤 3 次,即可得到载有甲硝唑的埃洛石纳米管;将上述载有甲硝唑的埃洛石纳米管干燥,研磨。

[0042] 2、将 1.08g 聚己内酯溶于 10g 六氟异丙醇中,室温磁力搅拌 24h 后得到溶液 C;

[0043] 3、将 0.72g 明胶溶于 8.8g 六氟异丙醇中,室温磁力搅拌 24h,得到溶液 D;

[0044] 4、将溶液 C 与溶液 D 充分混合后加入 0.24g 甲硝唑，室温磁力搅拌 12h 后加入 0.24g 载有甲硝唑的埃洛石纳米管，室温磁力搅拌 6h 后得到溶液 E，溶液 E 中聚己内酯和明胶总质量浓度为 6%，聚乳酸与明胶的质量比为 9:1，直接载入纤维中的甲硝唑质量与聚己内酯和明胶总质量的比为 20%，载有甲硝唑的埃洛石纳米管质量与聚己内酯和明胶总质量的比为 20%；

[0045] 5、室温用纺丝液 E 进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为 300rpm，纺丝液流动速率 1mL/h，电压 10kV，接收距离 15cm，纺丝 20h，得到厚度 250 μm 左右的电纺丝膜；

[0046] 6、将得到的纤维膜置于室温下通风橱中干燥 72h，使残余六氟异丙醇充分挥发。

[0047] 实施例 5

[0048] 1、取 4g 抗菌药物盐酸四环素加入到去离子水中，搅拌使其溶解以得到其饱和溶液 A；取 1g 埃洛石纳米管与溶液 A 混合，超声分散得到埃洛石纳米管的悬浮液 B；将悬浮液 B 抽真空直至液体表面不再有气泡出现，离心后取出上清液，用去离子水清洗沉淀。重复抽真空步骤 3 次，即可得到载有盐酸四环素的埃洛石纳米管；将上述载有盐酸四环素的埃洛石纳米管干燥，研磨。

[0049] 2、将 0.8g 聚己内酯溶于 9.2g 六氟异丙醇中，室温磁力搅拌 24h，得到聚己内酯质量浓度为 8% 的溶液 C；

[0050] 3、将 0.8g 壳聚糖溶于 9.2g 六氟异丙醇中，室温磁力搅拌 24h，得到壳聚糖质量浓度为 8% 的溶液 D；

[0051] 4、将 0.8g 明胶溶于 9.2g 六氟异丙醇中，室温磁力搅拌 24h，得到明胶质量浓度为 8% 纺丝液 E；

[0052] 5、将 7g 溶液 C 与 3g 溶液 D 充分混合后向其中加入 0.4g 甲硝唑，室温磁力搅拌 12h 后向其中加入 0.32g 载有盐酸四环素的埃洛石纳米管，室温磁力搅拌 6h 后得到纺丝液 F，纺丝液 F 中壳聚糖与聚己内酯的质量比 3:7，甲硝唑质量与壳聚糖和聚己内酯总质量的比为 50%，载有盐酸四环素的埃洛石纳米管质量与壳聚糖和聚己内酯总质量的比为 40%；

[0053] 6、将 8g 溶液 C 与 2g 溶液 E 充分混合，得到聚己内酯和明胶总质量浓度为 8% 的溶液 G，溶液 G 中聚己内酯与明胶的质量比为 4:1；

[0054] 7、室温用纺丝液 F 进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为 300rpm，纺丝液流动速率 2mL/h，电压 10kV，接收距离 15cm，纺丝 10h，得到厚度为 300 μm 左右的电纺丝致密层膜；

[0055] 8、更换纺丝液 G 继续进行静电纺丝于步骤 5 所纺的纤维膜上，纺丝条件为滚筒转动速率为 200rpm，纺丝液流动速率为 4mL/h，电压 12kV，接收距离 15cm，纺丝 5h，得到总厚度 500 μm 左右的双层引导组织再生膜。

[0056] 9、将纺丝纤维膜置于质量浓度为 0.5% 的京尼平的乙醇溶液中，使明胶发生交联，交联反应 30min 后，将膜在去离子水中浸洗 10 次，洗去未反应的京尼平及乙醇溶剂。

[0057] 10、将得到的纤维膜置于室温下通风橱中干燥 72h，使残余六氟异丙醇和乙醇充分挥发。

[0058] 实施例 6

[0059] 1、取 4g 抗菌药物甲硝唑加入到三氟乙醇中，搅拌使其溶解以得到其饱和溶液 A；

取1g 埃洛石纳米管与溶液A混合,超声分散得到埃洛石纳米管的悬浮液B;将悬浮液B抽真空直至液体表面不再有气泡出现,离心后取出上清液,用三氟乙醇清洗沉淀。重复抽真空步骤3次,即可得到甲硝唑的埃洛石纳米管;将上述载有甲硝唑的埃洛石纳米管干燥,研磨。

[0060] 2、将0.6g聚己内酯溶于9.4g六氟异丙醇中,室温磁力搅拌12h,得到聚己内酯质量浓度为6%的溶液C;

[0061] 3、将0.6g聚乳酸溶于9.4g六氟异丙醇中,室温磁力搅拌12h,得到聚乳酸质量浓度为6%的溶液D;

[0062] 4、将0.6g胶原溶于9.4g六氟异丙醇中,室温磁力搅拌12h,得到胶原质量浓度为6%的溶液E;

[0063] 5、将4g溶液C与6g溶液D充分混合后加入0.12g盐酸四环素,室温磁力搅拌12h后加入0.18g载有甲硝唑的埃洛石纳米管,室温磁力搅拌6h后得到纺丝液F,纺丝液F中聚己内酯和聚乳酸总质量浓度为6%,聚乳酸与聚己内酯的质量比2:3,盐酸四环素质量与聚己内酯和聚乳酸总质量的比为20%,载有甲硝唑的埃洛石纳米管质量与聚己内酯和聚乳酸总质量的比为30%;

[0064] 6、将5g溶液C与5g溶液E充分混合,磁力搅拌12h,得到胶原与聚己内酯的质量比1:1,胶原和聚己内酯总质量浓度为6%的纺丝液G;

[0065] 7、室温用纺丝液F进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为500rpm,纺丝液流动速率为3mL/h,电压12kV,接收距离18cm,纺丝5h,得到厚度为200μm左右的致密层膜;

[0066] 8、更换纺丝液G继续进行静电纺丝于步骤6所纺的纤维膜上,纺丝条件为滚筒转动速率为200rpm,纺丝液流动速率为1mL/h,电压12kV,接收距离18cm,纺丝15h,得到总厚度400μm左右的双层引导组织再生膜。

[0067] 9、将得到的纤维膜置于室温下通风橱中干燥72h,使残余六氟异丙醇充分挥发。

[0068] 实施例7

[0069] 1、取4g抗菌药物头孢霉素加入到去离子水中,搅拌使其溶解以得到其饱和溶液A;取1g埃洛石纳米管与溶液A混合,超声分散得到埃洛石纳米管的悬浮液B;将悬浮液B抽真空直至液体表面不再有气泡出现,离心后取出上清液,用去离子水清洗沉淀。重复抽真空步骤3次,即可得到载有头孢霉素的埃洛石纳米管;将上述载有头孢霉素的埃洛石纳米管干燥,研磨。

[0070] 2、将0.6g聚己内酯溶于9.4g六氟异丙醇中,室温磁力搅拌24h,得到聚己内酯质量浓度为6%的溶液C;

[0071] 3、将0.6g明胶溶于六氟异丙醇中,室温磁力搅拌24h,得到明胶质量浓度为6%的溶液D;

[0072] 4、向溶液C中加入0.3g甲硝唑,室温磁力搅拌12h后加入0.24g载有头孢霉素的埃洛石纳米管,室温磁力搅拌6h,得到溶液E,溶液E中甲硝唑与聚己内酯的质量比为50%,载有头孢霉素的埃洛石纳米管与聚己内酯质量比为40%;

[0073] 5、将5g溶液C与5g溶液D充分混合后加入0.12g纳米羟基磷灰石,超声1h,磁力搅拌12h,得到纺丝液F,纺丝液F中明胶和聚己内酯总质量浓度为6%,明胶与聚己内酯的质量比1:1,纳米羟基磷灰石质量与明胶和聚己内酯总质量的比为20%;

[0074] 6、室温用纺丝液 E 进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为 300rpm,纺丝液流动速率 1.5mL/h,电压 10kV,接收距离 18cm,纺丝 10h,得到厚度 200 μm 左右的电纺丝致密层膜;

[0075] 7、更换纺丝液 F 继续进行静电纺丝于步骤 5 所纺的纤维膜上,纺丝条件为滚筒转动速率为 300rpm,纺丝液流动速率为 1mL/h,电压 13kV,接收距离 18cm,纺丝 15h,得到总厚度 400 μm 左右的双层引导组织再生膜。

[0076] 8、将得到的纤维膜置于室温下通风橱中干燥 72h,使残余六氟异丙醇充分挥发。

[0077] 实施例 8

[0078] 1、取 4g 抗菌药物盐酸四环素加入到去离子水中,搅拌使其溶解以得到其饱和溶液 A;取 1g 埃洛石纳米管与溶液 A 混合,超声分散得到埃洛石纳米管的悬浮液 B;将悬浮液 B 抽真空直至液体表面不再有气泡出现,离心后取出上清液,用去离子水清洗沉淀。重复抽真空步骤 3 次,即可得到载有盐酸四环素的埃洛石纳米管;将上述载有盐酸四环素的埃洛石纳米管干燥,研磨。

[0079] 2、将 0.8g 聚己内酯溶于 9.2g 六氟异丙醇中,室温磁力搅拌 24h,得到聚己内酯质量浓度为 8% 的溶液 C;

[0080] 3、将 0.8g 胶原溶于 9.2g 六氟异丙醇中,室温磁力搅拌 24h,得到胶原质量浓度为 8% 的溶液 D;

[0081] 4、将 0.8g 壳聚糖溶于 9.2g 六氟异丙醇中,室温磁力搅拌 24h,得到壳聚糖质量浓度为 8% 的溶液 E;

[0082] 5、将 7g 溶液 C 与 3g 溶液 D 充分混合,加入 0.24g 氯霉素,室温磁力搅拌 12h 后加入 0.16g 载有盐酸四环素的埃洛石纳米管,磁力搅拌 6h,得到纺丝液 F,纺丝液 F 中胶原和聚己内酯总质量浓度为 8%,胶原与聚己内酯的质量比 3:7,氯霉素质量与胶原和聚己内酯总质量的比为 30%,载有盐酸四环素的埃洛石纳米管质量与胶原和聚己内酯总质量的比为 20%;

[0083] 6、将 6g 溶液 C 与 4g 溶液 E 混合,并加入 0.24g 生物活性玻璃,磁力搅拌 12h,得到纺丝液 G,其中壳聚糖与聚己内酯的质量比 2:3,壳聚糖和聚己内酯总质量浓度为 8%,生物活性玻璃质量与壳聚糖和聚己内酯总质量的比为 30%;

[0084] 7、室温用纺丝液 F 进行静电纺丝,以不锈钢滚筒为接收装置,滚筒转动速率为 300rpm,纺丝液流动速率 2mL/h,电压 12kV,接收距离 18cm,纺丝 5h,得到厚度 150 μm 左右的电纺丝致密层膜;

[0085] 8、更换纺丝液 G 继续进行静电纺丝于步骤 6 所纺的纤维膜上,纺丝条件为滚筒转动速率为 300rpm,纺丝液流动速率为 1mL/h,电压 12kV,接收距离 18cm,纺丝 10h,得到总厚度为 300 μm 左右的双层引导组织再生膜。

[0086] 9、将得到的纤维膜置于室温下通风橱中干燥 72h,使残余六氟异丙醇充分挥发。

[0087] 实施例 9

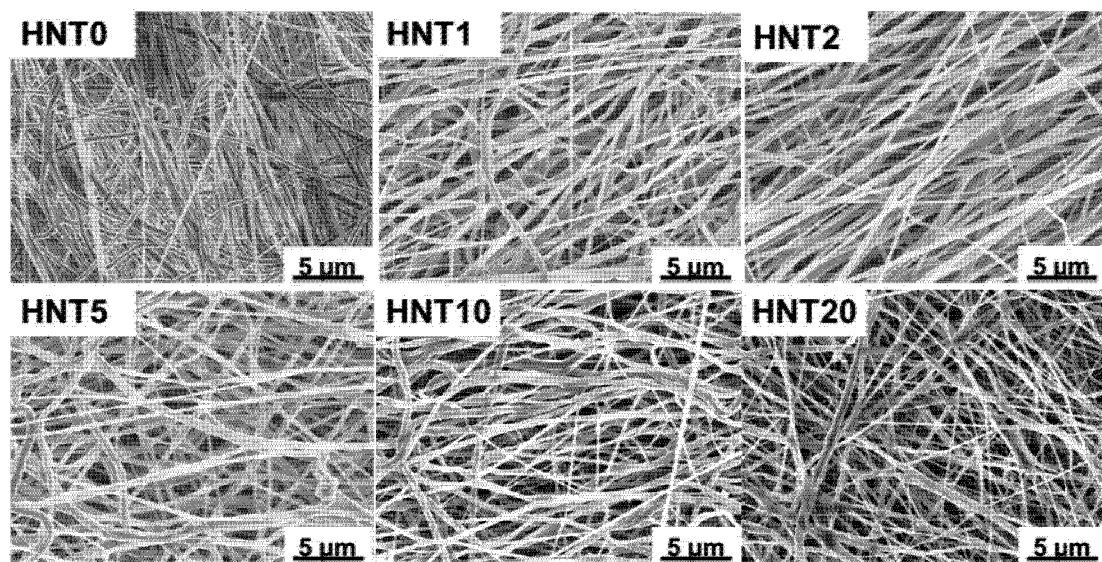
[0088] 1、取 4g 抗菌药物甲硝唑加入到三氟乙醇中,搅拌使其溶解以得到其饱和溶液 A;取 1g 埃洛石纳米管与溶液 A 混合,超声分散得到埃洛石纳米管的悬浮液 B;将悬浮液 B 抽真空直至液体表面不再有气泡出现,离心后取出上清液,用三氟乙醇清洗沉淀。重复抽真空步骤 3 次,即可得到甲硝唑的埃洛石纳米管;将上述载有甲硝唑的埃洛石纳米管干燥,研磨。

[0089] 2、将 1g 聚己内酯溶于 5.4g 二氯甲烷和 3.6g N,N 二甲基甲酰胺的混合有机溶剂中，室温磁力搅拌 24h，加入 0.4g 头孢霉素，室温磁力搅拌 12h 后加入 0.4g 载有甲硝唑的埃洛石纳米管，室温磁力搅拌 6h 后得到纺丝液 C，纺丝液 C 中聚己内酯质量浓度为 10%，头孢霉素与聚己内酯的质量比为 40%，载有甲硝唑的埃洛石纳米管与聚己内酯的质量比为 40%；

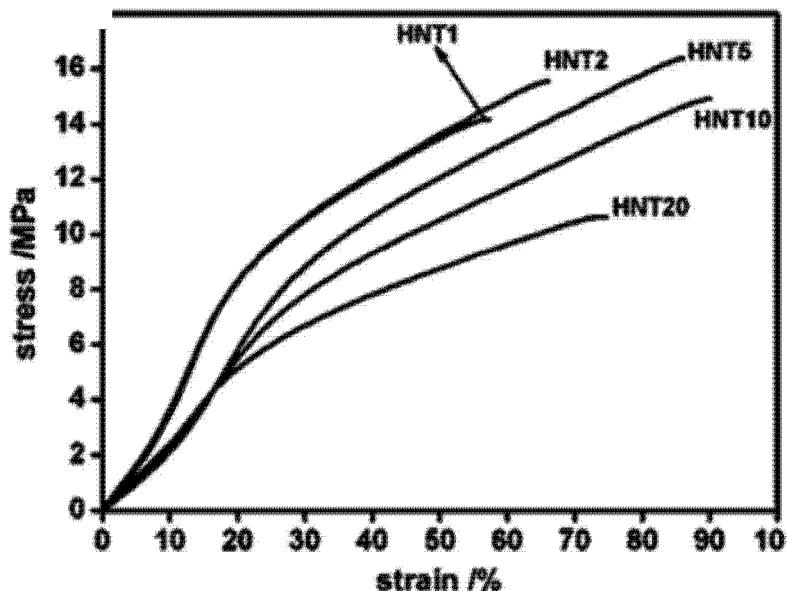
[0090] 3、室温用纺丝液 C 进行静电纺丝，以不锈钢滚筒为接收装置，滚筒转动速率为 500rpm，纺丝液流动速率为 3mL/h，电压 12kV，接收距离 15cm，纺丝 5h，得到厚度 200 μm 左右的电纺丝纤维膜。

[0091] 4、将得到的纤维膜置于室温下通风橱中干燥 72h，使残余二氯甲烷和 N,N 二甲基甲酰胺充分挥发。

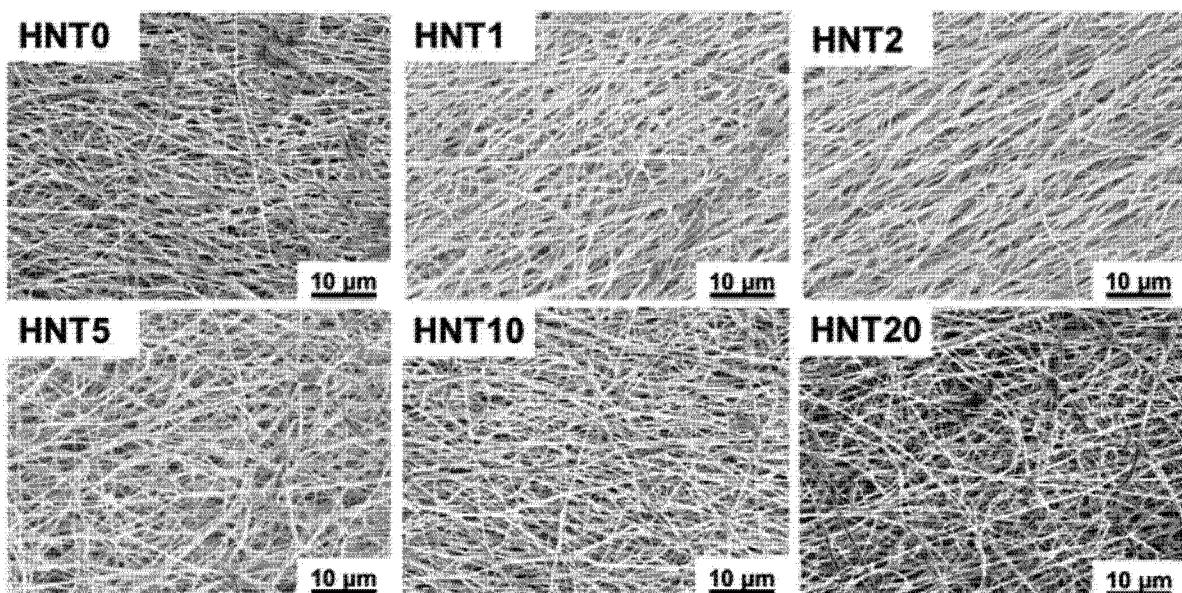
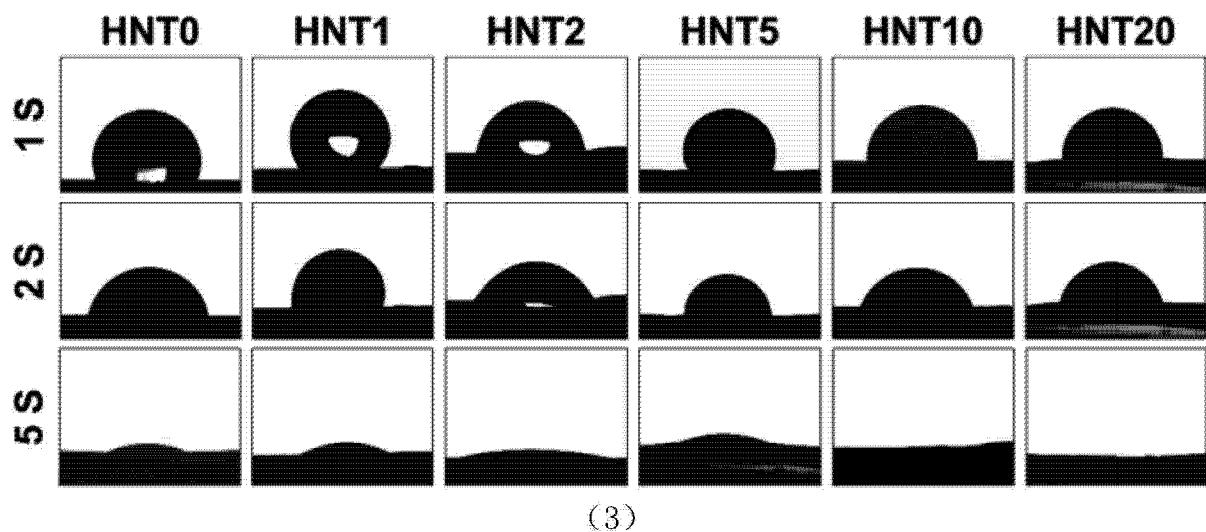
[0092] 5、将上述纤维膜平铺于培养皿底部，在其上浇铸质量浓度为 20% 的明胶水溶液，-40℃ 冷冻干燥 12h，得到双层膜。

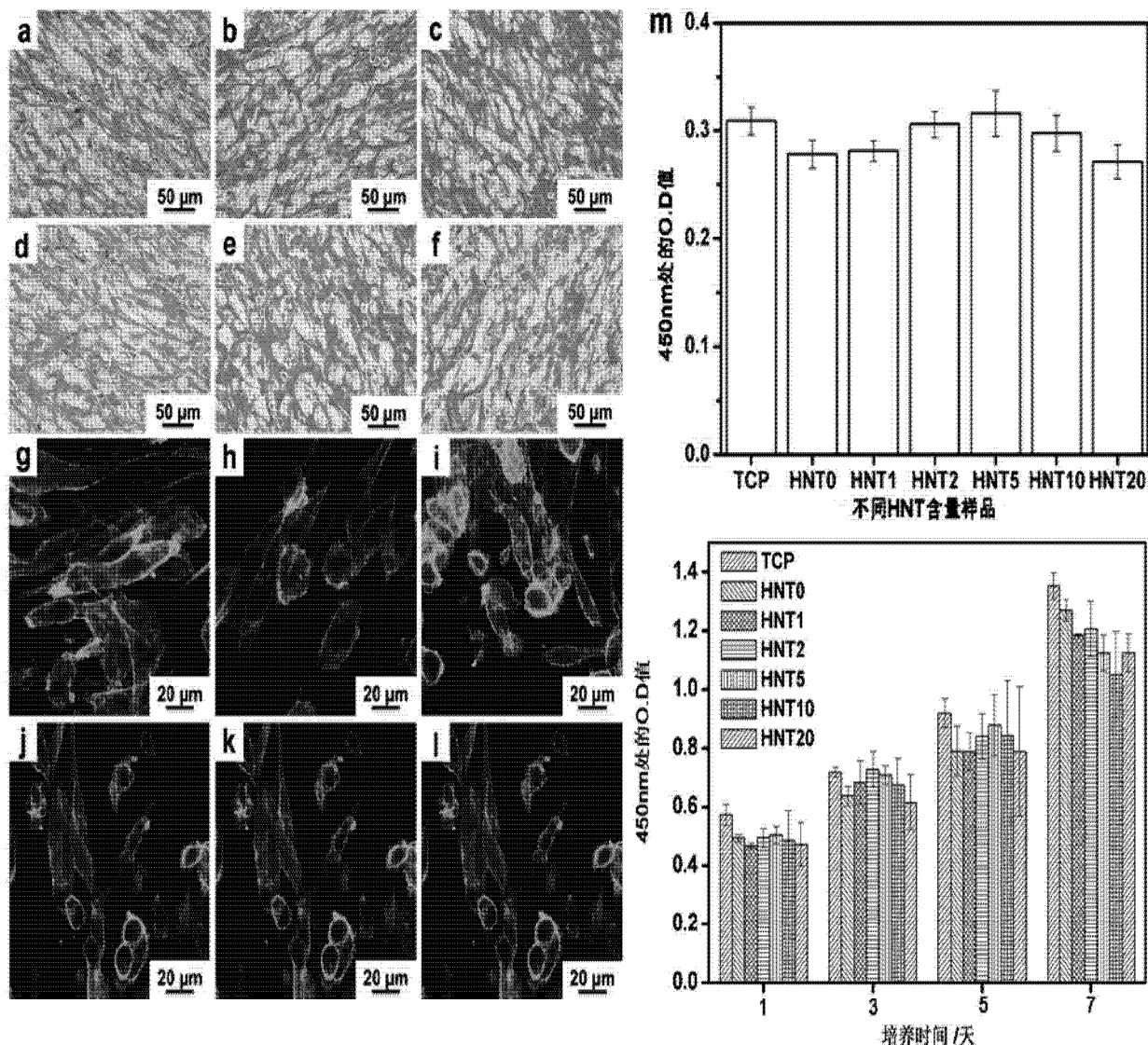


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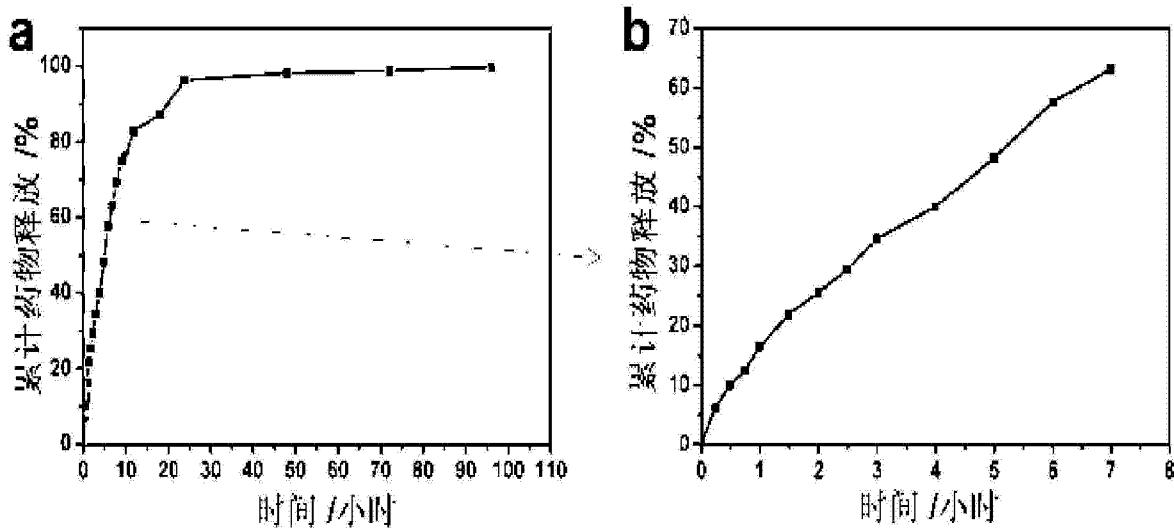
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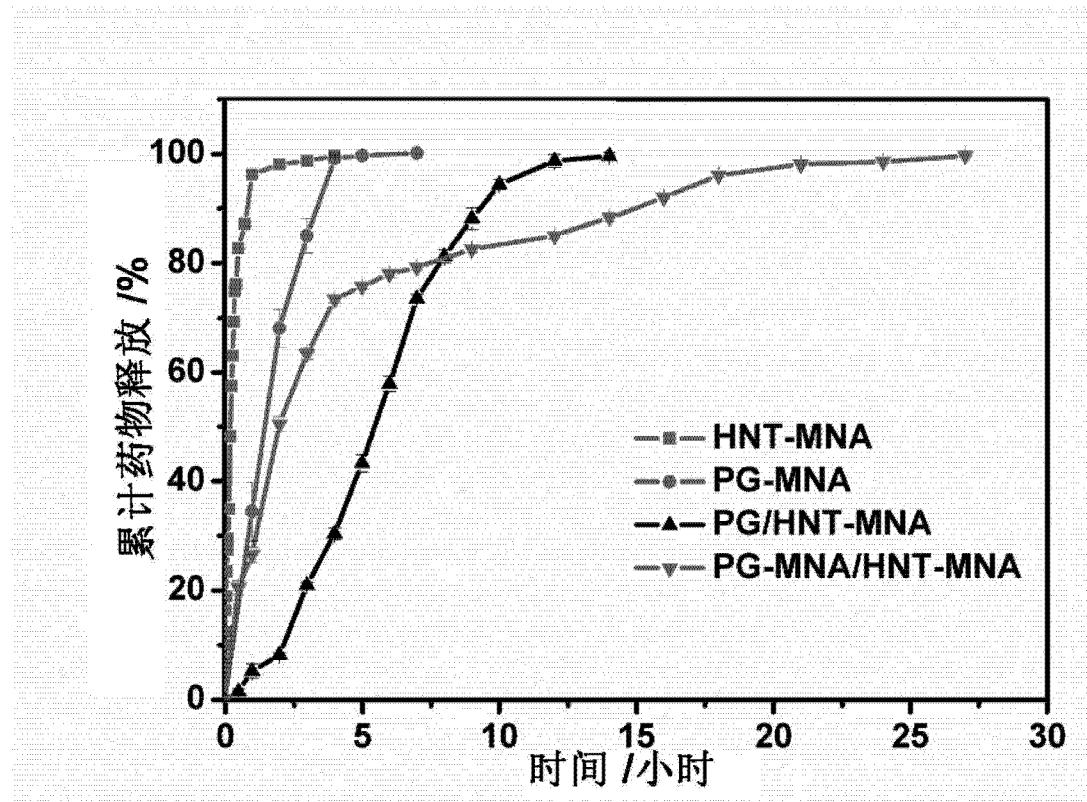


(5)

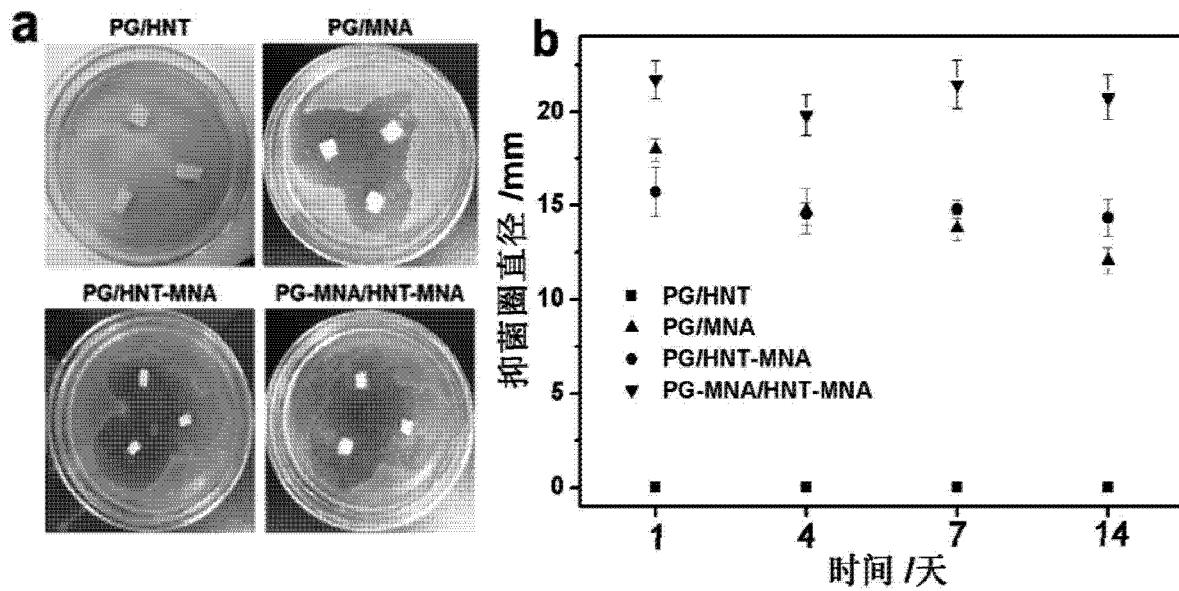
图 1



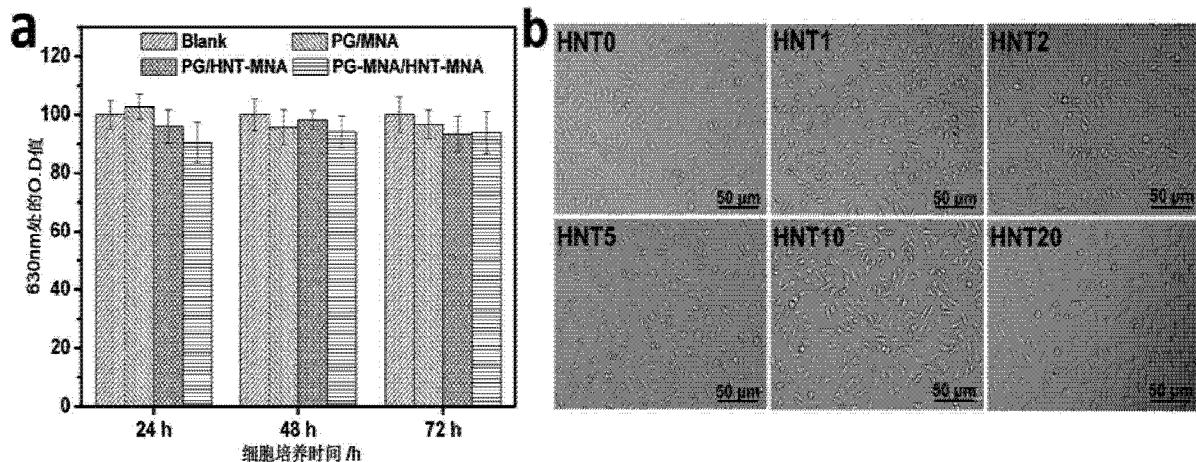
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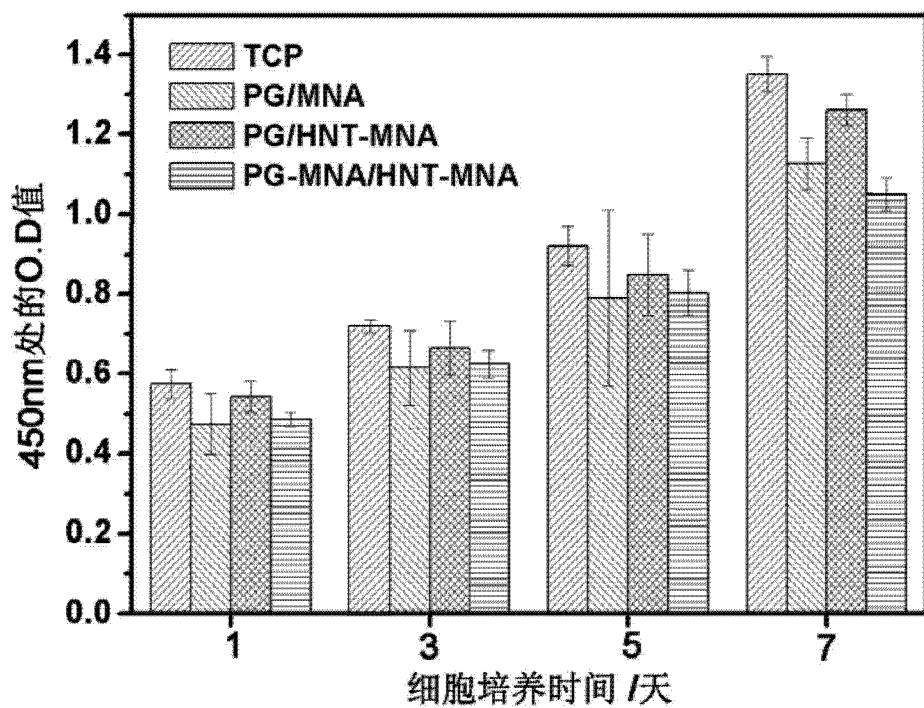
(2)



(3)



(4)



(5)

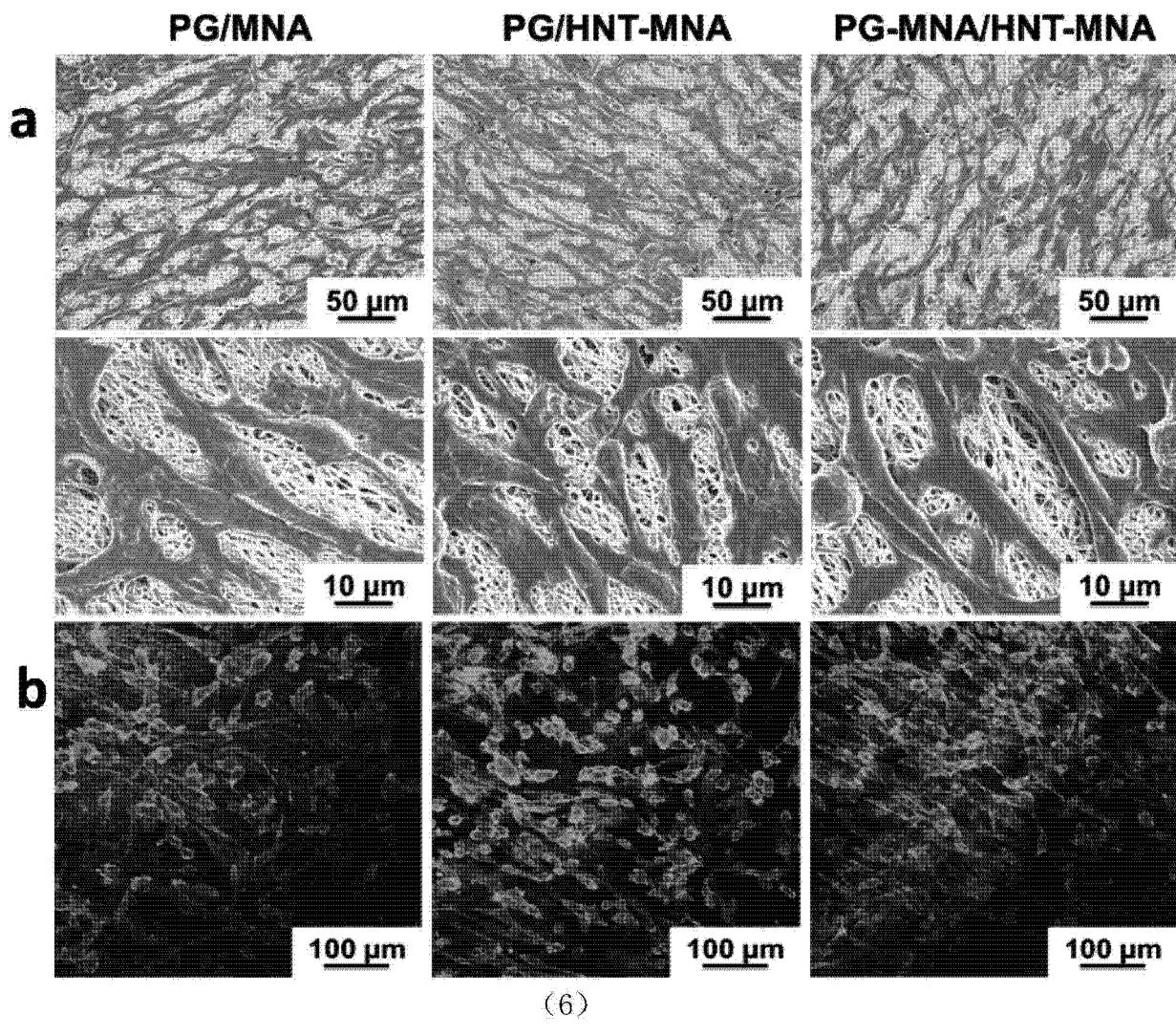


图 2

Patents

CN104524643A



> CN104524643A— Halloysite-nanotube-containing drug-loaded type guide tissue regeneration membrane and pre...

Halloysite-nanotube-containing drug-loaded type guide tissue regeneration membrane and preparation method thereof

Abstract

A halloysite-nanotube-containing drug-loaded type guide tissue regeneration membrane and a preparation method thereof are disclosed and belong to the field of biological materials. The membrane employs a degradable aliphatic polyester as a main raw material, and the raw material is added with an antibacterial medicine and a halloysite nanotube supporting the antibacterial medicine, so that a single-layer guide tissue regeneration membrane is prepared through electrostatic spinning; or the membrane employs a blended material of a degradable aliphatic polyester and a degradable natural high molecule as a compact layer, employs a degradable aliphatic polyester, a degradable natural high-molecular material and a bioactive particle as a loosen layer, and the compact layer is added with an antibacterial medicine and a halloysite nanotube supporting the antibacterial medicine, so that a double-layer guide tissue regeneration membrane possessing different pore structures and bioactivity is prepared through electrostatic spinning. The material possesses excellent biocompatibility, is capable of effectively preventing fiber cells and the like from growing into a tissue defect position, also helps to promote regeneration restore of tissue, does not need secondary operation, and is capable of inhibiting post-operation easily-happened bacterial infection and inflammation through controllable and long-term medicine release.

CN104524643A

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External links: Espacenet, Global Dossier, Discuss

Claims (9)

Hide Dependent ^

1. containing halloysite nanotubes medicine carrying type guide tissue regeneration film, it is characterized in that: using the fiber of degradable aliphatic polyester formation as major matrix material, and containing being loaded with the halloysite nanotubes of antibacterials, there is monolayer or double-layer porous structure, wherein:

(1) fiber that formed in electrostatic spinning using degradable aliphatic polyester or degradable aliphatic polyester and the high molecular mixture of degradable natural of monofil is as matrix material, and adds antibacterials and be loaded with the halloysite nanotubes of antibacterials; Degradable aliphatic polyester and the high molecular mass ratio of degradable natural are 10/90-90/10, be loaded with the halloysite nanotubes quality of antibacterials and the ratio of degradable aliphatic polyester and degradable natural macromolecule gross mass is 1/100-40/100, the quality being wherein loaded with the halloysite nanotubes of antibacterials is pure halloysite nanotubes and the gross mass being loaded into medicine, and the quality that following indication is loaded with the halloysite nanotubes of antibacterials is pure halloysite nanotubes and the gross mass being loaded into antibacterials; The ratio of the antibacterials quality in direct loading fiber and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100; Architectural feature is the fibre structure with random arrangement, and average bridging aperture is 2-6 μm, and fibre diameter is 200-1000nm, and film thickness is 50-500 μm;

(2) duplication is made up of compacted zone and weaker zone;

A) fiber that formed in electrostatic spinning with degradable aliphatic polyester and the high molecular mixture of degradable natural of compacted zone is for matrix material, and add antibacterials and be loaded with the halloysite nanotubes of antibacterials, wherein degradable aliphatic polyester and the high molecular mass ratio of degradable natural are 10/90-90/10, be loaded with the halloysite nanotubes quality of antibacterials and the ratio of degradable aliphatic polyester and degradable natural macromolecule gross mass is 1/100-40/100, the ratio of the quality of the antibacterials in direct loading fiber and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100, architectural feature is have fibre structure that is random or latticed array, and average pore size is 2-6 μm, and fibre diameter is 200nm-1200nm, and film thickness is 25-500 μm,

B) fiber that formed with degradable aliphatic polyester and inorganic bioactivity particle co-blended spinning of weaker zone, or co-blended spinning fiber prepared by degradable aliphatic polyester and degradable natural macromolecular material and inorganic bioactivity particle is matrix material, selectivity adopts cross-linking agent to be cross-linked degradable natural macromolecular material; Wherein degradable aliphatic polyester and the high molecular mass ratio of degradable natural are 10/90-90/10, and the ratio of inorganic bioactivity mass particle and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100; Weaker zone architectural feature is average pore size 5-100 μm, and fibre diameter is 200nm-7 μm, and thickness is 25-500 μm.

2. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, degradable aliphatic polyester comprises: polylactic acid, polycaprolactone, Poly(D,L-lactide-co-glycolide, polylactic acid-caprolactone copolymer, poly lactic-co-glycolic acid-caprolactone copolymer one or more mixture wherein; Degradable natural macromolecular material comprises: one or more the mixture in NTx, gelatin, chitosan, starch, cellulose, elastin laminin.

3. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, antibacterials, comprise penicillins, cephamycin class, Tetracyclines, chloromycetin, macrolide, lincomycin, fluoroquinolones, nitro glyoxaline, polypeptide class antimicrobial drug; Antibacterials are loaded into halloysite nanotubes, again the halloysite nanotubes of medicine carrying is joined in fiber by blending method, in order to improve drug loading, directly add another part medicine in the fibre, this some drugs directly added in fiber is identical or different from the medicine be loaded in galacteptide pipe simultaneously.

4. according to claim 1ly it is characterized in that containing halloysite nanotubes medicine carrying type guide tissue regeneration film, inorganic bioactivity particle comprises: particle size is one or more the mixture in the hydroxyapatite of 1-100nm, bata-tricalcium phosphate and bioactivity glass particle.

5. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, cross-linking agent comprises: the one in formaldehyde, glutaraldehyde, genipin, 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimides (EDC), N-hydroxy-succinamide (NHS).

6. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, the preparation method of monolayer guide tissue regeneration film has the following steps:

(1) getting antibacterials 1 joins in its good solvent, and fully stirring is ultrasonic makes it dissolve to obtain its saturated solution A;

(2) get halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B solution evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, by the good solvent washing and precipitating of antibacterials 1; Repeat vacuum step at least three times, the halloysite nanotubes being loaded with antibacterials 1 can be obtained;

(3) by the above-mentioned halloysite nanotubes drying being loaded with antibacterials 1, grinding;

(4) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains the solution C that degradable aliphatic polyester mass concentration is 0.04-0.2g/mL;

(5) in C solution, antibacterials 2 are added, the halloysite nanotubes being loaded with antibacterials 1 is added after room temperature magnetic agitation 6-12h, magnetic agitation 6-12h, obtain the solution D that degradable aliphatic polyester mass concentration is 0.04-0.2g/mL, be loaded with the halloysite nanotubes of antibacterials 1 and the mass ratio 1/100-40/100 of degradable aliphatic polyester in solution D, antibacterials 2 and degradable aliphatic polyester mass ratio be 5/100-50/100;

(6) carrying out electrostatic spinning by solution D, take stainless steel drum as receiving system, and cylinder slewing rate is 100-600rpm, spinning liquid flow rate is 0.5-10mL/h, voltage 7-20kV, receiving range 8-30cm, spinning 0.5-30h, obtains the electricity spinning fibre film of thickness 50-500 μm;

(7), after electrostatic spinning terminates, spinning film room temperature in fume hood is placed 2-7 days, package sterilization.

7. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, the preparation method of double-deck guide tissue regeneration film has the following steps:

(1) getting antibacterials 1 joins in its good solvent, stirs and makes it dissolve to obtain its saturated solution A;

(2) get halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By B solution evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, by the good solvent washing and precipitating of antibacterials 1, repeats above-mentioned vacuum step at least 3 times, can obtain the halloysite nanotubes being loaded with antibacterials;

(3) by the above-mentioned halloysite nanotubes drying being loaded with antibacterials 1, grinding;

(4) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains the solution C that degradable aliphatic polyester mass concentration is 0.04-0.2g/mL;

(5) be dissolved in organic solvent by degradable natural macromolecular material, room temperature magnetic agitation 6-24h, obtaining degradable natural high molecule mass concentration is the solution D of 0.04-0.2g/mL;

(6) solution C fully mixes with solution D by example according to a certain volume, room temperature magnetic agitation 6-12h, obtain degradable aliphatic polyester and degradable natural macromolecule total mass concentration is the solution E of 0.04-0.2g/mL, in solution E, the mass ratio of degradable aliphatic polyester and degradable natural macromolecular material is 10/90-90/10;

(7) in solution E, antibacterials 2 are added, room temperature lower magnetic force adds the halloysite nanotubes being loaded with antibacterials 1 after stirring 6-12h, room temperature magnetic agitation 6-12h, obtain the solution F that degradable aliphatic polyester and degradable natural macromolecule total mass concentration are 0.04-0.2g/mL, the quality of the halloysite nanotubes of antibacterials 1 is loaded with and the ratio of degradable aliphatic polyester and degradable natural macromolecule gross mass is 1/100-40/100 in solution F, the ratio of the quality of antibacterials 2 and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100,

(8) in solution F, bioactive particles is added, ultrasonic 30min-2h, room temperature lower magnetic force stirs 6-12h, obtain the solution G that degradable aliphatic polyester and degradable natural macromolecule total mass concentration are 0.04-0.2g/mL, wherein the ratio of inorganic bioactivity mass particle and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100;

(9) in solution E, bioactive particles is added, ultrasonic 30min-2h, room temperature lower magnetic force stirs 6-12h, obtain degradable aliphatic polyester and degradable natural macromolecule total mass concentration is the Solution H of 0.04-0.2g/mL, wherein the ratio of inorganic bioactivity mass particle and degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100;

(10) carrying out electrostatic spinning with solution F, take stainless steel drum as receiving system, and cylinder slewing rate is 100-600rpm, spinning liquid flow rate is 0.5-10mL/h, voltage 7-20kV, receiving range 8-30cm, spinning 0.5-15h, obtains the fine and close tunic of thickness 25-250 μm;

(11) on the basis of fine and close tunic, electrostatic spinning is carried out by solution G or Solution H, take stainless steel drum as receiving system, cylinder slewing rate is 100-600rpm, spinning liquid flow rate is 0.5-10mL/h, voltage 7-20kV, receiving range 8-30cm, spinning 0.5-20h, obtains the loose tunic of thickness 25-500 μm;

(12) after electrostatic spinning terminates, electricity spinning fibre film being immersed in mass concentration is crosslinked 10min-12h in the alcoholic solution of the cross-linking agent of 0.01%-3%, embathe 5-10 time in deionized water after immersion terminates, spinning film room temperature in fume hood is placed 2-7 days, package sterilization.

8. the halloysite nanotubes medicine carrying type guide tissue regeneration film that contains according to claim 1 is characterized in that, the another kind of preparation method of double-deck guide tissue regeneration film has the following steps:

(1) degradable aliphatic polyester is dissolved in organic solvent, room temperature magnetic agitation 6-24h, obtains the solution A that degradable aliphatic polyester mass concentration is 0.04-0.2g/mL, be dissolved in organic solvent by degradable natural macromolecule, room temperature magnetic agitation 6-24h, obtaining degradable natural high molecule mass concentration is the solution B of 0.04-0.2g/mL, solution A mixes with solution B by example according to a certain volume, add antibacterials 2, the halloysite nanotubes being loaded with antibacterials 1 is added after room temperature magnetic agitation 6-12h, room temperature magnetic agitation 6-12h, obtain degradable aliphatic polyester and degradable natural macromolecule total mass concentration is the solution C of 0.04-0.2g/mL, degradable aliphatic polyester and the high molecular mass ratio 10/90-90/10 of degradable natural in solution C, be loaded with the halloysite nanotubes quality of antibacterials 1 and the ratio of degradable aliphatic polyester and degradable natural macromolecule gross mass is 1/100-40/100, the ratio of the quality of antibacterials 2 and

degradable aliphatic polyester and degradable natural macromolecule gross mass is 5/100-50/100, carrying out electrostatic spinning by solution C, take stainless steel drum as receiving system, and cylinder slewing rate is 100-600rpm, spinning liquid flow rate is 0.5-10mL/h, voltage 7-20kV, receiving range 8-30cm, spinning 0.5-30h, obtains the fine and close tunic of thickness 25-500 μm,

(2) fine and close tunic is layered on bottom Flat bottom container, by the composition material degradable aliphatic polyester of weaker zone and degradable natural macromolecule and bioactive particles be dissolved in organic solvent, then the surface of fine and close tunic is cast in, by it at-60 DEG C-at 20 DEG C after freezing 6-12h, put into vacuum desiccator vacuum drying 4-12h, obtain double-deck guide tissue regeneration film.

9. it is characterized in that containing halloysite nanotubes medicine carrying type guide tissue regeneration film according to claim 6-8 any one, the good solvent of described antibacterials is water, ethanol, acetone, toluene, hexafluoroisopropanol, trifluoroethanol, chloroform, methanol, dichloromethane, N, one or more mixed solvents in N'-dimethyl Methanamide, the solvent of degradable aliphatic polyester and degradable natural macromolecular material is hexafluoroisopropanol, trifluoroethanol, chloroform, methanol, dichloromethane, N, one or more mixed solvents in N'-dimethyl Methanamide.

Description

Containing halloysite nanotubes medicine carrying type guide tissue regeneration film and preparation method thereof

Technical field

The invention belongs to technical field of biological material, being specifically related to a kind of fiber formed using degradable aliphatic polyester as major matrix material loads antibacterials and is loaded with the guide tissue regeneration film material and preparation method thereof of halloysite nanotubes of antibacterials.

Background technology:

Guide tissue regeneration (Guide Tissue Regeneration, GTR) technology is the new technique grown up the beginning of the nineties at the end of the eighties. Its principle utilizes the physical barriers function of film damaged part and surrounding tissue to be isolated, and creates the organizational environment of a relative closure, thus make osteoblast priority migration, growth. The treatment being applied as periodontal disease of GTR, Dental implantation district Bone mineral change and the reparation of other Cranial defect, the healing of fracture provide a new effective way.

Current GTR membrane material can be divided into nonabsorbable and the large class of Absorbable rod two. Nonabsorbable guide tissue regeneration film is prepared primarily of polytetrafluoroethyl material material, and main representative product is Gore-Tex. Although poly tetrafluoroethylene has good mechanical strength, can not degrade voluntarily, need second operation to remove, increase misery and the surgery cost of patient, and cellular affinity is poor, easily causes wound dehiscence, film exposes in early days, affects the healing of wound. Therefore, the R and D of Absorbable membrane material are just become to the important directions of GTR technical development.

The most general GTR film of current application is based on natural material collagen, and representative products is the Biomend that the Bio-Gide that produces of Geistlich company of Switzerland and Zimmer company of the U.S. produce, respectively with pigskin collagen and the preparation of cattle heel string collagen. Wherein Bio-Gide is also the goldstandard of current clinical practice. Although natural collagen GTR film has good biocompatibility, its mechanical strength is relatively weak, and degraded is very fast, easily causes premature degradation to subside after implantation. In order to strengthen and extend degradation time, usually need the thickness adding thick film, this is unfavorable with regard to providing for plantation and reparation. In addition, the defects such as GTR film prepared by natural material also exists product quality and limits by raw material sources, expensive. The defect that the GTR film prepared to overcome natural collagen material exists, GTR product prepared by some artificial synthesized degradable materials is also come out one after another.

Although GTR technology can obtain curative effect steady in a long-term, because operation neutralization is postoperative and in secondary taking-up process, being incorporated into the infection that the anaerobic bacteria of damage location and aerobe etc. cause will affect its neoblastic acquisition. At present also whole body system medication is mainly taked for postoperative infection, but utilization ratio of drug is lower, and easily causes gastrointestinal side effect. Local delivery of drug can strengthen therapeutic effect, reduces untoward reaction.

Electrostatic spinning technique is a kind of simple general-purpose method preparing nanofiber, because its medicine load mode is simple, in electrostatic spinning or spinning last handling process, different medicines and biomacromolecule are easy to be loaded into fibrous inside and surface, in addition, antibacterials are being loaded into after in fiber and performance change can not occurring, still its anti-microbial property can be kept, prevention of postoperative infection can be used for. Therefore, the nanofiber medicine carrying membrane that prepared by Electrospun has good potential applicability in clinical practice.

When being prepared medicine carrying type guide tissue regeneration film by electrostatic spinning, medicine joins fibrous inside and surface by blended mode, drug release rate is than very fast, existing dashes forward releases phenomenon, be transplanted to 5-7 days of human body at guide tissue regeneration film after, drug release reaches 80-90% substantially, although within postoperative one week, be the high-risk period occurring to infect, in process of tissue reparation, the generation of infection conditions will continue at least 2 weeks. In the process of tissue repair, infection mostly is mixed infection, i.e. anaerobe and aerobe doping, two kinds of mutual growth promoting effects of antibacterial, therefore, when Prevention and Curation guided tissue regeneration postoperative infection, must adopt and respectively curative effect be improved to the medicine of aerobe and anaerobe sensitivity. Therefore a kind of drug release rate thus the guide tissue regeneration film realizing the sustainable release of medicine but also can kill anaerobe and aerobe is simultaneously very important of not only can having slowed down is studied.

Halloysite nanotubes is a kind of aluminosilicate of tubulose, and its external diameter is about 50-80nm, and internal diameter is about 10-15nm, and length is approximately 1000nm, and load factor is approximately 15%-20%. The draw ratio of halloysite nanotubes is 20-50, and in polymer fiber, orientation can the mechanical property of remarkable fortifying fibre. Halloysite nanotubes has natural origin, to environment, and can not have good biocompatibility. As tubular container, halloysite nanotubes is used widely in cosmetics, drug release, medical transplanting (as dental filling) etc.

Antibacterials and the halloysite nanotubes being loaded with antibacterials is added in polymeric system, pass through electrostatic spinning technique, prepare monolayer and double-layer antimicrobial antiinflammatory type medicine carrying guide tissue regeneration film, if have higher requirement to the degradation rate of obtained guide tissue regeneration film or mechanical property and resistance to water, can also the natural polymer in polymeric matrix be cross-linked. The medicine carrying of this material is divided into two parts, and a part is the antibacterials being directly present in fibrous inside and fiber surface, and another part is present in the halloysite nanotubes of fibrous inside. The antibacterials being directly present in fibrous inside diffuse to fiber surface and then are transferred to damage location; First antibacterials in fibrous inside halloysite nanotubes will diffuse into fibrous inside from halloysite nanotubes, and then diffuse to fiber surface from fibrous inside, finally diffuse to damage location from fiber surface again, thus slow down the speed of drug release, alleviating dashes forward releases phenomenon. The design can improve the drug loading of nano fibrous membrane, slows down drug release rate, and alleviating dashes forward releases phenomenon; Meanwhile, fiber and halloysite nanotubes carrying medicament can be different, to play the effect killing anaerobe and aerobe simultaneously.

Accompanying drawing explanation

Fig. 1 is the relevant implementation result figure that monolayer polycaprolactone-gelatin (mass ratio is 9:1) prepared by the inventive method carries the monolayer guide tissue regeneration film of not commensurability halloysite nanotubes (HNT) (1%-20% mass ratio), in picture, the naming method of sample is: HNT0 represents that the ratio containing metronidazole halloysite nanotubes quality and polycaprolactone and gelatin gross mass in spinning PG/HNT nano fibrous membrane is 0%,

HNT20 represents that the ratio of halloysite nanotubes quality and polycaprolactone and gelatin gross mass in spinning PG/HNT nano fibrous membrane is 20%, other samples are analogized.

(1) be above-mentioned PG/HNT monolayer spinning fibre film SEM photo.

(2) be load-deformation curve under above-mentioned PG/HNT monolayer spinning fibre film hygrometric state, a is along catcher direction of rotation, the hot strength of not adding the nano fibrous membrane of halloysite nanotubes is about 7.3MPa, add material after halloysite nanotubes hot strength be improved significantly, elongation at break is without significant change.

(3) be above-mentioned PG/HNT monolayer spinning fibre film water contact angle schematic diagram.

(4) be contrary side form SEM photo after above-mentioned PG/HNT monolayer spinning fibre theca cell propagation.

(5) be the SEM of L929 cell when growing 5 days on above-mentioned PG/HNT monolayer spinning fibre film and laser confocal microscope photo, (a) (g) HNT0, (b) (h) HNT1, (c) (i) HNT2, (d) (j) HNT5, (e) (k) HNT 10, and (f) (l) HNT20, and L929 cell (m) on above-mentioned PG/HNT-MNA monolayer spinning fibre film adheres to the O.D value (O.D value tests use CCK-8 method) that 4h and (n) grow different time, test result shows the prolongation along with the time, cell can adhere to propagation on material.

Fig. 2 is material item performance test comparison diagram prepared by different medicine carrying mode, HNT-MNA refers to the halloysite nanotubes being loaded with antibacterials metronidazole preparing gained according to method described in embodiment 1, PG-MNA refers in polycaprolactone/gelatine nano fiber (mass ratio is 9:1) and is directly loaded into antibacterials metronidazole (mass ratio of 20%), PG/HNT-MNA refers in polycaprolactone/gelatin (mass ratio 9:1) fiber the halloysite nanotubes (mass ratio of 20%) containing metronidazole adding and prepare gained according to method described in embodiment 1, PG-MNA/HNT-MNA refers to the fibrous membrane prepared according to method described in embodiment 4, wherein polycaprolactone and gelatin mass ratio are 9:1, in fiber, directly the loading quality of metronidazole and the ratio of polycaprolactone and gelatin gross mass are 20%, be loaded with the halloysite nanotubes of metronidazole and the ratio of polycaprolactone and gelatin gross mass is 20%.

(1) be the drug release patterns of MNA release in the HNT being loaded with MNA.

(2) be that HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane is immersed in PBS buffer solution Chinese medicine release conditions.

(3) to be the bacteriostatic test situation of carrying out on a lbmc agar plate: a be when cultivating one under 37 DEG C of anoxia conditions, HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane inhibition zone situation; B is the time dependent situation of HNT-MNA, PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane antibacterial circle diameter.

(4) be cell toxicity test situation: a be that L929 cell grows 24h respectively in PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane lixiviating solution, the O.D value test result block diagram that 48h, 72h are later; B, c, d are the cellular morphology figure after L929 cell grows 72h respectively in blank and PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane lixiviating solution respectively.

(5) be cell proliferation experiment situation: L929 cell grows the O.D test result block diagram after 1 day, 3 days, 5 days, 7 days on blank and PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane.

(6) to be L929 cell be SEM photo after L929 cell grows 5 days on blank and PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane to a, and to be L929 cell be laser confocal microscope photo after L929 cell grows 5 days on blank and PG-MNA, PG/HNT-MNA, PG-MNA/HNT-MNA monolayer nano fibrous membrane to b.

Detailed description of the invention

Further illustrate the present invention below by embodiment, but the present invention is not limited to these examples.

Embodiment 1

1. getting 4g antibacterials metronidazole joins in trifluoroethanol, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, uses trifluoroethanol washing and precipitating, repeats vacuum step 3 times, can obtain the halloysite nanotubes being loaded with metronidazole; By the above-mentioned halloysite nanotubes drying being loaded with metronidazole, grinding.

2. 2g polycaprolactone is dissolved in 7.2g N, in the mixed solvent of N-dimethylformamide and 10.8g dichloromethane, 0.1g metronidazole is added after room temperature magnetic agitation 24h, the halloysite nanotubes that 0.01g is loaded with metronidazole is added after room temperature magnetic agitation 12h, solution C is obtained after room temperature magnetic agitation 6h, in solution C, polycaprolactone mass concentration is 10%, the mass ratio of the halloysite nanotubes and polycaprolactone that are loaded with metronidazole is 5%, get solution C and carry out electrostatic spinning, take stainless steel drum as receiving system, drum rotation speed is 200rpm, voltage 10KV, receiving range is 16cm, spinning liquid feed liquor speed 4mL/h, spinning 5h. Obtain the monolayer guide tissue regeneration film that thickness is about 400 μm .

3. the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, ensures that remaining DMF and dichloromethane fully volatilize.

Embodiment 2

1, getting 4g antibacterials quadraacycline joins in deionized water, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble occur that centrifugal rear taking-up supernatant, by washed with de-ionized water precipitation, repeats vacuum step 3 times, can obtain the halloysite nanotubes being loaded with quadraacycline; By the above-mentioned halloysite nanotubes drying being loaded with quadraacycline, grinding.

2,2g polycaprolactone is dissolved in 5.6g dichloromethane and 2.4gN, in the mixed organic solvents of N-dimethylformamide, 1g antibacterials metronidazole is added after room temperature magnetic agitation 24h, the halloysite nanotubes that 0.8g is loaded with quadraacycline is added again after room temperature magnetic agitation 12, solution C is obtained after room temperature magnetic agitation 6h, in solution C, polycaprolactone mass concentration is 20%, the mass ratio of metronidazole and polycaprolactone is 50%, and the mass ratio of the halloysite nanotubes and polycaprolactone that are loaded with quadraacycline is 40%.

3, room temperature is got solution C and is carried out electrostatic spinning, and take stainless steel drum as receiving system, cylinder slewing rate is 600rpm, spinning liquid flow rate is 3mL/h, voltage 12KV, receiving range 15cm, spinning 5h, obtains the monolayer guide tissue regeneration film of thickness about 300 μm .

4, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, residual dichloromethane and DMF are fully volatilized.

Embodiment 3

1, getting 6g antibacterials metronidazole joins in trifluoroethanol, stirs and makes it dissolve to obtain its saturated solution A; Get 1.5g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance,

centrifugal rear taking-up supernatant, uses trifluoroethanol washing and precipitating, repeats vacuum step 3 times, can obtain the halloysite nanotubes being loaded with metronidazole; By the above-mentioned halloysite nanotubes drying being loaded with metronidazole, grinding.

2,1.5g polycaprolactone is dissolved in 13.5g trifluoroethanol, after room temperature magnetic agitation 24h, obtains the solution C that polycaprolactone mass concentration is 10%;

3,1.5g polylactic acid is dissolved in 13.5g trifluoroethanol, room temperature magnetic agitation 24h, obtains the solution D that polylactic acid mass concentration is 10%;

4, solution C is fully mixed with solution D, add 0.6g quadraacycline, the halloysite nanotubes that 1.2g is loaded with metronidazole is added after room temperature magnetic agitation 12h, magnetic agitation 6h, obtain solution E, in solution E, polycaprolactone and polylactic acid total mass concentration are 10%, and the mass ratio of polycaprolactone and polylactic acid is 1:1, the ratio of quadraacycline quality and polycaprolactone and polylactic acid gross mass is 20%, is loaded with the halloysite nanotubes quality of metronidazole and the ratio of polycaprolactone and polylactic acid gross mass is 40%;

5, room temperature spinning liquid E carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 200rpm, spinning liquid flow rate is 2mL/h, voltage 13kV, receiving range 20cm, spinning 10h, obtains the monolayer electrospun fiber membrane of thickness about 300 μm .

6, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining trifluoroethanol is fully volatilized.

Embodiment 4

1, getting 4g antibacterials metronidazole adds in trifluoroethanol, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, uses trifluoroethanol washing and precipitating. Repeat vacuum step 3 times, the halloysite nanotubes being loaded with metronidazole can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with metronidazole, grinding.

2,1.08g polycaprolactone is dissolved in 10g hexafluoroisopropanol, after room temperature magnetic agitation 24h, obtains solution C;

3, be dissolved in 8.8g hexafluoroisopropanol by 0.72g gelatin, room temperature magnetic agitation 24h, obtains solution D;

4,0.24g metronidazole is added after solution C fully being mixed with solution D, the halloysite nanotubes that 0.24g is loaded with metronidazole is added after room temperature magnetic agitation 12h, solution E is obtained after room temperature magnetic agitation 6h, in solution E, polycaprolactone and gelatin total mass concentration are 6%, the mass ratio of polylactic acid and gelatin is 9:1, the ratio of the metronidazole quality in direct loading fiber and polycaprolactone and gelatin gross mass is 20%, is loaded with the halloysite nanotubes quality of metronidazole and the ratio of polycaprolactone and gelatin gross mass is 20%;

5, room temperature spinning liquid E carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 300rpm, spinning liquid flow rate 1mL/h, voltage 10kV, receiving range 15cm, spinning 20h, obtains the electrospinning cortina of thickness about 250 μm ;

6, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining hexafluoroisopropanol is fully volatilized.

Embodiment 5

1, getting 4g antibacterials quadraacycline joins in deionized water, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, precipitates by washed with de-ionized water. Repeat vacuum step 3 times, the halloysite nanotubes being loaded with quadraacycline can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with quadraacycline, grinding.

2,0.8g polycaprolactone is dissolved in 9.2g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution C that polycaprolactone mass concentration is 8%;

3,0.8g chitosan is dissolved in 9.2g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution D that chitosan mass concentration is 8%;

4, be dissolved in 9.2g hexafluoroisopropanol by 0.8g gelatin, room temperature magnetic agitation 24h, obtaining gelatin mass concentration is 8% spinning liquid E;

5,0.4g metronidazole is added wherein after 7g solution C fully being mixed with 3g solution D, the halloysite nanotubes that 0.32g is loaded with quadraacycline is added wherein after room temperature magnetic agitation 12h, spinning liquid F is obtained after room temperature magnetic agitation 6h, the mass ratio 3:7 of chitosan and polycaprolactone in spinning liquid F, the ratio of metronidazole quality and chitosan and polycaprolactone gross mass is 50%, is loaded with the halloysite nanotubes quality of quadraacycline and the ratio of chitosan and polycaprolactone gross mass is 40%;

6,8g solution C fully mixed with 2g solution E, obtain the solution G that polycaprolactone and gelatin total mass concentration are 8%, in solution G, the mass ratio of polycaprolactone and gelatin is 4:1;

7, room temperature spinning liquid F carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 300rpm, spinning liquid flow rate 2mL/h, voltage 10kV, receiving range 15cm, spinning 10h, obtains the fine and close tunic of Electrospun that thickness is about 300 μm ;

8, change spinning liquid G to proceed on the fibrous membrane that electrostatic spinning spins in step 5, spinning condition is cylinder slewing rate is 200rpm, and spinning liquid flow rate is 4mL/h, voltage 12kV, receiving range 15cm, spinning 5h, obtain the double-deck guide tissue regeneration film of gross thickness about 500 μm .

9, spinning fibre film is placed in the alcoholic solution that mass concentration is the genipin of 0.5%, makes cross-link gelatin, after cross-linking reaction 30min, film is embathed 10 times in deionized water, wash away unreacted genipin and alcohol solvent.

10, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining hexafluoroisopropanol and ethanol are fully volatilized.

Embodiment 6

1, getting 4g antibacterials metronidazole joins in trifluoroethanol, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, uses trifluoroethanol washing and precipitating. Repeat vacuum step 3 times, the halloysite nanotubes of metronidazole can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with metronidazole, grinding.

2,0.6g polycaprolactone is dissolved in 9.4g hexafluoroisopropanol, room temperature magnetic agitation 12h, obtains the solution C that polycaprolactone mass concentration is 6%;

3,0.6g polylactic acid is dissolved in 9.4g hexafluoroisopropanol, room temperature magnetic agitation 12h, obtains the solution D that polylactic acid mass concentration is 6%;

4.0.6g collagen is dissolved in 9.4g hexafluoroisopropanol, room temperature magnetic agitation 12h, obtains the solution E that collagen mass concentrations on collagen is 6%;

5.0.12g quadrcycline is added after 4g solution C fully being mixed with 6g solution D, the halloysite nanotubes that 0.18g is loaded with metronidazole is added after room temperature magnetic agitation 12h, spinning liquid F is obtained after room temperature magnetic agitation 6h, in spinning liquid F, polycaprolactone and polylactic acid total mass concentration are 6%, the mass ratio 2:3 of polylactic acid and polycaprolactone, the ratio of quadrcycline quality and polycaprolactone and polylactic acid gross mass is 20%, is loaded with the halloysite nanotubes quality of metronidazole and the ratio of polycaprolactone and polylactic acid gross mass is 30%;

6,5g solution C fully mixed with 5g solution E, magnetic agitation 12h, obtain the mass ratio 1:1 of collagen and polycaprolactone, collagen and polycaprolactone total mass concentration are the spinning liquid G of 6%;

7, room temperature spinning liquid F carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 500rpm, spinning liquid flow rate 3mL/h, voltage 12kV, receiving range 18cm, spinning 5h, obtains the fine and close tunic that thickness is about 200 μm ;

8, change spinning liquid G to proceed on the fibrous membrane that electrostatic spinning spins in step 6, spinning condition is cylinder slewing rate is 200rpm, and spinning liquid flow rate is 1mL/h, voltage 12kV, receiving range 18cm, spinning 15h, obtain the double-deck guide tissue regeneration film of gross thickness about 400 μm .

9, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining hexafluoroisopropanol is fully volatilized.

Embodiment 7

1, getting 4g antibacterials cephamicin joins in deionized water, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, precipitates by washed with de-ionized water. Repeat vacuum step 3 times, the halloysite nanotubes being loaded with cephamicin can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with cephamicin, grinding.

2,0.6g polycaprolactone is dissolved in 9.4g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution C that polycaprolactone mass concentration is 6%;

3,0.6g gelatin is dissolved in hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution D that gelatin mass concentration is 6%;

4, in solution C, 0.3g metronidazole is added, the halloysite nanotubes that 0.24g is loaded with cephamicin is added after room temperature magnetic agitation 12h, room temperature magnetic agitation 6h, obtain solution E, in solution E, the mass ratio of metronidazole and polycaprolactone is 50%, and the halloysite nanotubes and the polycaprolactone mass ratio that are loaded with cephamicin are 40%;

5,0.12g nanometer hydroxyapatite is added after 5g solution C fully being mixed with 5g solution D, ultrasonic 1h, magnetic agitation 12h, obtain spinning liquid F, in spinning liquid F, gelatin and polycaprolactone total mass concentration are 6%, the mass ratio 1:1 of gelatin and polycaprolactone, the ratio of nanometer hydroxyapatite quality and gelatin and polycaprolactone gross mass is 20%;

6, room temperature spinning liquid E carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 300rpm, spinning liquid flow rate 1.5mL/h, voltage 10kV, receiving range 18cm, spinning 10h, obtains the fine and close tunic of Electrospun of thickness about 200 μm ;

7, change spinning liquid F to proceed on the fibrous membrane that electrostatic spinning spins in step 5, spinning condition is cylinder slewing rate is 300rpm, and spinning liquid flow rate is 1mL/h, voltage 13kV, receiving range 18cm, spinning 15h, obtain the double-deck guide tissue regeneration film of gross thickness about 400 μm .

8, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining hexafluoroisopropanol is fully volatilized.

Embodiment 8

1, getting 4g antibacterials quadrcycline joins in deionized water, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance, centrifugal rear taking-up supernatant, precipitates by washed with de-ionized water. Repeat vacuum step 3 times, the halloysite nanotubes being loaded with quadrcycline can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with quadrcycline, grinding.

2,0.8g polycaprolactone is dissolved in 9.2g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution C that polycaprolactone mass concentration is 8%;

3,0.8g collagen is dissolved in 9.2g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution D that collagen mass concentrations on collagen is 8%;

4,0.8g chitosan is dissolved in 9.2g hexafluoroisopropanol, room temperature magnetic agitation 24h, obtains the solution E that chitosan mass concentration is 8%;

5,7g solution C is fully mixed with 3g solution D, add 0.24g chloromycetin, the halloysite nanotubes that 0.16g is loaded with quadrcycline is added after room temperature magnetic agitation 12h, magnetic agitation 6h, obtain spinning liquid F, in spinning liquid F, collagen and polycaprolactone total mass concentration are 8%, the mass ratio 3:7 of collagen and polycaprolactone, the ratio of chloromycetin quality and collagen and polycaprolactone gross mass is 30%, is loaded with the halloysite nanotubes quality of quadrcycline and the ratio of collagen and polycaprolactone gross mass is 20%;

6,6g solution C is mixed with 4g solution E, and add 0.24g bioactivity glass, magnetic agitation 12h, obtain spinning liquid G, the wherein mass ratio 2:3 of chitosan and polycaprolactone, chitosan and polycaprolactone total mass concentration are 8%, and the ratio of bioactivity glass quality and chitosan and polycaprolactone gross mass is 30%;

7, room temperature spinning liquid F carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 300rpm, spinning liquid flow rate 2mL/h, voltage 12kV, receiving range 18cm, spinning 5h, obtains the fine and close tunic of Electrospun of thickness about 150 μm ;

8, change spinning liquid G to proceed on the fibrous membrane that electrostatic spinning spins in step 6, spinning condition is cylinder slewing rate is 300rpm, and spinning liquid flow rate is 1mL/h, voltage 12kV, receiving range 18cm, spinning 10h, obtain the double-deck guide tissue regeneration film that gross thickness is about 300 μm .

9, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, remaining hexafluoroisopropanol is fully volatilized.

Embodiment 9

1, getting 4g antibacterials metronidazole joins in trifluoroethanol, stirs and makes it dissolve to obtain its saturated solution A; Get 1g halloysite nanotubes to mix with solution A, ultrasonic disperse obtains the suspension B of halloysite nanotubes; By suspension B evacuation until liquid surface no longer includes bubble appearance,

centrifugal rear taking-up supernatant, uses trifluoroethanol washing and precipitating. Repeat vacuum step 3 times, the halloysite nanotubes of metronidazole can be obtained; By the above-mentioned halloysite nanotubes drying being loaded with metronidazole, grinding.

2,1g polycaprolactone is dissolved in 5.4g dichloromethane and 3.6gN, in the mixed organic solvents of N-dimethylformamide, room temperature magnetic agitation 24h, add 0.4g cephalexin, add the halloysite nanotubes that 0.4g is loaded with metronidazole after room temperature magnetic agitation 12h, obtain spinning liquid C after room temperature magnetic agitation 6h, in spinning liquid C, polycaprolactone mass concentration is 10%, the mass ratio of cephalexin and polycaprolactone is 40%, and the mass ratio of the halloysite nanotubes and polycaprolactone that are loaded with metronidazole is 40%;

3, room temperature spinning liquid C carries out electrostatic spinning, take stainless steel drum as receiving system, and cylinder slewing rate is 500rpm, and spinning liquid flow rate is 3mL/h, voltage 12kV, receiving range 15cm, spinning 5h, obtains the electricity spinning fibre film of thickness about 200 μm.

4, the fibrous membrane obtained is placed in the dry 72h of fume hood under room temperature, residual dichloromethane and DMF are fully volatilized.

5, be laid in bottom culture dish by above-mentioned fibrous membrane, mass concentration of casting thereon is the aqueous gelatin solution of 20%, and-40 DEG C of lyophilization 12h, obtain duplicature.

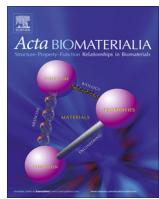
Patent Citations (5)

Publication number	Priority date	Publication date	Assignee	Title
CN101889972A *	2010-06-23	2010-11-24	东华大学	Preparation of TCH/HNTs/PLGA nanometer composite fibre medicine carrying system
CN102952385A *	2012-10-29	2013-03-06	暨南大学	Modified halloysite nanotube / biodegradable polyester composite material and preparation method thereof
CN103316376A *	2013-05-27	2013-09-25	暨南大学	Endellite composite chitosan haemostasis and wound restoration sponge material, preparation and applications
CN103736153A *	2013-12-30	2014-04-23	北京市创伤骨科研究所	Single-layer and double-layer polycaprolactone-based guided tissue regeneration membranes and preparation method thereof
CN103948974A *	2013-12-30	2014-07-30	北京化工大学	Drug-loading type guided tissue regeneration membrane and preparation method thereof
Family To Family Citations				

* Cited by examiner, † Cited by third party

Cited By (13)

Publication number	Priority date	Publication date	Assignee	Title
CN104971376A *	2015-06-29	2015-10-14	北京化工大学	Medicine-carrying type elastomer wound dressing containing halloysite nanotube and preparation method of wound dressing
CN105920587A *	2016-05-12	2016-09-07	扬州大学	PH-sensitive insulin sustained-release oral preparation and preparation method thereof
CN106166305A *	2016-06-27	2016-11-30	北京市创伤骨科研究所	A kind of broad-spectrum antiseptic type wound care film and preparation method thereof
CN106730035A *	2016-12-30	2017-05-31	北京化工大学	A kind of preparation method comprising overloading medicine slow-released system bone renovating material
CN106798950A *	2016-11-30	2017-06-06	浙江工业大学	A kind of preparation method of the guide tissue regeneration film repaired for periodontitis
CN106975106A *	2017-03-31	2017-07-25	北京化工大学	A kind of double-deck Bone Defect Repari membrane material and preparation method thereof
CN107625995A *	2017-08-18	2018-01-26	北京市创伤骨科研究所	A kind of coaxial fiber Bone Defect Repari membrane material of multilayer and preparation method thereof
CN107875453A *	2017-11-09	2018-04-06	上海纳米技术及应用国家工程研究中心有限公司	Carry preparation method of Types of Medicine electrostatic spinning guide tissue regeneration film and products thereof and application
CN107982579A *	2017-11-21	2018-05-04	上海纳米技术及应用国家工程研究中心有限公司	3D printing carries preparation method of the nano combined artificial bone of Types of Medicine and products thereof and application
CN110621354A *	2017-07-21	2019-12-27	矿物快速护理有限公司	Novel wound dressing for hemostasis
CN112999430A *	2021-04-13	2021-06-22	健诺维(成都)生物科技有限公司	Oral cavity repairing film and preparation method thereof
CN114288476A *	2022-01-05	2022-04-08	奥精医疗科技股份有限公司	Artificial dura mater and preparation method thereof
CN114788901A *	2022-06-24	2022-07-26	北京熵图医疗科技合伙企业(有限合伙)	Iodine-containing surgical membrane and preparation method thereof



Full length article

Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering[☆]

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ABSTRACT

Regeneration of injured tendon and ligament (T&L) remains a clinical challenge due to their poor intrinsic healing capacity. Tissue engineering provides a promising alternative treatment approach to facilitate T&L healing and regeneration. Successful tendon tissue engineering requires the use of three-dimensional (3D) biomimetic scaffolds that possess the physical and biochemical features of native tendon tissue. We report here the development and characterization of a novel composite scaffold fabricated by co-electrospinning of poly-ε-caprolactone (PCL) and methacrylated gelatin (mGLT). We found that photocrosslinking retained mGLT, resulted in a uniform distribution of mGLT throughout the depth of scaffold and also preserved scaffold mechanical strength. Moreover, photocrosslinking was able to integrate stacked scaffold sheets to form multilayered constructs that mimic the structure of native tendon tissues. Importantly, cells impregnated into the constructs remained responsive to topographical cues and exogenous tenogenic factors, such as TGF-β3. The excellent biocompatibility and highly integrated structure of the scaffold developed in this study will allow the creation of a more advanced tendon graft that possesses the architecture and cell phenotype of native tendon tissues.

Statement of Significance

The clinical challenges in tendon repair have spurred the development of tendon tissue engineering approaches to create functional tissue replacements. In this study, we have developed a novel composite scaffold as a tendon graft consisting of aligned poly-ε-caprolactone (PCL) microfibers and methacrylated gelatin (mGLT). Cell seeding and photocrosslinking between scaffold layers can be performed simultaneously to create cell impregnated multilayered constructs. This cell-scaffold construct combines the advantages of PCL nanofibrous scaffolds and photocrosslinked gelatin hydrogels to mimic the structure, mechanical anisotropy, and cell phenotype of native tendon tissue. The scaffold engineered here as a building block for multilayer constructs should have applications beyond tendon tissue engineering in the fabrication of tissue grafts that consist of both fibrous and hydrogel components.

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1. Introduction

Tendons and ligaments are prone to injuries such as rupture and laceration due to their load-bearing nature [1,2]. In cases of severe tendon injury, surgical intervention is employed to repair or replace the damaged tendon with autografts, allografts, xenografts, or prosthetic devices [3–5], for the natural healing process is slow and insufficient [6,7]. To date, the clinical outcomes of tendon repair remain limited and unsatisfactory due to donor site morbidity, high failure rates, risk of injury recurrence, and limited long-term

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function restoration [8–10]. These limitations have spurred the development of tendon tissue engineering approaches, which apply combination of cells, scaffolds and bioactive molecules, as a promising strategy to create functional tissue replacements or to enhance the innate healing process [11,12]. Ultimately, tendon tissue engineering aims at improving the quality of healing in order to fully restore tendon structure and function [13].

Tendon tissues are composed of densely packed aligned collagen fibrils that connect muscle to bone [7,14]. Therefore, aligned nano- and micro-fibrous scaffolds fabricated by electrospinning have been extensively explored in attempts to recapitulate the mechanical and topographical characteristics of native tendon tissue [15–17]. Electrospun poly- ϵ -caprolactone (PCL) scaffolds are frequently used in tendon tissue engineering as well as applications for other soft tissues. PCL is an aliphatic linear polyester approved by the U.S. Food and Drug Administration for clinical use [18]. It is biocompatible, bioresorbable and a low-cost synthetic polymer. Of equal importance, PCL exhibits low degradation rate due to its semi-crystalline and hydrophobic nature [19,20], making it a suitable graft material to facilitate the relatively slow healing process of injured tendons [21,22]. However, the hydrophobic nature of PCL often results in poor wettability [23], inadequate cell attachment, and poor tissue integration [24] when used in tissue engineering. Moreover, as a synthetic polyester, its lack of bioactivity is a major challenge for PCL to direct cell behavior after seeding due to the absence of cell-binding motifs found in natural extracellular matrix (ECM) proteins [25].

Hydrogels prepared from collagen and its derivative, gelatin, represent another class of scaffolds for regenerating and repairing a wide variety of tissues and organs [26,27]. Unlike other types of scaffolds, hydrogels retain a large volume of water and thus provide a highly hydrated environment similar to that in native tissues. Cells encapsulated within collagen/gelatin hydrogels can be easily distributed homogeneously by simple mixing during gel preparation [28,29]. Importantly, collagen and gelatin, as constituents of natural ECM, better mimic at least in part the native tissue microenvironment, as compared to synthetic polymers [30,31]. Nevertheless, improvement in the mechanical properties and introduction of topographical cues are needed to apply these hydrogels to tendon grafts that aim at reproducing the mechanical and structural features of native tendon tissues.

The organization of native ECM may be viewed as a cell-containing hydrogel reinforced by structural fibers. An engineered scaffold consisting of hydrogels and electrospun fibers may thus be considered as a biomimetic of the ECM. For example, a microfiber-reinforced silk hydrogel displayed a greatly improved modulus compared to a fiber-free hydrogel [32]. In addition, hydrogels composed of natural proteins could provide the bioactive motifs absent from synthetic polymeric scaffolds to enhance control of cell binding and fate determination [28,33]. In terms of tendon tissue engineering, an ideal composite scaffold consisting of hydrogel and fibrous scaffold has yet to be developed. Consequently, little is known about the effects such a composite scaffold may have on the activities of encapsulated cells.

In this study, we have developed a novel composite scaffold as a tendon graft consisting of electrospun PCL microfibers and methacrylated gelatin (mGLT). We have optimized the retention of mGLT by photocrosslinking and its integration with the fibrous scaffold. Simultaneous cell seeding and photocrosslinking between scaffold layers were performed to create cell-impregnated multi-layered constructs, and their mechanical properties and architecture and the activity of encapsulated cells were assessed. Our results show that this novel cell-scaffold construct combines the advantages of PCL nanofibrous scaffolds and gelatin hydrogels to mimic the mechanical feature, structure and cell phenotype of native tendon tissue.

2. Materials and methods

2.1. Synthesis of methacrylated gelatin

Methacrylated gelatin (mGLT) was synthesized using an established protocol with slight modification [29,34]. Gelatin (GLT, Sigma-Aldrich) was dissolved in deionized H₂O at 37 °C (30%, w/v). Methacrylic anhydride (Sigma-Aldrich) was then added dropwise into the mixture at 37 °C under mild agitation to react with amine groups on GLT for 24 h (*Supplementary Fig. S1 A*). Reacted mGLT solution was dialyzed against water to completely remove low molecular-weight byproducts using 3500 NMWCO dialysis cassettes (Thermo Scientific). Dialyzed mGLT was lyophilized and stored desiccated for future use. The methacrylation rate of the product was ~80% [34]. The visible light (VL)-activated photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was synthesized as described by Fairbanks et al. [35].

2.2. Fabrication of composite scaffolds

Composite scaffolds containing interspersed PCL and mGLT fibers were produced using dual electrospinning [36]. PCL particles (70–90 k, Sigma-Aldrich) were dissolved in 2,2,2-trifluoroethanol (TFE, Sigma-Aldrich, 18% w/v). Dehydrated mGLT was dissolved in 95% TFE (20% w/v). Each polymer solution was extruded through a 22-gauge blunt-tip needle at 2 mL/h for 1 h. The spinnerets were charged with an optimized DC potential (8 kV for PCL and 15 kV for mGLT, respectively) and aligned in opposing positions on each side of the collection mandrel with a distance of 15 cm between the needle tip and mandrel (*Fig. 1*). The scaffold was stored desiccated under vacuum to remove residual solvent. To retain mGLT in the composite scaffold, 0.5% LAP solution was cast onto dry composite scaffold surface at 15 µl/cm² and allowed to spread until the scaffold was completely wet. LAP was then activated by exposure to VL irradiation (450–490 nm) to photocrosslink the methacrylate groups of the dissolved gelatin in the scaffold (*Fig. 1, Supplementary Fig. S1 B*).

2.3. Imaging of composite scaffolds

Polymers were fluorescently labeled to track the presence and interspersion of the two distinct fiber populations: PCL solution was mixed with 0.1% (v/v) Vybrant® Dil Cell-Labeling Solution (Dil, Life Technologies), and non-methacrylated GLT was conjugated with fluorescein 5(6)-isothiocyanate (FITC, Sigma-Aldrich) before dissolution, respectively. Fibers were dual-electrospun onto glass slides for 5 min and imaged before and after wetting using an Olympus CKX41 inverted fluorescent microscope equipped with a CCD camera. Additionally, scaffold surface was examined by scanning electron microscopy (SEM, field emission, JEOL JSM6335F) operated at 3 kV accelerating voltage and 8 mm working distance.

2.4. Histological examination of composite scaffolds

Picosirius red staining was employed to assess gelatin retention and distribution within scaffolds. Composite scaffolds before and after photocrosslinking were washed in PBS at 37 °C overnight under mild agitation, frozen-embedded in OCT compound (4583 Scigen Scientific), and cryosectioned at 15 µm thickness using a Leica CM 1850 cryotome. Sections were washed in PBS and stained with 0.1% sirius red in saturated picric acid (Electron Microscopy Sciences) for 1 h. To visualize impregnated cells, cryosections of fixed, cell-seeded scaffolds were incubated with ethidium homodimer-1 (EthD-1, Life Technologies) to label cells via DNA binding.

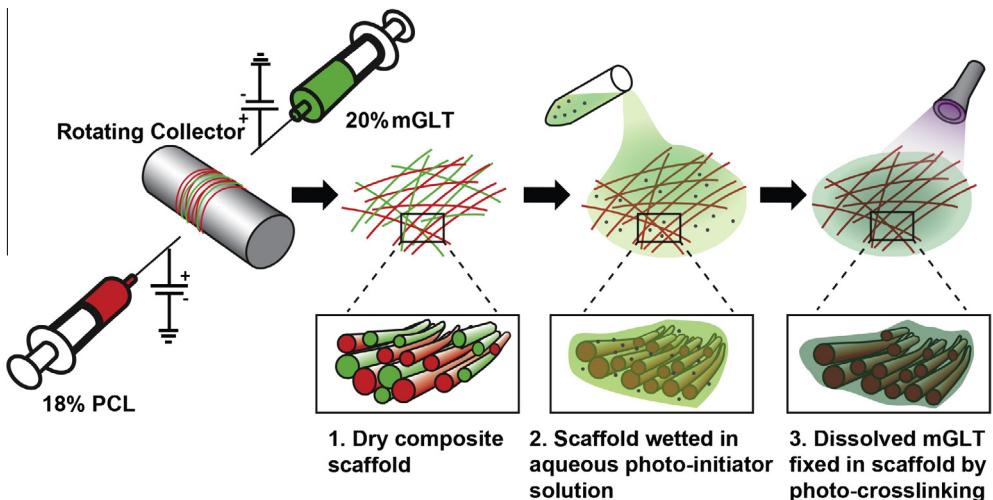


Fig. 1. Composite scaffold preparation. Dual electrospinning was employed to fabricate a scaffold containing PCL and mGLT fibers (Insert 1). Dry scaffold was wetted with aqueous photo-initiator solution (Insert 2) and then photocrosslinked by visible light (VL) to retain the gelatin (Insert 3).

2.5. Biochemical composition of composite scaffolds

Gelatin content of composite scaffolds was quantified by the Chloramine-T based hydroxyproline assay (Fisher Scientific and Sigma-Aldrich). Reaction product was measured spectrophotometrically at 550 nm using a microplate reader (BioTek). Relative gelatin retention rate was calculated as the reading of washed scaffold divided by that of dry scaffold.

2.6. Cell isolation and culture

Human adipose-derived stem cells (hASCs) were obtained from lipoaspirate-derived fat tissue of two donors (34 years old male and 38 years old female) using an automated cell isolation system (Tissue Genesis). The protocol was approved by the Institutional Review Board of the University of Pittsburgh. Isolated hASCs were cultured in growth medium (GM) consisting of DMEM-high glucose (Gibco), 10% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin and 100 mg/ml streptomycin (P/S, Gibco). Multipotency was confirmed by established differentiation tests [37].

2.7. Creation of multilayer constructs

Scaffold pairs were prepared by overlaying one third the area of a rectangular scaffold piece atop another scaffold piece of identical shape. The scaffold pair was then wetted with photo-initiator and crosslinked by VL irradiation (Fig. 4A), followed by gentle washing in PBS at 37 °C for up to 7 days. To create multilayered structures, 5 sheets of scaffold of identical rectangular shape were wetted with photo-initiator solution, stacked, and exposed to VL for 1 min on each side to crosslink adjacent scaffold layers (Fig. 5A). For these multilayer constructs, photo-initiator was dissolved in 8% mGLT solution to further reinforce the association between scaffold layers. Sandwich constructs made from alternative layers of fibrous PCL and mGLT solution were also prepared and subjected to the same photocrosslinking protocol. After 7 days of washing in PBS at 37 °C, scaffold integrity was examined by picrosirius red staining of orthogonal cryosections.

2.8. Mechanical testing

Tensile properties of single layer scaffold and multilayer constructs were analyzed by using the Bose 3230 mechanical tester. Samples were securely mounted and loaded with uniaxial force

applied at a displacement rate of 0.2 mm/s until 10 mm displacement. The tensile force and the displacement were recorded. The slope of the linear portion of the stress-strain curve was calculated as the elastic modulus.

2.9. Creation of a tendon-mimetic construct by multilayer scaffolds

Aligned composite scaffolds were fabricated by dual electrospinning. hASCs were detached and suspended in LAP-containing 8% mGLT solution and cast onto scaffold sheets for hydration before VL irradiation. Multilayer constructs consisting of 5 sheets of cell seeded, aligned scaffolds were then prepared as described above. To induce tendonogenic differentiation, constructs were maintained for 7 days in differentiation medium (DM) consisting of DMEM, 2% FBS, P/S, and 10 ng/ml TGF-β3 (PeproTech).

2.10. Cell proliferation tests

Cells encapsulated in multilayer constructs were maintained in standard GM and subject to MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega) at 1 day or 7 days after initial seeding. A₄₉₀ was determined spectrophotometrically using a microplate reader (BioTek). Double stranded DNA (dsDNA) content in each group was determined fluorimetrically by Picogreen assay (Quant-iT PicoGreen, Invitrogen; excitation 480 nm, emission 520 nm). All readings were normalized to the cell free control group at each time point.

2.11. Cytoskeleton fluorescent staining

Cell seeded, single layer scaffold sheets were washed in PBS, fixed in 4% paraformaldehyde and incubated with 1% bovine serum albumin (BSA). Cells were permeabilized in 0.1% Triton X-100 for 5 min and then incubated with phalloidin for 30 min at room temperature (Alexa Fluor 488 phalloidin, Life Technologies). Lastly, cells were rinsed with PBS, nuclear counterstained with DAPI (Life Technologies), and imaged using a confocal microscope (Olympus FluoView 1000).

2.12. Real-time PCR analysis

Total cellular RNA was isolated using an RNA extraction Kit (Qiagen) and first-strand cDNA synthesized with random hexamer primers (SuperScript III First-Strand cDNA synthesis kit, Invitrogen).

Quantitative real-time PCR was performed using SYBR green Supermix in a Step One Plus real-time PCR system (Applied Biosystem, Life Technologies) to analyze expression of tenogenic markers, scleraxis (SCX) and tenascin-C (TNC). The targets and sequences of primers are shown in **Table 1**. The relative level of each gene was normalized to that of 18S rRNA and calculated using the $\Delta\Delta\text{-}Ct$ method.

2.13. Statistical analysis

Data are presented as mean \pm standard deviation (SD). All quantitative assays were performed for no less than three times independently (n equals to the number of samples tested). One-way ANOVA with Bonferroni post hoc tests and Student's *t*-test was performed to determine statistical significance. Significance was considered at $p < 0.05$.

3. Results

3.1. Organization of fibers in composite scaffold

Microscopic observation of the co-electrospun scaffold showed that the fluorescently labeled fibers were interspersed within the dry scaffold (**Fig. 2A**, Composite Dry, red: PCL; green: mGLT). Hydration of the scaffold in an aqueous environment resulted in rapid dissolution of mGLT fibers, whereas PCL fibers remained intact (**Fig. 2A**, Composite Wet, red: PCL; green: mGLT). SEM revealed a fraction of fibers with distinct ribbon-like morphology, in addition to the cylindrically shaped fibers seen in the dry composite scaffold mesh (**Fig. 2B**, Composite Dry, indicated by arrow in insert). After hydration, the ribbon portion was no longer detectable; instead, adjacent fibers were found bridged or bundled by deposited sheaths (**Fig. 2B**, Composite Wet, indicated by arrows in insert). The alteration in scaffold organization is likely caused by the dissolution and deposition of incorporated gelatin during hydration, due to its high aqueous solubility. In contrast, scaffold made only of PCL showed no observable difference between the dry and wet state, exhibiting uniform shaped fibers without fused contacts (**Fig. 2B**, PCL Dry vs. PCL Wet).

3.2. Biochemical and mechanical analysis of composite scaffold

Hydrated composite scaffold was exposed to VL irradiation for crosslinking of mGLT in the presence of photo-initiator (**Fig. 1**). Picosirius staining of gelatin showed a pronounced difference in gelatin retention between non-crosslinked and crosslinked scaffolds (**Fig. 3A**). Without crosslinking, the scaffold displayed little red staining, indicating loss of the majority of incorporated gelatin after washing (**Fig. 3A**, Composite non-crosslinked). In contrast, a large amount of gelatin was retained and evenly distributed within the crosslinked scaffold mesh (**Fig. 3A**, Composite crosslinked, red), suggesting excellent integration between gelatin and PCL fibers. The control consisting of mGLT solution cast atop PCL scaffold resulted in a distinct boundary between gelatin hydrogel and PCL, suggesting separation of the two scaffold formats (**Supplementary Fig. S2**).

Scaffolds were dried in an oven and weighed before and after gentle washing in PBS for 1 day at 37 °C to estimate the gelatin loss (**Fig. 3B**, $p < 0.01$). After washing, non-crosslinked scaffolds lost one third of their initial weight ($32.87\% \pm 3.89$, n = 5), whereas cross-linked scaffolds lost only 16% ($15.89\% \pm 3.50$, n = 5). This finding is consistent with the gelatin content estimated by hydroxyproline assay: (**Fig. 3C**, $p < 0.01$) crosslinked scaffold retained half of its initial gelatin content ($44.53\% \pm 1.58$, n = 4), while non-crosslink scaffold preserved less than 3%. ($2.93\% \pm 0.46$, n = 4). We then analyzed

Table 1
Primer sequences of genes analyzed by real-time PCR.

Gene		Primer sequence (5'-3')	Product size (bp)
18S rRNA	Forward	GTAACCCGGTTGAACCCATT	151
	Reverse	CCATCCAATCGGTAGTAGCG	
SCX	Forward	ACACCCAGCCAAACAGA	65
	Reverse	GCGGTCTTGCTCAACTTC	
TNC	Forward	GGTGGATGGATTGTGTCTTGAGA	328
	Reverse	CTGTGTCCTGTCAAAGTGGAGA	

the impact of crosslinking on the mechanical strength of scaffolds, and found a 16% reduction in maximum load of non-crosslinked material after washing. In contrast, crosslinked scaffold demonstrated consistent maximum load with no significant alteration compared to dry composite scaffold (**Fig. 3D**).

3.3. Multilayered construct created by photocrosslinking

The crosslinkable nature of composite scaffold combined with the excellent integration between gelatin deposition and fibrous PCL suggests the possibility of preparing multilayered constructs by stacking and photocrosslinking the individual layers of composite scaffold. To test whether this approach could produce consistent integration between layered sheets, two layers were partially overlapped with or without subsequent photocrosslinking (**Fig. 4A**). Without photocrosslinking, some of the scaffold pairs no longer adhered to each other after washing, whereas all cross-linked scaffold layers remain intact throughout the test (**Fig. 4B**, areas between blue lines are overlapping regions). After 1 or 7 days of washing in PBS at 37 °C, unseparated scaffold pairs were securely mounted and subjected to uniaxial stretch force until separation of the scaffold layers. The load-displacement curve revealed markedly higher resistance to tension and higher maximum load in the crosslinked scaffold pair, indicating that the stacked layers of sheets can bond with each other (**Fig. 4C, D**).

To create multilayered constructs, 5 sheets of scaffold in rectangular shape (8 mm \times 4 mm) were wetted with photo-initiator solution, stacked and exposed to VL to crosslink adjacent scaffold layers (**Fig. 5A**). The average thickness of constructs was 0.367 mm (n = 12). After 7 days of washing in PBS, a substantial difference in scaffold integrity was identified by picrosirius red staining. As shown in **Fig. 5B**, without crosslinking (left, CN), the scaffold layers did not adhere to each other due to mGLT loss. Sandwich constructs made from alternative layers of fibrous PCL and mGLT showed partial failure (center, PC), while crosslinked multilayer composite scaffolds remained fully integrated with negligible separation between layers (right, CC). Mechanical testing showed significantly higher tensile strength of crosslinked scaffolds (1.55 ± 0.49 MPa) than non-crosslinked scaffolds (0.92 ± 0.38 MPa, **Fig. 5C**, CC vs. CN, $p < 0.05$, n = 6), while the integration between layers exerted little impact on overall tensile strength (**Fig. 5C**, PC vs. CC, n = 6).

3.4. Cell incorporation and activity in multilayered construct

hASCs were impregnated into the crosslinked PCL/mGLT sandwich constructs (PC) or crosslinked composite constructs (CC) to generate a cell-laden graft. After 7 days of culture, EthD-1 staining for cells showed that most hASCs were localized in the interstitial space between adjacent layers (**Fig. 6A**; red). In spite of the similarity in cell distribution, crosslinked composite constructs again demonstrated greater structural integrity than that of sandwich constructs, in which the individual scaffold layers had separated (**Fig. 6A**; dark red). Moreover, hASCs impregnated in composite

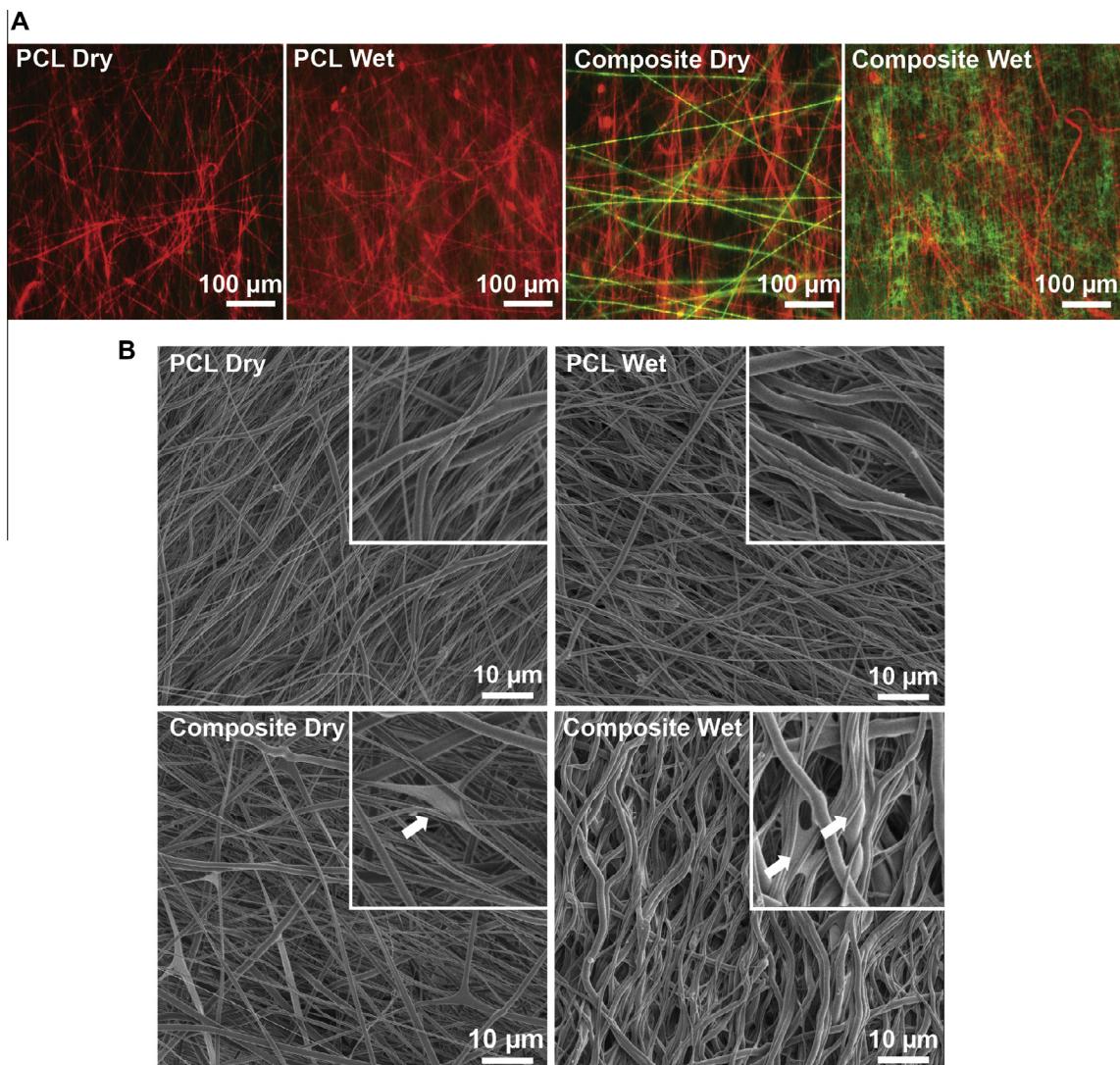


Fig. 2. Characterization of scaffold architecture. (A) Fluorescently labeled PCL (red) and gelatin (green) displayed a fibrous morphology and interspersed distribution within the dry composite scaffold prepared by dual electrospinning (Composite Dry). Wetting of the scaffold with an aqueous solution resulted in dissolution of the gelatin fibers (green), whereas the PCL fibers (red) remained intact (Composite Wet). PCL fibers alone showed no change with wetting. (B) SEM revealed distinct microscopic architecture of the composite scaffold, in which ribbon-like bands were seen in addition to the predominant cylindrical fibers (Composite Dry, indicated by arrow in insert). After wetting, adjacent fibers were bridged or wrapped with a sheath of gelatin (Composite Wet, indicated by arrows in insert). Unlike the composite mesh, scaffolds made of only PCL showed negligible differences in morphology before and after wetting (PCL Dry vs. PCL Wet). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

scaffold displayed significantly higher metabolic activity (Fig. 6B), while total cell number, reflected by the content of dsDNA, remained comparable between two construct formats (Fig. 6C). Taken together, these two findings suggest that constructs made from composite scaffolds promote cell metabolism but not proliferation.

3.5. Characterization of tendon phenotype induced by aligned multilayered constructs

To create tendon-mimicking constructs, aligned composite scaffolds were prepared and anisotropy confirmed by SEM (Fig. 7A) and mechanical testing along planes perpendicular (Cross) or parallel (Longi.) to the orientation of fibers (Fig. 7C, 0.12 ± 0.01 vs. 2.51 ± 1.33 MPa). F-actin fluorescent staining showed that impregnated hASCs adopted elongated morphology and were aligned in the direction of fibers (Fig. 7B; green, F-actin; blue, DAPI), whereas no uniformity in orientation was noted in non-aligned scaffolds

(Supplementary Fig. S3). For tendon cell lineage commitment, hASCs were treated with 10 ng/ml TGF- β 3 for 7 days. Real-time PCR assay showed pronounced upregulation of tendon markers SCX and TNC (Fig. 7D), indicating that encapsulated cells remained responsive to soluble tenogenic factors, and that the construct possessed sufficient porosity for cells to receive exogenous biochemical cues.

4. Discussion

Scaffolds are of critical importance in the context of tissue engineering, serving to provide a physical substrate that mimics the *in vivo* milieu of healthy tissues and thus orchestrate the activity of therapeutic cells in a tissue-specific fashion [38]. In terms of tendon tissue engineering, a number of scaffold designs have been developed to reproduce one or multiple structural/compositional characteristics of tendon tissues, among which aligned, electrospun fibrous scaffolds and the 3D hydrogels have been frequently

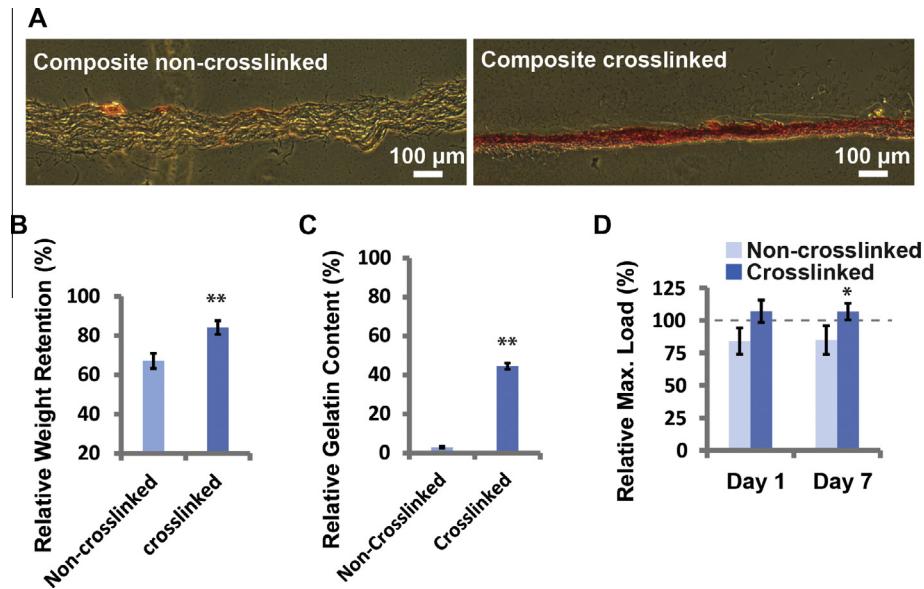


Fig. 3. Examination of gelatin retention. (A) Picosirius red staining showed that non-crosslinked scaffolds lost the majority of incorporated gelatin after washing (Composite non-crosslinked). In contrast, photo-crosslinked scaffolds retained a portion of incorporated gelatin within the scaffold mesh (Composite crosslinked, red). (B) Non-crosslinked scaffolds showed significantly higher weight loss compared to crosslinked scaffolds. (C) Crosslinked scaffolds retained ~45% of their initial gelatin content based on hydroxyproline assay, while non-crosslinked scaffolds retained less than 3%. (D) Mechanical testing of crosslinked scaffolds demonstrated equivalent maximum load to that of dry composite scaffolds, while non-crosslinked scaffolds showed approximately 16% reduction after washing. *p < 0.05; **p < 0.01, n = 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

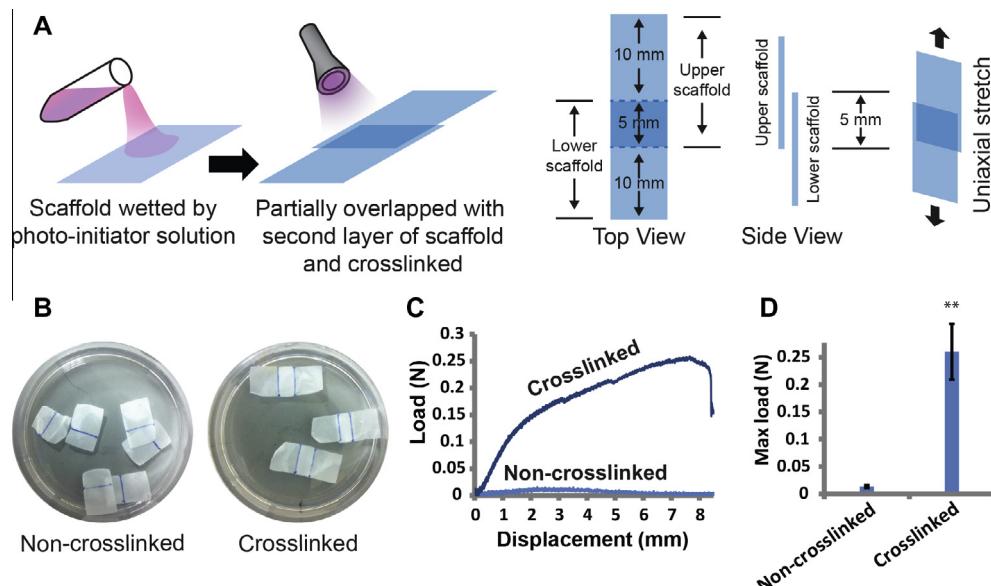


Fig. 4. Laminar integration of scaffold sheets. (A) Scaffold pairs were prepared by overlaying one scaffold sheet atop another sheet of identical rectangular shape, so that 1/3 of their lengths overlapped. The sheets were then wetted with photo-initiator and photocrosslinked. (B) Without crosslinking, some of the scaffold pairs fell apart after washing, whereas crosslinked scaffold pairs remained intact. Blue lines suggested the boundary of overlapping regions. (C) Representative load–displacement curve of crosslinked scaffold pairs showed higher resistance to separation. (D) Crosslinked scaffold pairs showed significantly higher maximum load before separation compared to non-crosslinked pairs. **p < 0.01; n = 6. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

implemented in preclinical models due to their capability of presenting the topographical cues and native biochemical cues to seeded therapeutic cells, respectively [15,16,28,39]. Therefore, use of a composite scaffold consisting of fibrous scaffold sheets and hydrogels is a potentially promising approach to mimic the physical and biological features of tendon tissue.

To achieve this goal, we first prepared PCL/mGLT composite scaffolds by dual electrospinning. Upon VL irradiation, the free radicals generated from the photo-initiator resulted in crosslinking via methacrylate on the gelatin backbone [40], thereby generating a

hydrogel network within the PCL fiber mesh (Fig. 3A). We chose gelatin rather than collagen as the bioactive building block of the construct for two reasons: (1) as a product of denatured collagen [41], gelatin possesses a portion of the amino acid sequence and bioactive motifs of the parent collagen and is consequently chemically similar to native collagen chains; and (2) gelatin is far less costly compared to purified collagen (~25 USD/100 g vs. 650 USD/100 mg; pricing from Sigma-Aldrich) while more stable to organic solvent dissolution [42]. These features make gelatin suitable for industry-scale scaffold manufacturing.

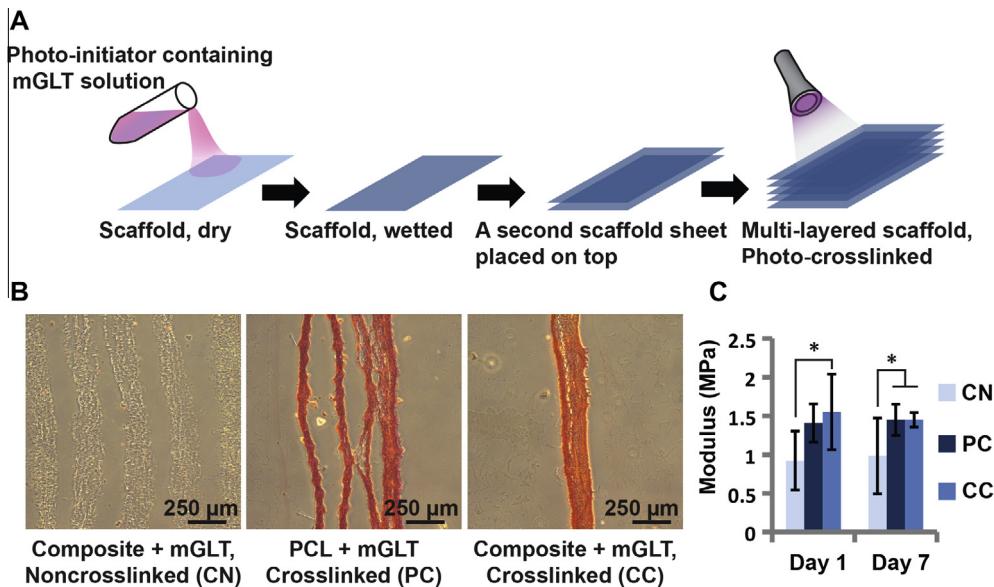


Fig. 5. Preparation and characterization of a multilayer construct. (A) Scaffold sheets were wetted, stacked and exposed to VL for crosslink formation between adjacent scaffold layers to create a complex multi-layered structure. (B) Picosirius red staining showed pronounced difference in scaffold integrity after washing among the three groups: stacked composite scaffold without crosslinking (CN, left), sandwich construct made from alternative layers of fibrous PCL and mGLT (PC, center), and crosslinked composite scaffold (CC, right). (C) Crosslinked scaffolds showed higher tensile strength than non-crosslinked scaffolds (CC vs. CN); the integration between fibrous scaffolds had little impact on overall tensile strength (CC vs. PC). * $p < 0.05$; $n = 6$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

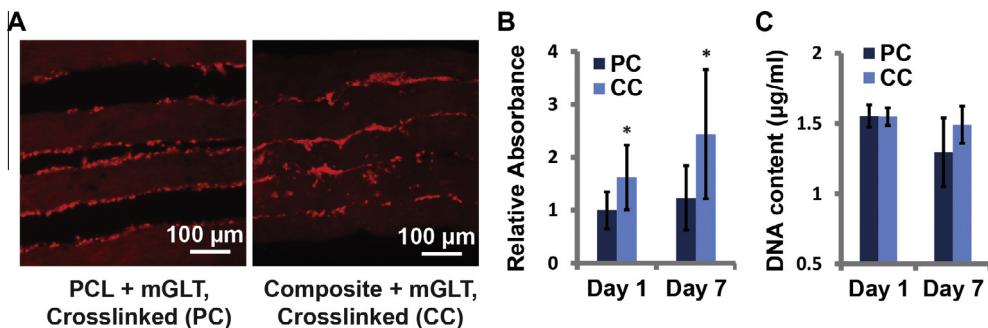


Fig. 6. Analysis of cell distribution and metabolic activity in multilayer constructs. (A) Ethidium homodimer-1 (EthD-1, red) staining indicated that most cells were localized between adjacent layers. Crosslinked composite constructs (CC) demonstrated evidently higher structural integrity than crosslinked sandwich constructs (PC). (B) MTS assay revealed significantly higher metabolic activity of cells impregnated in composite scaffold (CC), whereas the total cell number remained comparable to that of sandwich constructs (PC). * $p < 0.05$; $n = 8$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For electrospinning, PCL and mGLT were dissolved and extruded separately to create intercalated PCL and mGLT fibers so that the mechanical strength of the PCL scaffold mesh is not compromised by the change in mGLT structure upon hydration. In contrast, electrospinning of a mixed solution of gelatin and PCL led to a collection of fibers comprised of alternating segments of gelatin and PCL, in which the dissolution of gelatin will presumably lead to fiber dissociation at gelatin regions and sequential loss of overall mechanical strength [43]. Concentrations of PCL and mGLT ranging from 10% to 22% were tested, and 18% PCL and 20% mGLT were chosen based on the stability of the solution stream during electrospinning [44]. However, improvement in tensile strength is clearly needed if the scaffold is intended to be used to construct a clinical tendon graft (~ 550 MPa) [45], for the ultimate tensile strength (UTS) of multilayered PCL-mGLT sandwich construct and composite construct was only 1.41 ± 0.15 MPa and 1.45 ± 0.19 MPa, respectively ($n = 6$). One possible strategy to improve mechanical strength would be the inclusion of textile patterns, such as braiding or weaving [46]. Although mGLT fibers rapidly dissolved upon wetting and therefore possibly acted as sacrificial fiber to increase

the pore size of the scaffold, we did not observe improved cell infiltration into the interior region of the crosslinked scaffolds (Supplementary Fig. S4) [47]. The crosslinked gelatin hydrogel formed within the PCL fiber mesh may occupy the pores and impede cell migration. To address this potential issue, a peptide linker containing a matrix metalloproteinase (MMP)-sensitive motif might be incorporated to generate a more cell-cleavable hydrogel within a fibrous scaffold to improve cell infiltration [48,49].

In addition to presenting multiple environmental cues, multilayering of fibrous scaffold sheets can be employed to reconstitute the 3D architecture of a native tissue [38]. To achieve this goal, the rapid, robust integration between scaffold layers and sufficient incorporation of therapeutic cells must be carefully balanced [46,50,51]. In the scaffold described here, the gelatin component was found evenly distributed throughout the depth of PCL mesh, and therefore enabled integration of multiple sheets along the z axis upon photocrosslinking. However, the constructs used in this study were made of no more than five layers of scaffold sheet to ensure the orthogonal penetration of light into the construct for sufficient crosslinking. Higher degree of stacking may result in

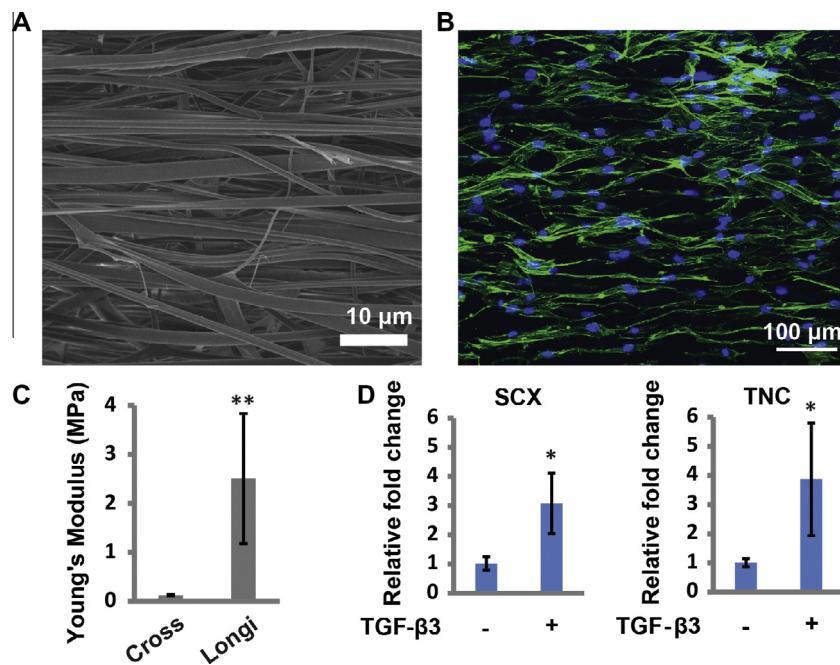


Fig. 7. Tendon-like features in cell-impregnated multilayer constructs. (A) Fiber alignment observed by SEM. (B) Elongated morphology of human adipose stem cells (hASCs) aligned in the direction of fibers (green, F-actin; blue, nuclei). (C) Anisotropy based on tensile strength properties measured by mechanical testing along two directions (Longi vs. Cross). (D) Significant upregulation of tendon markers scleraxis (SCX) and tenascin C (TNC) upon treatment with exogenous tenogenic factor TGF- β 3, measured by real-time PCR analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inadequate crosslinking in the center zone of the construct due to the accumulated opacity. To address such restriction on construct thickness, thermo-responsive or chemically initiated crosslinkers may be considered. The VL-based photocrosslinking technique employed in our study is biocompatible, as our previous study found less than 6% cell death in the encapsulated cell population [29]. By combining the two aforementioned characteristics, we have achieved a robust and instant integration of scaffold layers that is compatible with simultaneous, sufficient cell encapsulation.

For future study, the composite scaffold is open to further modifications that aim at eliciting the tenogenesis of impregnated cells. For instance, heparin may be conjugated to the gelatin backbone to sequester and subsequently deliver exogenous tenogenic growth factors into the constructs [52]. Tendon tissue derived-ECM may also be incorporated into the hydrogel portion of the constructs to recapitulate the proteinaceous microenvironment of native tendon tissue [28]. Moreover, given the inflammatory milieu of damaged tendons and the co-morbidities possible in patients, the response of native cells derived from diseased tendon tissues to the scaffold created in this study is worthy of future exploration in order to further elucidate the scaffold's potential as the biomaterial component of a tendon implant. It is noteworthy that Hakimi et al. reported excellent attachment and alignment of tendon cells derived from chronic tendinopathy patients on the electrospun component of a layered electrospun and woven scaffold, along with retention of tenogenic cell phenotype [46]. Finally, we believe that the scaffold engineered in this study as a building block for multilayer constructs should have applications beyond tendon tissue engineering in the fabrication of tissue grafts that contain both a fibrous component and a hydrogel portion.

5. Conclusion

In this study, we have developed a novel composite scaffold consisting of fibrous PCL and methacrylated gelatin (mGLT) interspersed by dual-electrospinning. The crosslinkable nature of the

composite scaffold, together with the excellent integration of the gelatin within the PCL mesh, allowed the creation of a multilayered construct as a tendon graft through photo-crosslinking of stacked scaffold sheets. Human ASCs were impregnated into the scaffold to generate a cell-laden construct and were seen to align along the orientation of the fibers. Seeded cells adopted tendon cell phenotype upon treatment with TGF- β 3. These findings provide information for the development of more advanced tendon grafts that can mimic both structural and cellular characteristics of native tendon tissues.

Disclosure statement

No competing financial interests exist.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.actbio.2016.03.004>.

References

- [1] E. Pennisi, Tending tender tendons, *Science* 295 (2002) 1011.
- [2] D.L. Butler, N. Juncosa, M.R. Dressler, Functional efficacy of tendon repair processes, *Annu. Rev. Biomed. Eng.* 6 (2004) 303–329.
- [3] M. Griffin, S. Hindocha, D. Jordan, M. Saleh, W. Khan, An overview of the management of flexor tendon injuries, *Open Orthop. J.* 6 (2012) 28–35.
- [4] M. Calleja, D.A. Connell, The Achilles tendon, *Semin. Musculoskelet. Radiol.* 14 (2010) 307–322.
- [5] K.A. Derwin, A.R. Baker, R.K. Spragg, D.R. Leigh, J.P. Iannotti, Commercial extracellular matrix scaffolds for rotator cuff tendon repair. Biomechanical, biochemical, and cellular properties, *J. Bone Joint Surg. Am.* 88 (2006) 2665–2672.
- [6] M. O'Brien, Structure and metabolism of tendons, *Scand. J. Med. Sci. Sports* 7 (1997) 55–61.
- [7] P. Kannus, Structure of the tendon connective tissue, *Scand. J. Med. Sci. Sports* 10 (2000) 312–320.
- [8] B.R. Bach Jr., S. Tradonsky, J. Bojchuk, M.E. Levy, C.A. Bush-Joseph, N.H. Khan, Arthroscopically assisted anterior cruciate ligament reconstruction using patellar tendon autograft. Five- to nine-year follow-up evaluation, *Am. J. Sports Med.* 26 (1998) 20–29.
- [9] T.R. Duquin, C. Buyea, L.J. Bisson, Which method of rotator cuff repair leads to the highest rate of structural healing? A systematic review, *Am. J. Sports Med.* 38 (2010) 835–841.
- [10] N. Maffulli, P. Sharma, K.L. Luscombe, Achilles tendinopathy: aetiology and management, *J. R. Soc. Med.* 97 (2004) 472–476.
- [11] C.K. Kuo, J.E. Marturano, R.S. Tuan, Novel strategies in tendon and ligament tissue engineering: advanced biomaterials and regeneration motifs, *Sports Med. Arthrosc. Rehabil. Ther. Technol.* 2 (2010) 20.
- [12] M.T. Rodrigues, R.L. Reis, M.E. Gomes, Engineering tendon and ligament tissues: present developments towards successful clinical products, *J. Tissue Eng. Regen. Med.* 7 (2013) 673–686.
- [13] G. Yang, B.B. Rothrauff, R.S. Tuan, Tendon and ligament regeneration and repair: clinical relevance and developmental paradigm, *Birth Defects Res. C. Embryo Today* 99 (2013) 203–222.
- [14] M. Lavagnino, M.E. Wall, D. Little, A.J. Banes, F. Guilak, S.P. Arnoczky, Tendon mechanobiology: current knowledge and future research opportunities, *J. Orthop. Res.* 33 (2015) 813–822.
- [15] Z. Yin, X. Chen, J.L. Chen, W.L. Shen, T.M. Hieu Nguyen, L. Gao, et al., The regulation of tendon stem cell differentiation by the alignment of nanofibers, *Biomaterials* 31 (2010) 2163–2175.
- [16] K.L. Moffat, A.S. Kwei, J.P. Spalazzi, S.B. Doty, W.N. Levine, H.H. Lu, Novel nanofiber-based scaffold for rotator cuff repair and augmentation, *Tissue Eng. Part A* 15 (2009) 115–126.
- [17] Z. Yin, X. Chen, H.X. Song, J.J. Hu, Q.M. Tang, T. Zhu, et al., Electrospun scaffolds for multiple tissues regeneration in vivo through topography dependent induction of lineage specific differentiation, *Biomaterials* 44 (2015) 173–185.
- [18] A. Cipitria, A. Skelton, T.R. Dargaville, P.D. Dalton, D.W. Hutmacher, Design, fabrication and characterization of PCL electrospun scaffolds—a review, *J. Mater. Chem.* 21 (2011) 9419–9453.
- [19] J.C. Middleton, A.J. Tipton, Synthetic biodegradable polymers as orthopedic devices, *Biomaterials* 21 (2000) 2335–2346.
- [20] L.S. Nair, C.T. Laurencin, Biodegradable polymers as biomaterials, *Prog. Polym. Sci.* 32 (2007) 762–798.
- [21] H. Miyashita, M. Ochi, Y. Ikuta, Histological and biomechanical observations of the rabbit patellar tendon after removal of its central one-third, *Arch. Orthop. Trauma Surg.* 116 (1997) 454–462.
- [22] M. Hope, T.S. Saxby, Tendon healing, *Foot Ankle Clin.* 12 (2007) 553–567. v.
- [23] Z.W. Ma, Z.W. Mao, C.Y. Gao, Surface modification and property analysis of biomedical polymers used for tissue engineering, *Colloids Surf., B-Biointerfaces* 60 (2007) 137–157.
- [24] P.B. Vanwachem, T. Beugeling, J. Feijen, A. Bantjes, J.P. Detmers, W.G. Vanaken, Interaction of cultured human-endothelial cells with polymeric surfaces of different wettabilities, *Biomaterials* 6 (1985) 403–408.
- [25] E. Ruoslahti, M.D. Pierschbacher, New perspectives in cell-adhesion – Rgd and integrins, *Science* 238 (1987) 491–497.
- [26] T. Billiet, M. Vandenhauwe, J. Schelfhout, S. Van Vlierberghe, P. Dubrule, A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering, *Biomaterials* 33 (2012) 6020–6041.
- [27] I.M. El-Sherbiny, M.H. Yacoub, Hydrogel scaffolds for tissue engineering: progress and challenges, *Global Cardiol. Sci. Pract.* 2013 (2013) 316–342.
- [28] G. Yang, B.B. Rothrauff, H. Lin, R. Gottardi, P.G. Alexander, R.S. Tuan, Enhancement of tenogenic differentiation of human adipose stem cells by tendon-derived extracellular matrix, *Biomaterials* 34 (2013) 9295–9306.
- [29] H. Lin, A.W.M. Cheng, P.G. Alexander, A.M. Beck, R.S. Tuan, Cartilage tissue engineering application of injectable gelatin hydrogel with in situ visible-light-activated gelation capability in both air and aqueous solution, *Tissue Eng. Part A* 20 (2014) 2402–2411.
- [30] E.E. Antoine, P.P. Vlachos, M.N. Rylander, Review of collagen I hydrogels for bioengineered tissue microenvironments: characterization of mechanics, structure, and transport, *Tissue Eng. Part B Rev.* 20 (2014) 683–696.
- [31] L. Gasperini, J.F. Mano, R.L. Reis, Natural polymers for the microencapsulation of cells, *J. R. Soc. Interface* 11 (2014) 20140817.
- [32] S. Yodmuang, S.L. McNamara, A.B. Nover, B.B. Mandal, M. Agarwal, T.A. Kelly, et al., Silk microfiber-reinforced silk hydrogel composites for functional cartilage tissue repair, *Acta Biomater.* 11 (2015) 27–36.
- [33] Z. Yin, X. Chen, T. Zhu, J.J. Hu, H.X. Song, W.L. Shen, et al., The effect of decellularized matrices on human tendon stem/progenitor cell differentiation and tendon repair, *Acta Biomater.* 9 (2013) 9317–9329.
- [34] J.W. Nichol, S.T. Koshy, H. Bae, C.M. Hwang, S. Yamanlar, A. Khademhosseini, Cell-laden microengineered gelatin methacrylate hydrogels, *Biomaterials* 31 (2010) 5536–5544.
- [35] B.D. Fairbanks, M.P. Schwartz, C.N. Bowman, K.S. Anseth, Photoinitiated polymerization of PEG-diacylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility, *Biomaterials* 30 (2009) 6702–6707.
- [36] B.M. Baker, A.O. Gee, R.B. Metter, A.S. Nathan, R.A. Marklein, J.A. Burdick, et al., The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers, *Biomaterials* 29 (2008) 2348–2358.
- [37] R. Tuli, S. Tuli, S. Nandi, M.L. Wang, P.G. Alexander, H. Haleem-Smith, et al., Characterization of multipotential mesenchymal progenitor cells derived from human trabecular bone, *Stem Cells* 21 (2003) 681–693.
- [38] K. Atesok, M.N. Doral, J. Karlsson, K.A. Egol, L.M. Jazrawi, P.G. Coelho, et al., Multilayer scaffolds in orthopaedic tissue engineering, *Knee Surg. Sports Traumatol. Arthrosc.* (2014).
- [39] H.A. Awad, G.P. Boivin, M.R. Dressler, F.N.L. Smith, R.G. Young, D.L. Butler, Repair of patellar tendon injuries using a cell-collagen composite, *J. Orthop. Res.* 21 (2003) 420–431.
- [40] K. Yue, G. Trujillo-de Santiago, M.M. Alvarez, A. Tamayol, N. Annabi, A. Khademhosseini, Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels, *Biomaterials* 73 (2015) 254–271.
- [41] A. Veis, J. Anesey, J. Cohen, The long range reorganization of gelatin to the collagen structure, *Arch. Biochem. Biophys.* 94 (1961) 20–31.
- [42] D.I. Zeugolis, S.T. Khew, E.S. Yew, A.K. Ekaputra, Y.W. Tong, L.Y. Yung, et al., Electro-spinning of pure collagen nano-fibres – just an expensive way to make gelatin?, *Biomaterials* 29 (2008) 2293–2305.
- [43] Y.Z. Zhang, H.W. Ouyang, C.T. Lim, S. Ramakrishna, Z.M. Huang, Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds, *J. Biomed. Mater. Res. Part B-Appl. Biomater.* 72B (2005) 156–165.
- [44] M.K. Leach, Z.Q. Feng, S.J. Tuck, J.M. Corey, Electrospinning fundamentals: optimizing solution and apparatus parameters, *J. Vis. Exp.* (2011).
- [45] J. Louis-Ugbio, B. Leeson, W.C. Hutton, Tensile properties of fresh human calcaneal (Achilles) tendons, *Clin. Anat.* 17 (2004) 30–35.
- [46] O. Hakimi, P.A. Mouthuy, N. Zargar, E. Lostis, M. Morrey, A. Carr, A layered electrospun and woven surgical scaffold to enhance endogenous tendon repair, *Acta Biomater.* 26 (2015) 124–135.
- [47] B.M. Baker, R.P. Shah, A.M. Silverstein, J.L. Esterhai, J.A. Burdick, R.L. Mauck, Sacrificial nanofibrous composites provide instruction without impediment and enable functional tissue formation, *Proc. Natl. Acad. Sci. U.S.A.* 109 (2012) 14176–14181.
- [48] A.K. Jha, K.M. Tharp, J.Q. Ye, J.L. Santiago-Ortiz, W.M. Jackson, A. Stahl, et al., Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels, *Biomaterials* 47 (2015) 1–12.
- [49] S. Khetan, M. Guvendiren, W.R. Legant, D.M. Cohen, C.S. Chen, J.A. Burdick, Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels, *Nat. Mater.* 12 (2013) 458–465.
- [50] M.B. Fisher, E.A. Henning, N. Soegaard, M. Boström, J.L. Esterhai, R.L. Mauck, Engineering meniscus structure and function via multi-layered mesenchymal stem cell-seeded nanofibrous scaffolds, *J. Biomech.* 48 (2015) 1412–1419.
- [51] R. Omi, A. Gingery, S.P. Steinmann, P.C. Amadio, K.N. An, C. Zhao, Rotator cuff repair augmentation in a rat model that combines a multilayer xenograft tendon scaffold with bone marrow stromal cells, *J. Shoulder Elbow Surg.* (2015).
- [52] C.N. Manning, A.G. Schwartz, W. Liu, J. Xie, N. Havlioglu, S.E. Sakiyama-Elbert, et al., Controlled delivery of mesenchymal stem cells and growth factors using a nanofiber scaffold for tendon repair, *Acta Biomater.* 9 (2013) 6905–6914.



Electrospun gelatin/poly(ϵ -caprolactone) fibrous scaffold modified with calcium phosphate for bone tissue engineering

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ABSTRACT

In this study gelatin (Gel) modified with calcium phosphate nanoparticles (SG5) and polycaprolactone (PCL) were used to prepare a 3D bi-layer scaffold by collecting electrospun PCL and gelatin/SG5 fibers separately in the same collector. The objective of this study was to combine the desired properties of PCL and Gel/SG5 in the same scaffold in order to enhance mineralization, thus improving the ability of the scaffold to bond to the bone tissue. The scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and the wide angle X-ray diffraction (WAXD) measurements confirmed that SG5 nanoparticles were successfully incorporated into the fibrous gelatin matrix. The composite Gel/SG5/PCL scaffold exhibited more enhanced mechanical properties than individual Gel and Gel/SG5 scaffolds. The presence of SG5 nanoparticles accelerated the nucleation and growth of apatite crystals on the surface of the composite Gel/SG5/PCL scaffold in simulated body fluid (SBF). The osteoblast response in vitro to developed electrospun scaffolds (PCL and Gel/SG5/PCL) was investigated by using normal human primary NH0st cell lines. NH0st cell culture studies showed that higher alkaline phosphatase (ALP) activity and better mineralization were obtained in the case of composite materials than in pure PCL scaffolds. The mechanically strong PCL scaffold served as a skeleton, while the Gel/SG5 fibers facilitated cell spreading and mineralization of the scaffold.

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1. Introduction

Fibrous scaffolds produced by an electrospinning process have attracted considerable interest in bone tissue engineering [1]. Randomly electrospun fibrous scaffolds possess a characteristic microstructure with a high specific surface area, high porosity and high interconnectivity of pores. Moreover, the mechanical and biological properties, as well as the degradability kinetics of the electrospun scaffold, can be manipulated by changing the composition of the polymer solution and processing parameters [2,3]. Using electrospinning, it is possible to produce scaffolds which are similar to the natural extracellular matrix (ECM), which can thus enhance the adhesion, proliferation and growth of cells [4,5]. In order to more accurately mimic the natural ECM, the electrospinning of natural materials, such as gelatin, has gained much attention during the past few years [6–8]. Gelatin has been widely examined as a tissue engineering scaffold because it has a high biocompatibility, and biodegradability, identical composition and almost the same biological properties as collagen [9,10]. Gelatin has also many integrin-

binding sites for cell adhesion and differentiation [11,12]. However, the main disadvantage of using gelatin as the main structure component in a scaffold is its rapid degradation rate and weak mechanical properties [13].

The bone ECM is a type of organic–inorganic nanocomposite. Specifically, 70% of the bone matrix is composed of nanocrystalline hydroxyapatite, which is deposited in an orderly manner within a nanofibrous collagen matrix [14,15]. Recent research efforts have been focused on the incorporation of bioactive inorganic particles within the polymeric matrix in order to mimic both the physical architecture and chemical composition of natural bone ECM [16–18]. Many fibrous constructs involve the use of hydroxyapatite nanoparticles or silicon-based glasses with silicon contents over 40–45% (Bloglass®). However, their degradation is poor and their functionality is based on the stable precipitation of a hydroxyapatite layer on their surfaces in order to be linked to the bone. In this work, titanium based nanoparticles with a low content of titanium were developed in order to favor degradation and, thus, ion release competing with hydroxyapatite precipitation. Cells are able to directly detect this extracellular ion concentration such as calcium, which is able to act as an osteoinduction promoter through the regulation of a calcium-sensing receptor (CaSR) [19,20]. Calcium also acts as a cell

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homing recruiting cells from the bone marrow suggesting a CaSR-mediated chemotaxis and a further bone resorption [21,22].

Polycaprolactone (PCL) is a biocompatible and biodegradable polyester with excellent mechanical properties [23]. PCL exhibits more prolonged mechanical strength than other bioresorbable polymeric materials, and degrades at a rate compatible with bone regeneration [24,25]. However, PCL has an intrinsic hydrophobic chemical nature, and its poor surface wetting and poor interaction with biological fluids make cell adhesion and proliferation less efficient.

The ideal scaffold for bone tissue engineering should have good cell affinity, bioactive properties and enough mechanical strength to serve as an initial support. Scaffold inspired by natural bone ECM that combines a natural origin polymer (such as gelatin, which contains nanoparticles of a bioactive calcium phosphate), with a synthetic polymer (such as poly(ϵ -caprolactone)), could provide optimal physicochemical and biological properties. Applications of such materials are multiple. However, guided bone healing through the regeneration of the periosteum membrane would be an option, especially for osteoporotic bone fractures. To date, numerous studies confirmed that the existence of a relatively high amount of stem cells located in the periosteum make this option as a very promising and potential approach for the initial bone ingrowth [26–28].

The aim of the present work was to prepare electrospun bi-layer fibrous scaffolds, including both the poly(ϵ -caprolactone) for the backbone of the scaffold and the gelatin modified with calcium phosphate for better mineralization. A hierarchically designed composite material is presented, using a sol-gel method to prepare a bioactive phase as an ion release agent, and electrospinning to mimic the extracellular matrix (ECM), adapting their features to biological requirements. The properties of the composite Gelatin/Calcium phosphate/Polycaprolactone scaffold obtained were characterized by SEM, FTIR, WAXD, TGA and DSC methods. Scaffold bioactivity was examined in SBF as well as in cell culture.

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin, type A) and polycaprolactone (PCL) with a molecular mass of 70,000–90,000 g/mol were purchased from Sigma-Aldrich. 2,2,2-Trifluoroethanol (TFE, ARCOS, Poland) for gelatin and a

mixture of chloroform and methanol 1:1 (POCH, Poland) for PCL were used as solvents. Calcium phosphate nanoparticles (SG5) were produced at the Institute of Bioengineering of Catalonia (IBEC, Spain) by the sol-gel method, which was described previously [29]. Briefly, it consists in the controlled mix in a molar ratio: 44.5 P₂O₅–44.5 CaO–5 TiO₂–6 Na₂O of calcium and sodium 2-methoxyethoxide solutions, phosphorus pentoxide ethanolic solution and titanium tetraisopropoxide solution previously prepared in the laboratory under nitrogen atmosphere. Solutions were aged for one week at 70 °C in vigorous stirring. Finally, nanoparticles were collected by centrifugation. To prepare the spinning solutions, 3 g of gelatin was dissolved in 30 ml of 2,2,2-trifluoroethanol (TFE) and 2.5 g of PCL in 40 ml of chloroform/methanol (1:1) mixture. 0.2 g of calcium phosphate (SG5) powder was added into the gelatin solution. Stable dispersion of SG5 powder was achieved by sonicating the slurry. Three solutions were prepared: (1) gelatin/TFE; (2) gelatin/SG5/TFE; and (3) PCL/chloroform/methanol.

2.2. Scaffold fabrication by electrospinning

Scaffolds were produced by electrospinning, whose set-up consisted of a high voltage supply, an infusion pump and a collector (rotary drum). For scaffold fabrication, each sample of prepared slurries was placed in a syringe (10 ml) topped with a needle whose diameter was 0.7 mm and then connected to 30 kV voltage. The solution flow rate was 1.5 ml/h, and the distance between the tip and the collector was 15 cm. The rotary drum was wrapped in silica-coated paper. In order to produce a hybrid nonwoven fabric (two-layer composite), gelatin/SG5 fibers were spun onto a nanofibrous PCL substrate. Four electrospun scaffolds were prepared: (1) gelatin (Gel); (2) gelatin modified with calcium phosphate (Gel/SG5); (3) polycaprolactone (PCL) and (4) composite Gelatin/Calcium phosphate/Polycaprolactone (Gel/SG5/PCL).

2.3. Scaffold characterization

The electrospun scaffold samples were covered by a sputtered gold coating and analyzed by a scanning electron microscope (JEOL JSM 5500). Fiber diameters were determined based on SEM images. The average diameter of the fibers was determined by performing measurements on 100 fibers.

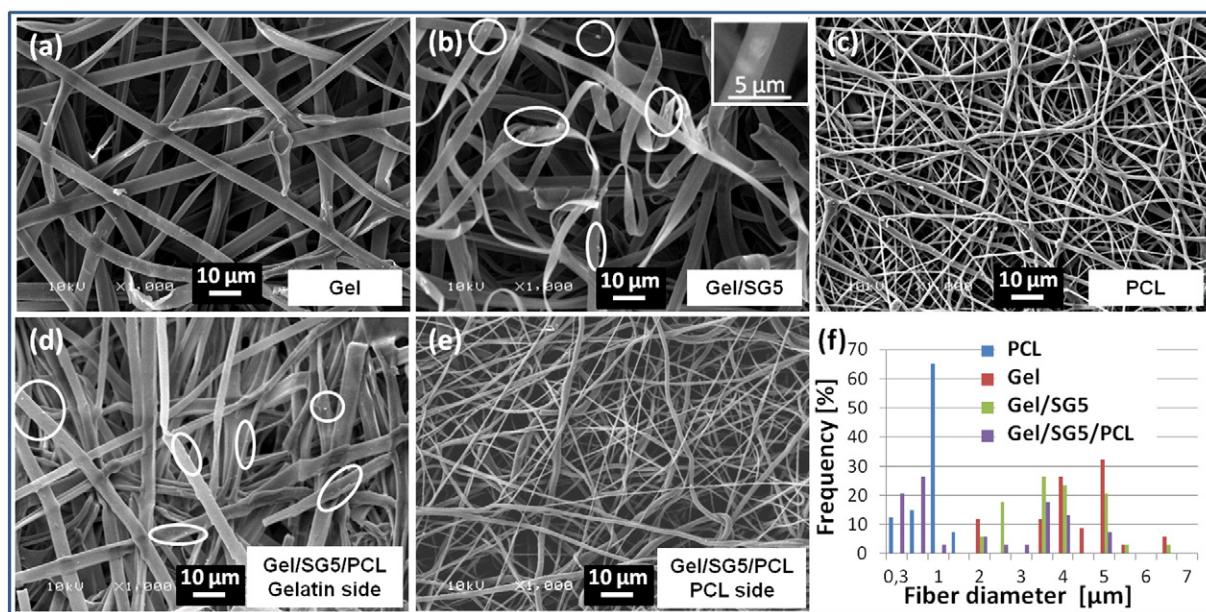


Fig. 1. SEM images of the fibrous scaffold: (a) gelatin (Gel); (b) calcium phosphate-modified gelatin (Gel/SG5); (c) polycaprolactone (PCL); (d–e) composite Gel/SG5/PCL; and (f) fiber diameter distribution in electrospun scaffolds.

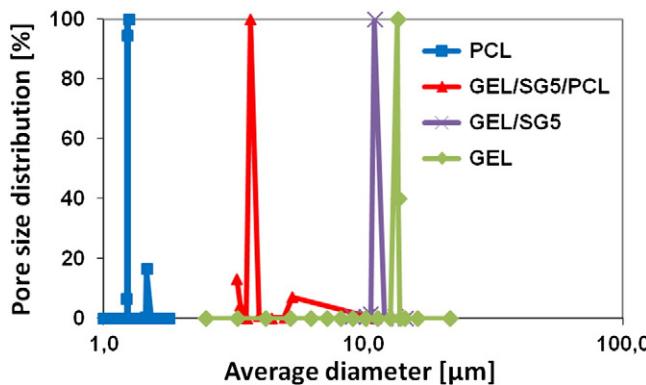


Fig. 2. Pore size distributions for electrospun scaffolds.

A PMI capillary flow porosimeter (USA) was applied to evaluate the pore size distribution of electrospun materials. Porosity of the scaffolds was determined based on SEM images using Image J software. The average porosity of the scaffolds was determined by performing measurements on 5 SEM images.

FTIR spectra were recorded using an FTS Digilab 60 BioRad spectrophotometer (ATR technique, in the 400–4000 cm⁻¹ range).

The WAXD measurements were carried out on the URD-63 Seifert diffractometer, equipped with a graphite monochromator and a copper target sealed X-ray tube operated at: U = 40 kV and I = 30 mA (Cu K α radiation, $\lambda = 1.542 \text{ \AA}$). The step scanning mode was employed in the range of the 2 θ scattering angle from 2.5° to 60° with the step of 0.1°.

Tensile tests were performed using a Zwick-Roell Z 2.5 testing machine. The thickness of the electrospun scaffolds was determined using the thickness gauge TILMET 73. The thickness test was measured on eight samples of each type of fibrous scaffold. The values of the average thickness and standard deviation were determined. The tensile tests were conducted at a speed of 1.0 mm/min. Five samples of each type of electrospun scaffold were tested. Tensile strength and work of fracture for all obtained materials were determined.

The thermal gravimetric analysis (TGA) measurements were carried out on the Mettler TOLEDO (TA) Instrument, up to 800 °C at a heating rate of 10 °C/min in an inert gas atmosphere (nitrogen, flow rate: 40 ml/min).

The differential scanning calorimetry (DSC) measurements were carried out using the TA Instruments 5100 analytical system, at a heating rate of 10 °C/min in an inert gas atmosphere (nitrogen, flow rate: 40 ml/min).

In order to evaluate bioactivity of the scaffold in vitro, the mineralization test was performed using simulated body fluid (SBF) [30]. Electrospun scaffolds were incubated for 14 days in 14 ml of 1.5x SBF

Table 1
Assignment of FTIR bands for polycaprolactone.

Wavenumber [cm ⁻¹]	Type of vibration
2949	CH ₂ , asymmetric, stretching
2865	CH ₂ , asymmetric, stretching
1727	C=O, stretching
1293	C—O—C, stretching
1240	C—O—C, asymmetric, stretching
1175	C—O—C, symmetric, stretching
1157	C—O, C—C, stretching

(pH 7.4; 37 °C). SBF solution was replaced every 2.5 days. After each soaking period, the samples were washed with distilled water and then dried at 37 °C. The surface morphology of the samples and ability of apatite formation were examined by the SEM and EDS methods.

2.4. Cell study

Cell culture experiments were carried out with two types of electrospun samples: (1) pure PCL, and (2) composite Gel/SG5/PCL scaffolds. The selected materials were cut into disks (diameter 14 mm) and were sterilized by exposure to ultraviolet (UV) light for half an hour on both sides and placed in a 24 well culture plate. Cell culture studies were carried out using human normal osteoblast (NHOst) cells obtained from Lonza (USA). Cells were cultured in OGM culture medium (Lonza, USA) containing 10% FBS, 5% gentamicin and amphotericin-B, under an atmosphere of 5% CO₂ at 37 °C. Cells were seeded on the scaffold in the concentration 2.0 × 10⁴ cells/ml. An empty polystyrene well was constituted as a positive control. The morphology of the cells on the surface of the scaffolds was observed by SEM (JEOL JSM 5400) on the 3rd and 7th days of culture.

Mineralization was assessed by the OsteoImage mineralization test (Lonza, USA). The test was performed after 7, 14 and 21 days of the cell culture in differentiation medium with the addition of OGM™ differentiation SingleQuots (Lonza, USA). The alkaline phosphatase (ALP) activity was determined using 4-Methylumbelliferyl phosphate as a substrate and was measured on the 7th and 14th days of culture. The hydrolysis of MUP was determined by fluorescence detection on a POLARStar Omega microplate reader (BMG Labtech, Germany) with settings for excitation at 360 nm and emission at 440 nm.

3. Results and discussion

3.1. Morphological, chemical and mechanical characterization of electrospun scaffold

The microstructure of electrospun fibrous scaffolds is presented in Fig. 1. Microscopic observation shows that materials made of gelatin

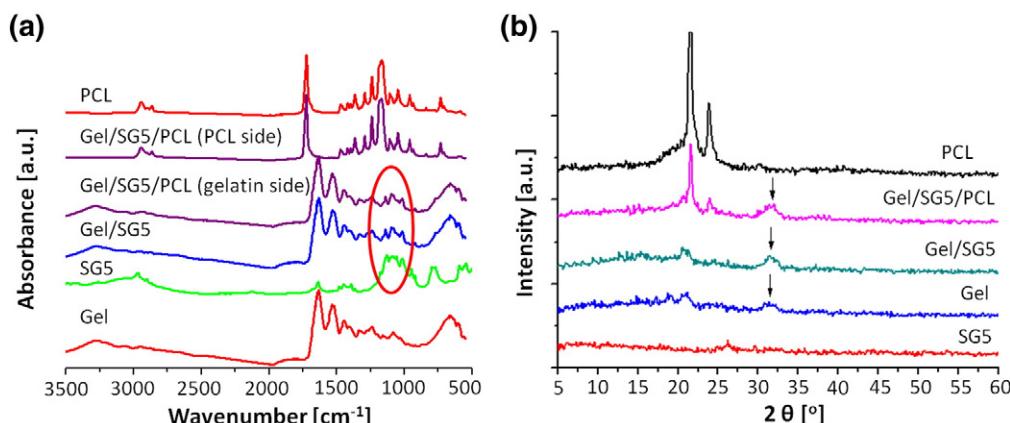


Fig. 3. (a) FTIR spectra of electrospun scaffolds; (b) WAXD patterns of electrospun scaffolds.

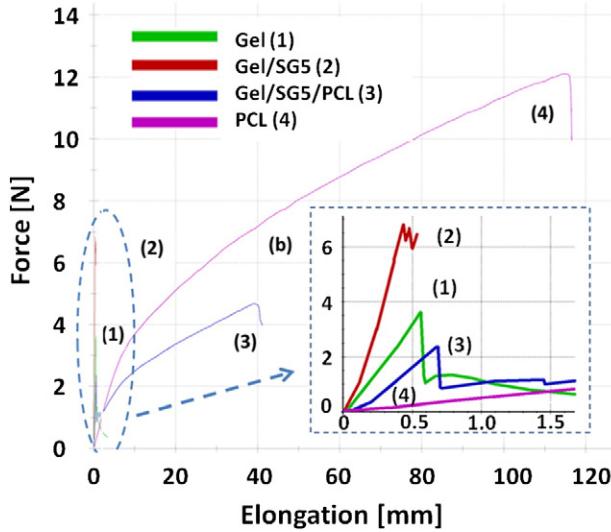


Fig. 4. Force–elongation curves for electrospun scaffolds: (1) Gel; (2) Gel/SG5; (3) Gel/SG5/PCL; and (4) PCL.

and calcium phosphate-modified gelatin have fibers with a flat shape, so-called ribbon fibers (Fig. 1a). Fibers with such morphology are obtained by the rapid evaporation of solvent from the surface of the stream during the electrospinning process which forms a crust in the form of a hollow tube (so-called Kirkendall effect) [31]. As a result of the further evaporation of the solvent, the crust collapses and wet fibers flatten during the clash with the collector, forming a flat ribbon. In the case of the modified gelatin scaffold (Gel/SG5), some calcium phosphate particles are clearly visible on the surface of the fibers (Fig. 1b). The polycaprolactone (PCL) scaffolds are made up of randomly arranged and mutually entangled smooth fibers (Fig. 1c). Microscopic images of the composite scaffold consist of two layers: (1) gelatin-modified calcium phosphate, and (2) polycaprolactone are shown in Fig. 1d, e. The microstructure of each side of the composite nonwoven fabric is very similar to the corresponding reference samples: Gel/SG5 and PCL. Fiber diameter distribution in nonwoven electrospun scaffolds is shown in Fig. 1f. The diameter of the ribbon fibers made of gelatin and calcium phosphate-modified gelatin is in the range of 2.0–6.5 μm. The addition of calcium phosphate lowers the average gelatin fiber diameter from 4.3 μm for pure nonwoven fabric to 3.8 μm for modified electrospun scaffolds (Gel/SG5). The decrease in the diameter of the fiber is probably due to the increase of the Coulomb interaction forces during electrospinning, caused by the presence of calcium phosphate powder in the spinning solution. The massive increase in electrostatic

repulsion tends to increase the surface area and leads to decreased fiber diameter [32]. In addition, microscopic observation shows that the ribbon fibers have a thickness of about 2 μm and a width up to 6.5 μm, so the distribution of diameters shown in Fig. 1f is also the result of twisting the gelatin ribbons. Polycaprolactone fiber diameter is in the range of 0.3–1.5 μm, while the calculated average diameter of the polycaprolactone fibers is 0.77 μm ± 0.34 μm. In the case of composite nonwoven fabrics, the fiber diameter is in the range of 0.3–5 μm. The results of scaffold porosity (Fig. 2) show that the PCL scaffold has the main fraction of pores about 1.2 μm, while the average pore diameter of the second fraction is 1.5 μm. Unmodified gelatin scaffold (Gel) has the largest average pore diameter and the main pore fraction is in the range 12.7–14.5 μm. Modified Gel/SG5 nonwoven fabric has smaller pore diameters, whose main fraction is in the range 10.2–11.3 μm. For the composite nonwoven fabric the main fraction of pores is in the range 3.9–4.2 μm. These low values of composite pore size correspond to the PCL layer of the composite; however, pores with bigger diameters (up to 11 μm) are also present. The presence of gelatin in the composite increases the pore size of the scaffold compared with pure PCL material. Porosity of the scaffolds was determined based on SEM images using Image J software. The challenge with determining the threshold of histogram for micrographs was to segment this type of grayscale image into regions associated with the fibers and interfiber spaces respectively. To determine threshold the “eye approach” technique was used. Once a threshold value was defined the grayscale image was changed to binary black and white. The binary image which was unambiguously divided, was analyzed for the interfiber spaces. The resulted average porosity was: 78.3 ± 4.9% for pure Gel scaffolds, 69.4 ± 6.1% for Gel/SG5 samples, 81.3 ± 6.4% for pure PCL scaffolds and in the case of composite scaffold: 74.3 ± 7.0% for the Gel/SG5 side of scaffold and 86.7 ± 2.3% for the PCL side of composite scaffold. FTIR spectra recorded for all electrospun nonwoven samples and SG5 powder are presented in Fig. 3a. The band characteristic for peptide bonds can be seen in the FTIR spectra of gelatin (Gel): amide-A band in the wavenumber range 3200–3500 cm⁻¹ corresponding to N–H stretching vibrations, amide-I band at 1632 cm⁻¹ corresponding to C=O stretching vibrations and the amide II and III bands, respectively at 1534 cm⁻¹ and 1239 cm⁻¹, mainly corresponding to NH bending vibration and CN stretching vibrations. Additionally, in the case of the modified nonwoven fabric (Gel/SG5) and composite nonwoven fabric on the gelatin side there are bands in the range 900–1140 cm⁻¹ derived from SG5 powder, corresponding to the vibrations of PO₄ groups. The most important characteristic bands for polycaprolactone are shown in Table 1. FTIR studies confirmed that SG5 nanoparticles were successfully incorporated into the fibrous gelatin matrix. The results of WAXD diffraction curves of the electrospun scaffolds are shown in Fig. 3b. WAXD diffraction curve for SG5 powder looks as a noise line in the whole range of the 2θ scattering angle. On the polycaprolactone scaffold WAXD pattern, two strong

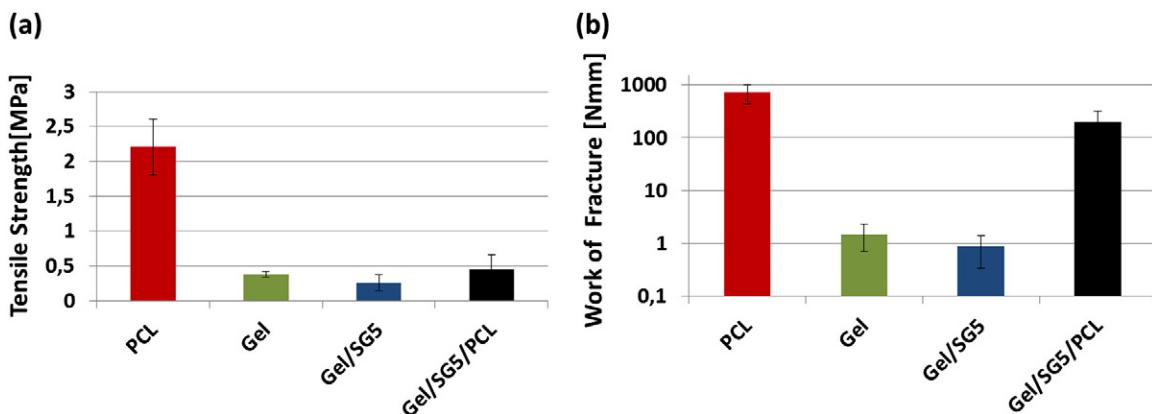


Fig. 5. (a) Tensile strength of electrospun scaffolds; (b) work of fracture for electrospun fibrous scaffolds.

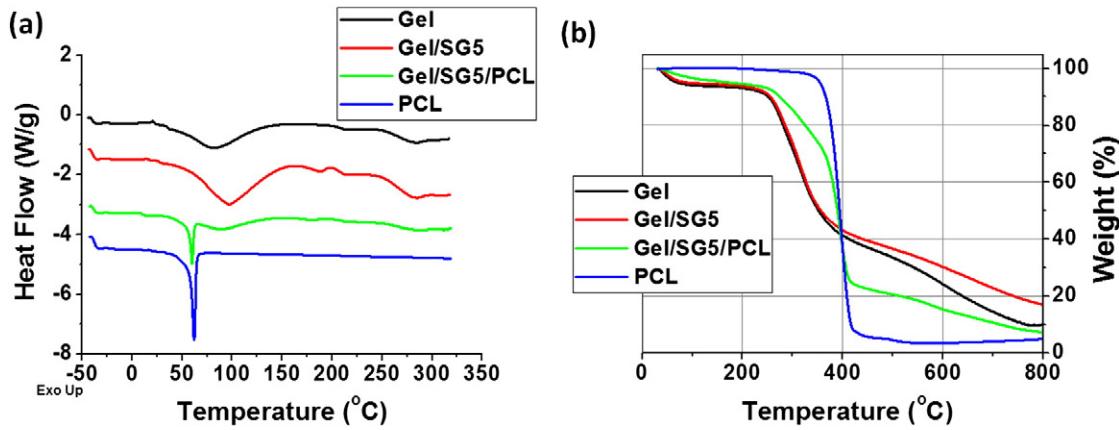


Fig. 6. (a) DSC thermograms of Gel/SG5/PCL, Gel/SG5, Gel and PCL electrospun scaffolds; (b) TGA curves of Gel/SG5/PCL, Gel/SG5, Gel and PCL electrospun scaffolds.

diffraction peaks appeared at 2θ: 21.60° and 23.95°. The diffraction curve for gelatin is characterized by two very weak and relatively broad diffraction peaks centered at the 2θ diffraction angle: 20.90° and 31.50°. These diffraction peaks appear as well on WAXD patterns assigned to Gel/SG5 scaffold. In the case of Gel/SG5/PCL hybrid composite, there are diffraction peaks characteristic for both composite layers (PCL; Gel/SG5). No structural changes in the layers of the composite scaffold, in relation to the structure of the original components of the composite, were observed. Typical force–elongation curves for the electrospun nonwoven fabrics are presented in Fig. 4. The highest value of tensile force (12 N) and the largest elongation (about 115 mm) were observed in the case of polycaprolactone (PCL) nonwoven fabric. The tensile force of the gelatin nonwoven fabric modified with calcium phosphate (Gel/SG5) was lower by about 42%. This scaffold is also characterized by the lowest elongation of about 0.6 mm. The composite scaffold showed about a 60% lower tensile force compared to an unmodified polycaprolactone nonwoven fabric, and elongation was lower by about 65%. Tensile strengths for all obtained electrospun scaffolds are shown in Fig. 5a. Polycaprolactone material had the highest tensile strength. The tensile strength for the unmodified gelatin nonwoven scaffold was about 0.4 MPa. The addition of calcium phosphate caused a deterioration of mechanical properties of gelatin nonwoven fabrics by about 32%. The decrease in the tensile strength of modified Gel/SG5 scaffold is probably due to a tendency of the SG5 powder to agglomerate and the creation of pores due to a bad wetting and different hydrophilicity. Electrospinning of gelatin–polycaprolactone composite material (Gel/SG5/PCL) allowed for the production of scaffold (containing gelatin) with a tensile strength of about 43% higher than the strength of Gel/SG5 nonwoven fabric. Similar changes are

observed in the results of work of fracture (Fig. 5b). PCL scaffold has the greatest work of fracture (725 Nmm). For the composite scaffold, the work of fracture is almost 3.5 times lower (202 Nmm). The lowest values of the work of fracture were recorded for gelatin nonwoven fabrics (0.5 and 0.8 Nmm). Compared with the composite scaffold and unmodified polycaprolactone nonwoven fabrics, gelatin electrospun scaffold is characterized by relatively low mechanical properties. The creation of a composite material made it possible to significantly improve the mechanical properties of gelatin substrates. The DSC results are shown in Fig. 6a. The curve obtained for the gelatin sample reveals exothermic peaks at temperatures of 82.4 °C, 215.6 °C and 283.7 °C. Gelatin is composed mainly of denatured collagen, and in about 10% of renaturated collagen. Thermal degradation of the gelatin molecules is accompanied by disorganization of the helical structure. The first exothermic peak is related to the evaporation of water from the sample, and the second with the transition from the helical structure into the statistical coil (a less ordered structure). The endothermic effect on the DSC curve for the composite nonwoven fabric at 58 °C is associated with the melting temperature of polycaprolactone. In addition, on the same curve we observe peaks characteristic of gelatin. The TGA curves are shown in Fig. 6b. In the case of the gelatin scaffold and gelatin modified with calcium phosphate, the initial weight loss occurring in the temperature range 25–150 °C is related to the evaporation of water. Weight loss in this temperature range is about 5–6%. The main weight loss for gelatin samples was observed in the temperature range 250 °C–400 °C, which is likely accompanied by breaking of peptide bonds. Weight loss in this temperature range is about 55% for gelatin nonwoven fabric, 53% for modified nonwoven fabric (Gel/SG5) and 73% for the composite sample.

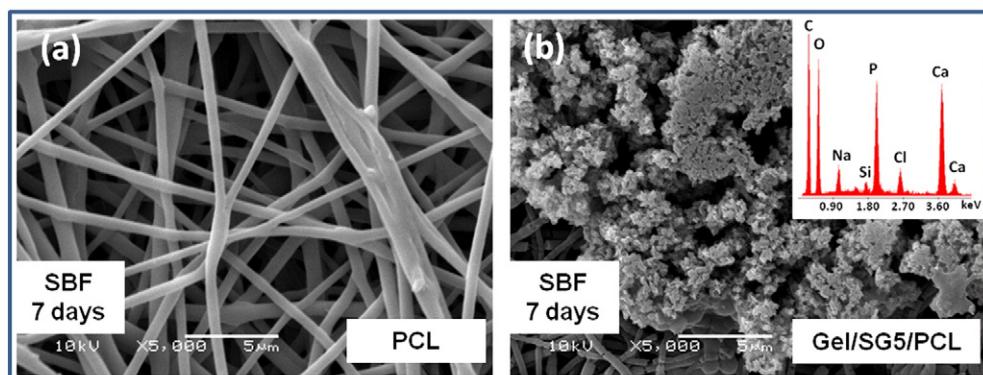


Fig. 7. (a) PCL and (b) Gel/SG5/PCL electrospun scaffolds after 7 days of incubation in SBF.

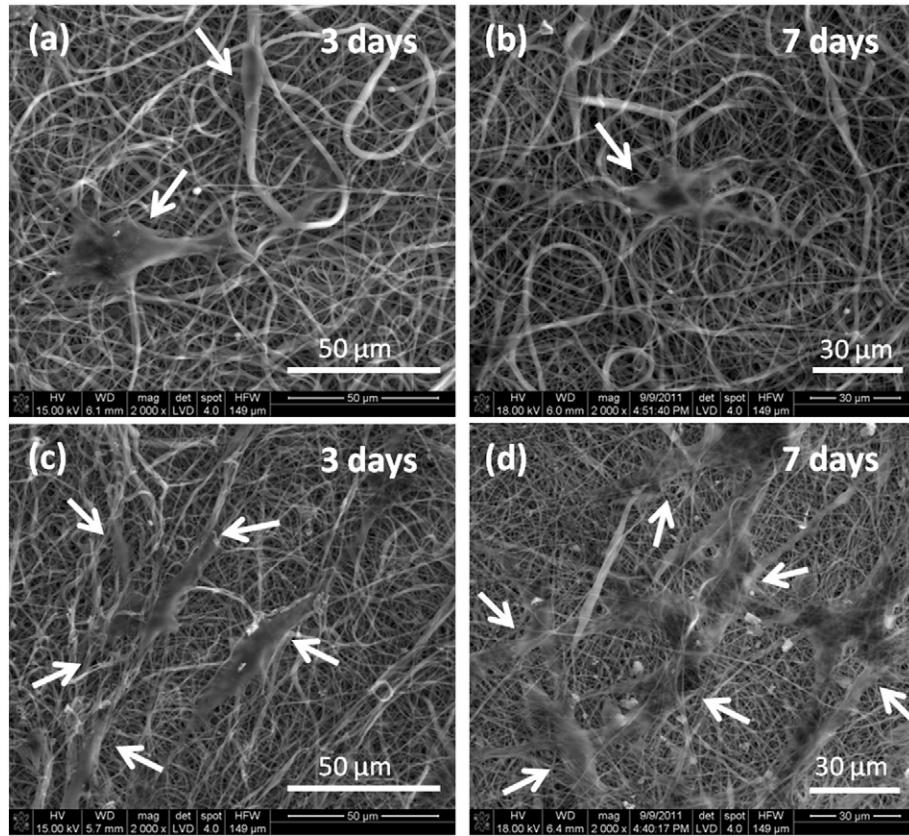


Fig. 8. Morphology of NHOst cells cultured on pure PCL (a, b) and composite Gel/SG5/PCL (c, d) scaffolds after 3 days and 7 days.

To assess the bioactivity of the composite scaffold, obtained materials were incubated in SBF. The surface morphology of the PCL and Gel/SG5/PCL samples after 7 days of immersion in 1.5x SBF solution is shown in Fig. 7. SEM micrographs of samples at day 7 showed the growth of an apatite-like layer on the composite Gel/SG5/PCL sample surface, whereas the surface of pure PCL sample remained unchanged.

3.2. Mineralization test in cell culture

In order to estimate the composite capability in promoting mineralization in the cell culture, NHOst cells were seeded onto PCL and Gel/SG5/PCL scaffolds. Fig. 8 presents the morphology of NHOst cells cultured on PCL and Gel/SG5/PCL scaffolds after 3 and 7 days. After 3 days of cell culture it can be observed that cells had different interactions with both used materials. NHOst cells spread well on the surface of

composite sample, showing that the gelatin and calcium phosphate (SG5) contents have a positive effect on the cell adhesion. After 7 days numerous cells covered the surface of composite Gel/SG5/PCL scaffolds. Some of the cells entered the material through the pores, what confirmed a good biocompatibility of the composite samples. Progress of mineralization was assessed by ALP activity and OsteoImage tests. The alkaline phosphatase activity has a crucial role in the initiation of the mineralization process. The ALP activity is believed as a marker of the early stages of osteogenic differentiation because its level increases with maturation of osteoblast [33]. ALP hydrolyses phosphate and increases the local phosphate concentration, further enhancing the mineralization of ECM [34]. Fig. 9a shows the ALP activity of NHOst cells cultured on PCL and composite samples for 7 and 14 days. A hydrophobic surface leads to a lower cell adhesion in the initial step of cell culture [35]. Gelatin layer allows a better adhesion of cells onto the scaffold

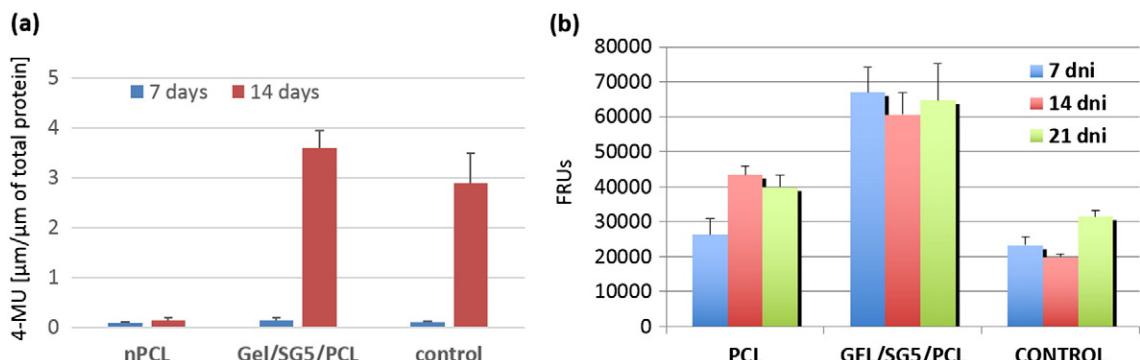


Fig. 9. (a) Alkaline phosphatase activity test; (b) mineralization progress measured by the concentration of HAp on the 7th, 14th and 21st days of NHOst cell culture on the TCPS control surface and on the surface of Gel/SG5/PCL and pure PCL scaffolds. RFUs – relative fluorescence units. Statistically significant differences compared to the control (according to Tukey's t-test for $p < 0.05$) are exhibited by Gel/SG5/PCL.

surface and, therefore, after 7 days of cell culture, ALP activity in the gelatin layer is already higher than that in pure PCL, and much higher after 14 days. The higher ALP activity of the composite samples compared to the pure PCL material also indicates a better cell differentiation on the Gel/SG5/PCL surface. There are also other studies confirming the differentiation ability of bioactive glass additives [36,37]. Cell differentiation is also confirmed by OsteoImage (OI) test results (Fig. 9b). The progress of mineralization was better pronounced on composite materials. Bioactive glasses are known for its influence on mineralization [38–40]. Although the high OI test values for Gel/SG5/PCL composite in 7 day series might be caused by the SG5 content, the 21 day series confirmed a high mineralization capability of the obtained scaffolds. The lower value of OI in a 14 day series was probably the result of SG5 component dissolution, but the increase of OI values after 21 days indicates the influence of cell activity.

In this study Gel/SG5/PCL composite scaffolds were fabricated without the use of any cross-linking agent, through collecting separately electrospun poly(ϵ -caprolactone) (PCL) and calcium phosphate modified gelatin (Gel/SG5) fibers on the same collector. It is well known that commonly used cross-linking agents (e.g. glutaraldehyde) are not relevant for gelatin scaffold as they decrease the hydrophilicity and the bioactive character of this polymer [2,9]. Moreover the cross-linking process changes the microstructure of electrospun gelatin fibers and inhibits cell adhesion and proliferation [41]. In order to enhance the hydrophobic character of polycaprolactone and mechanical properties of gelatin, composite bi-layered scaffolds were obtained in this study. Microscopic SEM observation demonstrated the similarity of both composite layers to pure PCL or Gel/SG5 scaffolds. SG5 nanoparticles were clearly visible on the surface of the gelatin component of the scaffold and their presence was confirmed by FTIR study. No structural changes in the layers of the composite scaffold, in relation to the structure of the original components of the composite, were observed on the WAXD diffraction curves. Combining gelatin with polycaprolactone, a new material with increased porosity compared with pure PCL sample was obtained. However, further modification of the production process is required in order to provide optimal porosity of scaffold for bone tissue regeneration. The composite Gel/SG5/PCL scaffold exhibited an enhanced tensile strength and elevated work of fracture than individual Gel and Gel/SG5. The bioactive character of the obtained material was confirmed by the SBF mineralization study. Both ALP and OI results prove the ability of Gel/SG5/PCL to promote the osteogenic activity of cells i.e. their differentiation and, in consequence, the mineralization.

4. Conclusions

In this study Gel/SG5/PCL composite scaffolds were fabricated by collecting electrospun poly(ϵ -caprolactone) (PCL) and calcium phosphate modified gelatin (Gel/SG5) fibers separately on the same collector. The introduction of PCL improved the mechanical properties of the gelatin scaffold remarkably, and the addition of calcium phosphate nanoparticles (SG5) enhanced the bioactivity of the composite scaffold. The composite Gel/SG5/PCL material had better pore size distribution than individual PCL samples. In vitro biological evaluation showed that the presence of gelatin and calcium phosphate nanoparticles in the scaffold offered higher activity of ALP and better NH₃Ost cell mineralization.

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References

- [1] J.-H. Jang, O. Castaño, H.-W. Kim, Electrospun materials as potential platforms for bone tissue engineering, *Adv. Drug Deliv. Rev.* 61 (2009) 1065–1083.
- [2] Z.X. Meng, Y.S. Wang, C. Ma, W. Zheng, L. Li, Y.F. Zheng, Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering, *Mater. Sci. Eng. C* 30 (2010) 1204–1210.
- [3] T.J. Sill, H.A. Von Recum, Electrospinning: applications in drug delivery and tissue engineering, *Biomaterials* 29 (2008) 1989–2006.
- [4] K. An, H. Liu, S. Guo, K. D.N.T., Q. Wang, Preparation of fish gelatin and fish gelatin/poly(l-lactide) nanofibers by electrospinning, *Int. J. Biol. Macromol.* 47 (2010) 380–388.
- [5] I. Rajzer, E. Menasze, R. Kwiatkowski, W. Chrzanowski, Bioactive nanocomposite PLDL/nano-hydroxyapatite electrospun membranes for bone tissue engineering, *J. Mater. Sci. Mater. Med.* 25 (2014) 1239–1247.
- [6] Z.-M. Huang, Y.Z. Zhang, S. Ramakrishna, C.T. Lim, Electrospinning and mechanical characterization of gelatin nanofibers, *Polymer* 45 (2004) 5361–5368.
- [7] Q. Jiang, H. Xu, S. Cai, Y. Yang, Ultrafine fibrous gelatin scaffolds with deep cell infiltration mimicking 3D ECMs for soft tissue repair, *J. Mater. Sci. Mater. Med.* (2014), <http://dx.doi.org/10.1007/s10856-014-5208-2>.
- [8] J.-P. Chen, C.-H. Su, Surface modification of electrospun PLLA nanofibers by plasma treatment and cationized gelatin immobilization for cartilage tissue engineering, *Acta Biomater.* 7 (2011) 234–243.
- [9] T.H. Nguyen, B.T. Lee, Fabrication and characterization of cross-linked gelatin electrospun nanofibers, *J. Biomed. Sci. Eng.* 3 (2010) 1117–1124.
- [10] J.S. Mao, L.G. Zhao, Y.J. Yin, K.D. Yao, Structure and properties of bilayer chitosan-gelatin scaffolds, *Biomaterials* 24 (2003) 1067–1074.
- [11] J. Lee, G. Tae, Y.H. Kim, I.S. Park, S.-H. Kim, S.H. Kim, The effect of gelatin incorporation into electrospun poly(L-lactide-co- ϵ -caprolactone) fibers on mechanical properties and cytocompatibility, *Biomaterials* 29 (2008) 1872–1879.
- [12] X. Liu, P.X. Ma, Phase separation, pore structure and properties of nanofibrous gelatin scaffolds, *Biomaterials* 30 (2009) 4094–4103.
- [13] S. Panzavolta, M. Gioffre, M.L. Focarete, C. Gualandi, L. Foroni, A. Bigi, Electrospun gelatin nanofibers: optimization of genipin crosslinking to preserve fiber morphology after exposure to water, *Acta Biomater.* 7 (4) (2011) 1702–1709.
- [14] X. Liu, L.A. Smith, J. Hu, P.X. Ma, Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering, *Biomaterials* 30 (2009) 2252–2258.
- [15] I. Rajzer, J. Grzybowska-Pietras, J. Janicki, Fabrication of bioactive carbon nonwovens for bone tissue regeneration, *Fibres Text. Eur.* 19 (1) (2011) 66–72.
- [16] M.O. Choi, Y.-J. Kim, Fabrication of gelation/calcium phosphate composite nanofibrous membranes by biomimetic mineralization, *Int. J. Biol. Macromol.* 50 (2012) 1188–1194.
- [17] I. Rajzer, M. Rom, M. Błażewicz, Production and properties of modified carbon fibers for medical applications, *Fibers Polym.* 11 (4) (2010) 615–624.
- [18] I. Rajzer, R. Kwiatkowski, W. Piekarzky, W. Biniaś, J. Janicki, Carbon nanofibers produced from modified electrospun PAN/hydroxyapatite precursors as scaffolds for bone tissue engineering, *Mater. Sci. Eng. C* 32 (2012) 2562–2569.
- [19] E.M. Brown, R.J. MacLeod, Extracellular calcium sensing and extracellular calcium signaling, *Physiol. Rev.* 81 (1) (2001) 239–297.
- [20] R. Mentaverri, S. Yano, N. Chattopadhyay, L. Petit, O. Kifor, S. Kamel, E.F. Terwilliger, M. Brazier, E.M. Brown, The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis, *FASEB J.* 20 (14) (2006) 2562–2564.
- [21] G.B. Adams, K.T. Chabner, I.R. Alley, D.P. Olson, Z.M. Szczepiorkowski, M.C. Poznansky, C.H. Kos, M.R. Pollak, E.M. Brown, D.T. Scadden, Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor, *Nature* 439 (7076) (2006) 599–603.
- [22] M. Tommila, A. Jokilammi, P. Terho, T. Wilson, R. Penttilä, E. Ekholm, Hydroxyapatite coating of cellulose sponges attracts bone-marrow-derived stem cells in rat subcutaneous tissue, *J. R. Soc. Interface* 6 (39) (2009) 873–880.
- [23] L. Van der Schueren, B. De Schoenmaker, O.I. Kalaoglu, K.D. De Clerck, An alternative solvent system for the steady state electrospinning of polycaprolactone, *Eur. Polym. J.* 47 (2011) 1256–1263.
- [24] P. Fabbri, F. Bondioli, M. Messori, C. Bartoli, D. Dinucci, F. Chiellini, Porous scaffolds of polycaprolactone reinforced with in situ generated hydroxyapatite for bone tissue engineering, *J. Mater. Sci. Mater. Med.* 21 (2010) 343–351.
- [25] I. Rajzer, Fabrication of bioactive polycaprolactone/hydroxyapatite scaffolds with final bilayer nano-/micro-fibrous structures for tissue engineering application, *J. Mater. Sci.* 49 (2014) 5799–5807.
- [26] M.R. Allen, J.M. Hock, D.B. Burr, Periosteum: biology, regulation, and response to osteoporosis therapies, *Bone* 35 (5) (2004) 1003–1012.
- [27] X. Zhang, H.A. Awad, R.J. O'Keefe, R.E. Goldberg, E.M. Schwarz, A perspective: engineering periosteum for structural bone graft healing, *Clin. Orthop. Relat. Res.* 466 (2008) 1777–1787.
- [28] Z. Lin, A. Fateh, D.M. Salem, G. Intini, Periosteum: biology and applications in craniofacial bone regeneration, *J. Dent. Res.* (Oct 2 2013), <http://dx.doi.org/10.1177/0022023413506445>.
- [29] O. Castaño, M. Navarro, J.A. Planell, E. Engel, A. Aguirre, Patent EP2386525 (Institute for Bioengineering of Catalonia-IBEC and Technical University of Catalonia-UPC), 2012.
- [30] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* 27 (15) (2006) 2907–2915.
- [31] H.J. Fan, M. Knez, R. Scholz, D. Hesse, K. Nielsch, M. Zacharias, U. Gösele, Influence of surface diffusion on the formation of hollow nanostructures induced by the Kirkendall effect: the basic concept, *Nano Lett.* 7 (2007) 993–997.

- [32] L. Ji, A.J. Medford, X. Zhang, Electrospun polyacrylonitrile/zinc chloride composite nanofibers and their response to hydrogen sulfide, *Polymer* 50 (2009) 605–612.
- [33] E.E. Golub, K. Boesze-Battaglia, The role of alkaline phosphate in mineralization, *Curr. Opin. Orthop.* 18 (2007) 444–448.
- [34] Z.X. Meng, H.F. Li, Z.Z. Sun, W. Zheng, Y.F. Zheng, Fabrication of mineralized electrospun PLGA and PLGA/gelatin nanofibers and their potential in bone tissue engineering, *Mater. Sci. Eng. C* 33 (2013) 699–706.
- [35] L. Ghasemi-Mobarakeh, M.P. Prabhakaran, M. Morshed, M.H. Nasr-Esfahani, S. Ramakrishna, Electrospun poly(epsilon-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering, *Biomaterials* 29 (2008) 4532–4539.
- [36] E. Pamula, J. Kokoszka, K. Cholewa-Kowalska, M. Laczka, et al., Degradation, bioactivity, and osteogenic potential of composites made of PLGA and two different sol-gel bioactive glasses, *Ann. Biomed. Eng.* 39 (8) (2011 Aug) 2114–2129.
- [37] J. Pawlik, M. Widziołek, K. Cholewa-Kowalska, M. Łączka, A.M. Osyczka, New sol-gel bioactive glass and titania composites with enhanced physico-chemical and biological properties, *J. Biomed. Mater. Res. Part A* 102 (7) (2014 Jul) 2383–2394, <http://dx.doi.org/10.1002/jbm.a.34903>.
- [38] O. Tsigkou, J.R. Jones, J.M. Polak, M.M. Stevens, Differentiation of fetal osteoblasts and formation of mineralized bone nodules by 45S5 Bioglass conditioned medium in the absence of osteogenic supplements, *Biomaterials* 30 (21) (2009 Jul) 3542–3550.
- [39] O. Tsigkou, LL. Hench, A.R. Boccaccini, J.M. Polak, M.M. Stevens, Enhanced differentiation and mineralization of human fetal osteoblasts on PDLLA containing Bioglass composite films in the absence of osteogenic supplements, *J. Biomed. Mater. Res. Part A* 80 (4) (2007 Mar 15) 837–851.
- [40] O. Castano, N. Sachot, E. Xuriguera, E. Engel, J.A. Planell, J.-H. Park, et al., Angiogenesis in bone regeneration: tailored calcium release in hybrid fibrous scaffolds, *ACS Appl. Mater. Interfaces* 6 (10) (2014) 7512–7522.
- [41] Y.Z. Zhang, J. Venugopal, Z.M. Huang, C.T. Lim, Crosslinking of the electrospun gelatin nanofibers, *J. Biomed. Mater. Res. B Appl. Biomater.* 47 (8) (2006) 2911–2917.