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Experimental models and perfusion assessments in vascularized bone grafts: a systematic review.

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Background and Objectives

Vascularized bone grafts (VBGs) are considered superior to non-VBGs due to their presumed advantages in preserving cell viability, resisting infection, and promoting faster osteogenesis. They are commonly used in cases involving large bone defects, poor vascularity, or previous radiation. However, definitive evidence for their superiority remains limited, and ethical constraints hinder direct comparative studies in human. This systematic review examines anatomical animal and cadaveric models of VBGs with a special focus on different perfusion assessment techniques. Only by ensuring perfusion is maintained in the grafts, can we expect to find any true difference to non-VBG comparison groups in subsequent experiments and eventually clinical practice.

Methods

The review was conducted according to PRISMA 2020 guidelines in February 2025. Databases searched included PubMed, Embase, and the Cochrane Library, using terms related to vascularized bone grafts and anatomical models. Studies were included if they involved original research on animal or cadaveric models assessing bone graft perfusion using contrast agents. Systematic reviews, clinical outcome reports, and studies lacking perfusion assessment were excluded. Data were extracted on specimen and graft type, arterial supply, contrast agent used, and perfusion assessment methods.

Results

25 out of 520 identified studies met the inclusion criteria. These involved cadaveric and animal models, examining a range of pedicled and free VBGs from various donor sites. Most studies used latex perfusion to facilitate dissection. Perfusion was assessed using diverse techniques: **Histology** was used to confirm osteocyte viability and marrow status, showing variable recovery timelines, with full viability reported as early as 3 weeks or as late as 14 weeks. Barium-based **CT** tracked vessel patency and proliferation, while nano-CT was used to quantify the vascular volume. **MRI** usually showed decreased T1 and increased T2 signals during early revascularization, yet these results had inconsistent correlation with actual flow. **Technetium-based scintigraphy** offers an indirect assessment as calcium uptake correlates with blood flow, leading to increased uptake in viable grafts. **Near-infrared fluorescence imaging** is used to visualize perfusion intraoperatively, while **angiography** and microangiography were used to identify proliferating periosteal vessels, a vessel density comparable to controls and corrected scintigraphic false-positives postoperatively. Cerium-141-labeled, **radioactive microspheres** quantified regional blood flow, revealing a pattern of initial hypervascularization of 3 weeks followed by stabilization at the level of untreated controls after 3 months. Furthermore, **fluorochromes** can be administered sequentially, the presence or absence of which in subsequent histology informs about vessel patency at different timepoints.

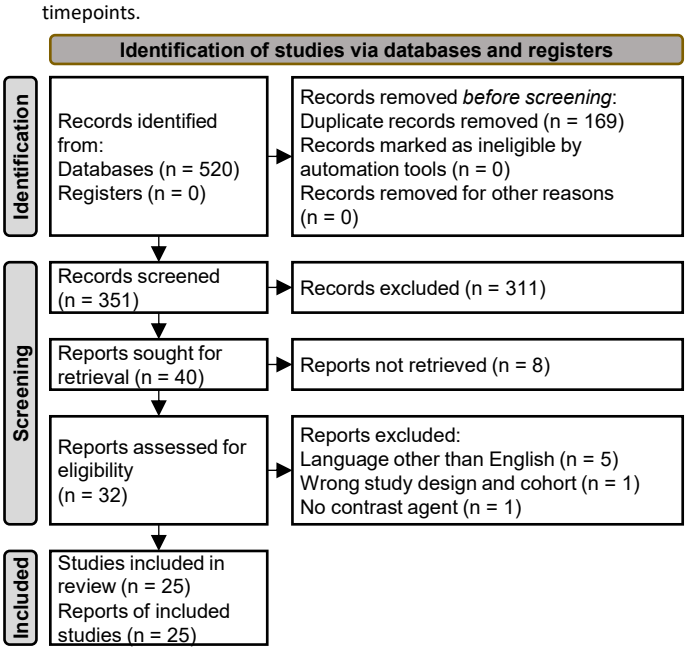

Figure 1: PRISMA 2020 flow diagram on the database search.

Discussion

Preservation of blood perfusion results in **VBGs** remaining viable and allows for accelerated osteogenesis without creeping substitution. The observed initial phase of hypervascularization is attributed to a reaction to ischemia due to new hemodynamic conditions, the sympathectomy achieved by skeletonizing the pedicle, or the inflammatory response. The physiological centrifugal **blood flow** from medullary vessels is reversed in ischemic situations. VBGs usually rely on periosteal perfusion and establish a circulation to medullary vessels. Monitoring these processes in experiments is difficult. Pure **latex** injections are used as guide during dissection, while radiopaque injections allow visualization with CT. Relationships between vessels and surrounding tissue are preserved, which is valuable in anatomical studies. However, polymerization, vessel ruptures due to over-perfusion, and inability to perfuse the capillary bed are limitations necessitating other modalities to assess in vivo perfusion. Yet, no single technique effectively and reliably monitors viability of VBGs, which is why most studies used a combination of several **perfusion assessment methods**. The advantages and limitations of these are demonstrated in Table 1. Spontaneous **neoangiogenesis** was identified as a confounding factor in assessing true vascular supply. Strategies to prevent this during experiments included chemical coatings or placing grafts in avascular environments.

	Advantages	Disadvantages
Histology	Reliable correlation with blood flow, spatial resolution, functional information	Time and personnel-intensive, destructive, incomplete 3D visualization, falsifications due to preparation techniques
Nano-CT	High resolution, non-destructive, 3D, no contrast agents	Ex vivo, time-intensive, lack of standardization
MRI	Non-invasive, high diagnostic accuracy, quantification of perfusion	Reliable only with contrast, operator-dependent, false-positive enhancement by capillary leakage, availability
Scintigraphy	Non-invasive, early detection of perfusion issues	Nonspecific, false-positives (inflammation, osteoradionecrosis, beginning creeping substitution of new periosteal bone), inhomogeneous uptakes, limited resolution, resource- and time-intensive
NIRFI	Real-time, time-efficient, high resolution, sensitivity	Operator-dependent, postoperative assessment restricted to skin perfusion, slow and incomplete clearance
Angiography	Direct visualization, measurement of vessel density, quantifiable comparison. *Microangiography*: precise postmortem analysis	Invasive, no information on bone metabolism, operator-dependent, risk of thrombosis
Microspheres	Precise, multiple measurements without direct access	Restricted to larger capillaries, complex, isotope handling, prerequisites (thorough mixing with blood, avoid shunting and reaching a sufficient threshold of radioactivity)
Fluorochomes	Highly specific, quantitative measurements, dynamic studies	Fluorescence overlaps, insufficient intensity, specialized equipment and expertise, failure of deposition (inactive osteons)

Table 1: Advantages and disadvantages of perfusion assessment techniques.

Conclusions

We provide an overview of perfusion assessment techniques used in research on VBGs. Characteristics between studies differed significantly. Diverse techniques are employed and each was shown to have its limitations. A multimodal approach is necessary to provide a comprehensive understanding of graft perfusion and viability in experimental models.

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