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· 综述 ·

含铜钛合金调控巨噬细胞极化的研究进展

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【摘要】 含铜钛合金作为一种新型抗菌材料,具有良好的生物学性能和机械性能,有望广泛应用于临床骨科和口腔种植等领域。巨噬细胞是合金植入人体后介导免疫反应的主要细胞,并且直接影响合金长期服役的稳定性。含铜钛合金由于铜的添加,赋予了合金抗菌性能,一方面促进巨噬细胞吞噬和杀灭细菌,另一方面促进巨噬细胞极化激活,表达多种细胞因子,引发体内炎症反应。然而,目前关于含铜钛合金调控巨噬细胞极化的类型尚无定论,铜离子调控巨噬细胞极化的机制仍未统一。本文对已发表的关于含铜钛合金调控巨噬细胞极化的研究进行总结,分别从材料类型、表面处理、加工方式和细胞的培养方式、培养密度等方面,对相关文献进行综述,并且对医用含铜钛合金的应用进行了展望,希望通过改变含铜钛合金的性能,如加工方式或表面处理等,调控巨噬细胞的极化方向,以期为医用含铜钛合金的设计及临床应用提供参考。

【关键词】 巨噬细胞; 极化; M1型巨噬细胞; M2型巨噬细胞; 细胞因子; 含铜钛合金; 抗菌材料; 铜元素; 细菌感染; 免疫反应; 抗菌

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Research progress on Ti-Cu alloys regulating macrophage polarization LOU Jingyang¹, GENG Xinrong¹, GAO Huimeng¹, FAN Dongyang², ZHAO Xin³, WANG Qiang⁴. 1. School and Hospital of Stomatology, China Medical University, Shenyang 110002, China; 2. Department of General Dentistry, School and Hospital of Stomatology, China Medical University, Shenyang 110002, China; 3. Department of Prosthodontics, School and Hospital of Stomatology, China Medical University, Shenyang 110002, China; 4. Department of Dental Materials, School and Hospital of Stomatology, China Medical University, Shenyang 110002, China

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【Abstract】 As a new type of antibacterial material, copper-containing titanium alloys have good biological and mechanical properties and are expected to be widely used in clinical orthopedics and dental implants. Macrophages are the main cells that mediate the immune response after the alloy is implanted into the human body, and they directly affect the stability of the alloy in long-term service. Due to the addition of copper, the copper-containing titanium alloy gives the alloy antibacterial properties. On the one hand, this promotes the phagocytosis of macrophages and kills bacteria. On the other hand, copper promotes the polarization activation of macrophages, which then express a variety of cytokines and trigger inflammation in the body. However, at present, there is no definitive conclusion about the type of copper-containing titanium alloys that regulate the polarization of macrophages, and the mechanism of copper ions regulating the polarization of macrophages is still not fully clear. This article summarizes the published studies on the regulation of macrophage polarization by copper-containing titanium alloys and reviews the relevant literature in terms of mate-

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rial types, surface treatments, processing methods, cell culture methods, and culture density. The application of medical copper-containing titanium alloys has prospects. It is hoped that by changing the properties of copper-containing titanium alloys, such as the processing methods or surface treatments, the polarization direction of macrophages can be adjusted, with a view to the design and clinical application of medical copper-containing titanium alloys.

【Key words】 macrophage; polarization; M1 macrophage; M2 macrophage; cytokine; copper-containing titanium alloys; antibacterial material; cuprum; bacterial infection; immunoreaction; antibacterial

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植入合金常引起感染且难以控制,通常是由细菌在合金表面定植所引起。细菌通过黏附素黏附于种植体表面后,产生胞外聚合物,在合金表面形成细菌生物膜^[1]。生物膜中的细菌会引起慢性感染,表现为持续的炎症和组织损伤^[2]。近年来,合金在植入机体后所引起的免疫反应备受关注,有关钛合金免疫效应的研究报道数量呈逐年上升的趋势。目前发现,铜元素的添加可以赋予钛合金抗细菌感染的功能^[3-4]。微观结构为 α -Ti+Ti₂Cu的钛合金具有最佳的综合性能,包括理想的延展性、抗菌性和耐腐蚀性,具有良好的临床应用价值^[4]。巨噬细胞作为机体主要的免疫细胞,在合金植入体内后率先反应。巨噬细胞不仅能与细菌生物膜接触,激活杀菌能力,还能发生极化、增殖、产生炎性细胞因子等,诱导发生局部炎症反应^[5]。本文对含铜钛合金调控巨噬细胞极化的相关研究进行综述。

1 巨噬细胞对合金的响应行为

合金植入时不可避免地引起组织损伤。损伤组织周围微环境释放募集、定位和触发炎性细胞功能的信号,协调炎症与修复进程。巨噬细胞率先响应微环境刺激信号,进行极化激活^[6]。极化的巨噬细胞主要分M1型和M2型,M1型巨噬细胞主要与急性炎症发生有关,表达大量炎性细胞因子,而M2型巨噬细胞主要促进组织再生和愈合,表达抗炎因子^[7]。

巨噬细胞不仅参与合金植入后的反应,且对合金能否长期稳定地存在体内也有影响。一方面,巨噬细胞融合形成异物巨细胞,试图吞噬合金,并释放降解介质,如活性氧中间体、降解酶和酸等^[8]。另一方面,合金在体内被磨损产生碎片。碎片不仅会受到机体的免疫攻击,引发局部组织

不良反应,还可以通过激活巨噬细胞等免疫细胞来抑制成骨细胞活性,促进破骨细胞生成^[9]。研究发现在髋关节置换术中植入物周围常常发生骨溶解,最终导致植入失败^[9]。

2 含铜钛合金的免疫反应

2.1 铜元素的生物性能

铜是人体必需的微量元素,具有良好的抗菌性。研究表明,含铜量大于5 wt%的钛合金均表现出较理想的抗菌性能^[10]。随着铜含量的增加,合金的强度和硬度也逐渐提高。但是,过高的铜含量会降低钛合金的延展性,使其易发生脆性断裂,并且当铜含量增加到7.5 wt%时,合金腐蚀速率增加,铜离子释放增多,产生细胞毒作用^[11]。研究表明,过量的铜会激活凋亡调节蛋白p53介导的凋亡通路^[12],当Cu²⁺造成的氧化损伤严重且无法修复时,p53启动凋亡通路,促进细胞凋亡^[13]。

另外,铜的杀菌能力与氧化应激相关。细胞内发生氧化应激反应: $\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \cdot\text{OH} + \text{OH}^-$,这个过程被称为Fenton反应^[14]。铜通过促进活性氧(reactive oxygen species, ROS)的生成,打破Fenton反应平衡,产生氧化损伤^[15]。受到氧化损伤后细胞会产生膜脂化、蛋白质不可逆的羰基化、DNA的断裂或突变等,从而影响RNA的转录、翻译过程^[16]。细胞内过多的ROS积累会使细胞发生不可逆转的损伤和死亡^[17]。研究表明,巨噬细胞在含铜钛合金表面生长时,吞噬的细菌数量远远大于在不含铜钛合金上的细菌数量^[18],因此,推测巨噬细胞内积累的Cu²⁺不仅通过Fenton反应对细胞产生直接损伤,还能增强细胞内活性氧的氧化还原循环^[19],这种循环会产生更多的氧化还原活性物质,并消耗关键的细菌抗氧化剂(如:硫醇),从而导致细菌死亡^[15]。

2.2 含铜钛合金对巨噬细胞的极化调控

Huang等^[18]通过Cu-Hier-Ti表面对巨噬细胞极化的研究,发现巨噬细胞在培养48 h后,表达了较高的促炎标志物,如:一氧化氮合成酶(iNOS)和较低水平的抗炎标志物,如:精氨酸酶(Arg1);Cu-Hier-Ti表面刺激肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)产生,抑制白细胞介素-2(interleukin-2, IL-2)产生;另外,Cu-Hier-Ti表面M1表面标志物CD11c和促炎因子(如:TNF- α , iNOS和IL-1 β)增多。以上结果表明,Cu-Hier-Ti表面促进巨噬细胞向M1极化,这是由于材料中释放出来的Cu²⁺激活了巨噬细胞中的铜转运信号CTR1和ATP7。其中,CTR1负责细胞摄取铜,ATP7负责细胞内转运铜^[20],使巨噬细胞表达了较高的iNOS,极化为M1型^[18]。

Xu等^[21]通过Ti6Al4V-6Cu对巨噬细胞极化的研究,发现Ti6Al4V-6Cu可显著抑制巨噬细胞的活化增殖,抑制巨噬细胞炎性基因表达,促进向M2型极化。PCR检测结果显示,Ti6Al4V-6Cu抑制巨噬细胞表达促炎细胞因子(TNF- α 、IL-1 β 和IL-6);Ti6Al4V-6Cu表面的巨噬细胞产生大量的抗炎细胞因子。Ti6Al4V-6Cu表面的巨噬细胞表达更低水平的M1型巨噬细胞标志物基因iNOS和更高水平的M2型巨噬细胞标志物Arg1。

Hoene等^[22]与Walschus等^[23]改变含铜钛合金的加工方式,分别植入大鼠颈部肌肉进行体内实验,所得结果相同,含铜钛合金均促进巨噬细胞增殖和上调炎症反应,合金的加工方式似乎对巨噬细胞的免疫调控效果并无影响。Huang等^[18,24]先后进行两次实验,将小鼠RAW264.7巨噬细胞以相同孔密度接种于相同工艺处理的含铜钛合金表面,尽管合金表面孔径不同,但所得实验结果一致,含铜钛合金均促进巨噬细胞增殖,促进巨噬细胞M1极化和炎性表达,合金的表面孔径并不影响巨噬细胞的免疫调控效果;同时研究中还发现,铜的添加并不会改变合金的表面形貌和粗糙度,但可以增强表面润湿性,而巨噬细胞的形态并没有因表面润湿性的改变而发生明显变化。Chen等^[25]使用等离子体浸没离子注入的方法,将铜元素引入钛基板,使铜沉积在钛表面形成稳定的含铜氧化物层,以降低合金表面粗糙程度,然后对小鼠巨噬细胞RAW264.7进行体外实验,发现无论是巨噬细胞单独接种,还是与小鼠骨髓间充质干细胞mBMSCs共同接种于合金表面,含铜钛合金对巨噬细胞的增殖都没有不利影响,而且巨噬细胞均

呈抗炎表达趋势,更倾向于M2极化,下调炎症反应;另外,研究者还进行了小鼠的体内植入合金实验,含铜钛合金周围的免疫细胞显示出与体外实验一致的抗炎趋势。

对比Xu等^[21]和Chen等^[24]的实验,改变含铜钛合金的加工方式与表面结构,将巨噬细胞RAW264.7以不同孔密度接种至不同的含铜钛合金表面,均得到含铜钛合金抑制巨噬细胞炎性表达的结果。

含铜钛合金作为新型抗菌材料,植入体内后可以调控免疫细胞的活性,促进成骨发生,在骨科和口腔医学领域表现出了良好的应用前景。Xu等^[21]提出,含铜钛合金对牙龈成纤维细胞和成骨细胞均无毒性,并且能抑制巨噬细胞介导的炎症反应,促进血管生成,有望应用于促进牙槽骨再生的钛合金网片。在口腔修复领域,含铜钛合金的抗菌性能使得它不仅抑制口腔细菌如变形链球菌和牙龈卟啉单胞菌的增殖,抑制细菌生物膜的形成,还可以抑制细菌感染引起的骨吸收,有望用作新型义齿材料^[4]。

用钛合金制成的常规接骨板也被广泛应用于外科固定骨折,但由于合金表面生物膜的形成和细菌定植常导致临床使用受限^[26]。铜的添加能增加材料的抗菌性能,抑制炎症反应,巨噬细胞介导含铜钛合金植入后的免疫反应,当其极化激活为M2型时,可表达大量抗炎因子,下调炎症反应,促进组织愈合和再生。

然而,当前含铜钛合金的免疫效应研究仍存在一些问题,关于铜离子调控巨噬细胞极化的机制尚未统一定论,含铜钛合金如何调控巨噬细胞极化激活仍有待深入研究,同时缺乏足够的体内实验验证含铜钛合金的免疫调控效果,这都是含铜钛合金在临床进一步应用时必须考量的问题。

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